ABSTRACT

Title of Dissertation: ASSESSMENT OF FOODBORNE PATHOGEN SURVIVAL DURING PRODUCTION AND PRE-HARVEST APPLICATION OF COMPOST AND COMPOST TEA

David T. Ingram, Ph.D. 2009

Directed By: Sam W. Joseph, Professor (Emeritus), CBMG and Director, Maryland Institute for Applied Environmental Health

Jianghong Meng, Professor, NFSC, and Interim Director of the Joint Institute for Food Safety and Nutrition

The use of compost in crop production systems as a soil amendment is recognized by both conventional and organic plant production practitioners as a means to increase yields and reduce the incidence of foliar diseases. Compost tea (CT), an aqueous extract of the biological components of compost, is also recognized as a means to broadcast the phytopathogen-reducing components of compost directly to the surfaces of plants where many foliar diseases become established. CT has been shown to control the proliferation of a variety of foliar diseases in many turf, crop and horticulture production systems when applied directly to the foliar surfaces of plants. This dissertation research was designed to address several pre-harvest food safety

issues concerning compost and compost teas. Three objectives were pursued to establish whether the use of compost and compost teas as pre-harvest practices may introduce foodborne pathogens into the food supply and, therefore, contribute to the incidence of foodborne illness. The first objective involved a microbiological survey of commercially available compost in the U.S. to determine the prevalence of fecal coliforms, *Escherichia coli*, *Salmonella* and enterococci that might be reaching consumers through contaminated fruit and vegetables. The second objective was to investigate the ability of these foodborne pathogens to propagate during the production of CT. The third objective involved a field study examining the potential of CT to disseminate *E. coli* into organic and conventional strawberry production systems. The effects of CT on the fruit yield, phytopathogen suppression, as well as the potential for foodborne pathogen survival on the fruit surfaces were examined. This project provided important information and recommendations for the safe production and pre-harvest application of compost and compost teas. It was contended that, with proper attention to the manufacture and storage of compost and with simple modification of current trends in CT production systems, the current threshold of pre-harvest introduction of foodborne pathogens could be significantly reduced.

ASSESSMENT OF FOODBORNE PATHOGEN SURVIVAL DURING PRODUCTION AND PRE-HARVEST APPLICATION OF COMPOST AND COMPOST TEA

By

David Thomas Ingram

Dissertation submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
2009

Advisory Committee:

Professor Sam W. Joseph, Co-Chair
Professor Jianghong Meng, Co-Chair
Professor Thomas W. Castonguay
Dr. Mark A. Kantor
Dr. Patricia D. Millner

DEDICATION

This dissertation is dedicated first and foremost to my family who, through the years have provided the strength, security and unconditional love every professional graduate student needs to nurture his dreams, cultivate his ideas, wrestle with his demons, and find his own path in life and career (with gentle prodding). I know that no one will be more pleased than my father when he places a copy of this dissertation on his bookshelf next to his own, also earned at the University of Maryland. Dad, you will never know how much I have relied on your always sound advice, guidance and much needed camaraderie to provide me with direction -- sometimes with a kick in the gluteus maximus. Your pursuit of excellence is second to none, and our entire family is all the stronger for your leadership.

Mom, you have no idea how often I have tapped into your unbounded energy and amazing ability to remember the important things in life. Just thinking about you always puts a smile on my face.

Kirsten, my sister, thank you for your patience and positive encouragement that has kept me focused over the years. You have no idea how much I have enjoyed watching you become a wonderful mother for my niece, Ella and nephew, Thomas.

If not for my fabulous wife, Shannon Leigh Kondrad, I could not have accomplished this piece of work because of her selflessness and support. Words cannot describe my gratitude for her love. You are truly the woman of my dreams.

Finally, the individual who probably most inspired me to complete this dissertation is my daughter who will arrive this spring. I eagerly await your arrival so

that I can share with you all that I have learned about life, love and the pursuit of dreams.

This dissertation is dedicated to you all.

ACKNOWLEDGEMENTS

"It may be poop to you, but it's my bread and butter"

Sam W. Joseph, Ph.D.

I would first like to acknowledge Sam W. Joseph, Ph.D., my long-time mentor, counselor, professor, and friend who recognized the potential microbiologist in me back in the days when *E. coli* O157:H7 and other foodborne pathogens were mostly implicated in red meat and poultry. I cherished our time together and mentally processed every bit of advice that you gave me over the many research projects, "coffee house" discussions, golf outings, manuscript drafting, the completion of my M.S. Thesis, and the ups and downs associated with completing, finally, this dissertation. I would not be where I am today in my career without your guidance on coping with the many pitfalls and entrapments associated with putting pen-to-paper. My father repeatedly said, "David, you owe Sam Joseph much more than you realize right now." He was right. Your efforts to help me to earn the Ph.D. enable me to continue tinkering in the laboratory and exploring a science that I have learned to love. Thank you for your patience and all you have done for me.

Dr. Jianghong Meng, your interest in me as one of your first lab-technicians in the NFSC Department and later your support as I embarked on this Ph.D. journey will never be forgotten. Thank you. I hope that this dissertation will mark the beginning of more opportunities to collaborate with you.

Before I met Patricia Millner, Ph.D., I thought compost was simply a pile of poop, and that there was only one type of strawberry. Bless you for introducing me to the incredibly diverse and wonderful world of agriculture. Giving me the

opportunity, freedom and financial support to pursue my experiments, (even some you surely knew would fail), strengthened my instincts and methods and enabled me to find my scientific legs. Engaging me in your diverse and fruitful research programs in the USDA-ARS family has helped me to understand the interactions of medical, food and environmental microbiology. Thank you for all your support, guidance and friendship that enabled a seed, planted in a cooperative research agreement with the University, to grow through the medium of a Student-Career Experience Program into a full-bloom government career. In my book, you are a renaissance leader.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Overview

Food-borne bacterial pathogens cause significant morbidity in the United States, resulting in a notable monetary drain on the U.S. economy and medical resources. Gastroenteritis affects between 250 and 350 million Americans each year and 30% of these are attributed to foodborne pathogens (154). Historically, red meat and poultry products have been implicated as the principal food source harboring the bacterial pathogens traced to these outbreaks, namely *E. coli*, *Salmonella*, *Campylobacter* and *Listeria*. During the past three decades, however, fresh fruits and vegetables have become implicated in an increasing number of outbreaks involving these foodborne pathogens, thereby beginning to rival the number caused by meat and poultry (148, 149). Greig and Ravel, in 2009, analyzed all U.S. reported foodborne outbreaks that occurred in a ten year period between 1996-2005 (n=978, Table 1.1) and determined that 48.4% of the outbreaks were from meat sources and 24.5% were from produce (81). In a recent survey of 190 produce-associated foodborne illnesses, 48% of them implicated *Salmonella* as the major adulterant (226). During this study period the foods most often implicated in the produce-related outbreaks were leafy greens, juice, melon, sprouts and berries. This increase in produce-associated outbreaks has greatly affected all areas of the farm-to-fork spectrum. The USDA and FDA have recognized this trend and are in the process of organizing and mobilizing the support of joint research efforts to combine the latest technologies of industrial, academic and government institutions. It is well documented that foodborne

pathogens implicated in produce outbreaks have the ability to survive and even propagate during post-harvest processing, e.g. washing, packing, shipping and storing (120, 225, 248, 249). While significant attention has been applied to sanitation technology for post-harvest processing and consumer education, there is considerable room for improvement in advancing food safety on-farm through pre-harvest practices. Once microbial contamination has occurred on the farm, the existing safeguards in packing houses and fresh-cut processing facilities designed to prevent dissemination of these pathogens into the food supply are not always completely successful.

Table 1.1 : Number of reported outbreaks in the U.S. and associated food vehicles for the most frequently isolated pathogens between 1996 and 2005 (Adapted from J.D. Greig, 2009) (81)[a]

Foodborne Pathogen	Beef	Pork	Poultry[b]	Eggs	Dairy Products	Seafood	Produce
Escherichia coli	79		3		8	2	51
***Salmonella* spp.**	55	34	115	91	52	29	104
***Campylobacter* spp.**	5		34	3	9		2
Clostridium perfringens	14	2	6			2	5
Staphylococcus aureus	21	37	15	4	3	3	2
Norovirus	24	10	20	1	12	45	76
Total	**198**	**83**	**193**	**99**	**84**	**81**	**240**

[a] Information compiled from publicly available reports
[b] Includes sources from chicken, turkey and other poultry products

There is relatively little information on the potential for in-field contamination of produce from pathogen sources from air- (184), water- (233, 236, 240), manure- (178) or vector-borne- (126, 127) pathogens (e.g. transmission via insects and

nematodes). It has been shown that produce grown in soil or compost and/or irrigated with water contaminated with foodborne pathogens (e.g. *Salmonella*) can become infected as the organisms establish themselves within the soil, rhizosphere and even vascular plant tissue (115, 118, 233, 262, 263).

There is a substantiated concern that any production system utilizing manure products as fertilizers are vulnerable to microbial contamination. Since organic farmers are historically the main users of manure and manure-based composts as fertilizers, the quality of compost becomes an issue. The land and market-share of organic farming practices has increased: the estimation for world-wide land area dedicated for organic farming at the end of 2003 was 26.3 million hectares, which was 69% higher than in 1998 (163). In the first published on-farm microbiological assessment of produce, researchers found a prevalence of *E. coli* in produce samples that was 19 times greater on farms which used manure or compost aged less than 12 months (165).

National certification guidelines for organic producers that use manure and manure-based compost require either demonstrative evidence that their compost does not contain human pathogens or they must follow a 90/120 day rule after application of the (untested) compost or manure. This requirement means that the harvesting of produce may not occur until at least 90 days post-application of the manure or manure-based compost for crops that do not contact the soil (e.g., tomatoes) and harvest must occur at least 120 days after manure or manure-based compost application for crops that have direct contact with the soil (e.g., cantaloupes, carrots, lettuce). Further information is needed to substantiate or modify these ”safety”

regulations as in some studies the persistence of *E. coli* O157:H7 and *Salmonella* in the soil inoculated with artificially contaminated irrigation water and/or composts lasted over 120 days for *E. coli* O157:H7(113) and 231 days for *Salmonella* (114).

This research addressed the mission of NP 206 (Manure and Byproduct Utilization) and NP 108 (Food Safety) by assessing the potential for contamination of fresh market produce prior to harvest via compost and gain further insight into safe production and utilization of compost tea, a common method of applying beneficial properties of compost to fresh produce prior to harvest.

Although the United States regulatory agencies spend considerable effort and resources to maintain the integrity and safety of the food supply, problems or breaches in this extremely broad food-production system persist and can cause serious human illness or death. The detection and control of foodborne pathogens was an important thrust area that began with President Clinton's Food Safety Initiative in 1997 and continued with the Bush administration's National Integrated Food Safety Initiative (NIFSI). The latter was assigned to the CSREES (Cooperative State Research Education and Extension Service) branch of the USDA: (http://www.csrees.usda.gov/nea/food/in_focus/safety_if_national.html). Research programs funded under the CSREES-NIFSI use an integrated approach (research, education and extension or outreach) to address potential risks to the food supply from farm to fork. This direction is an integral component of the government's effort to reduce the incidence of foodborne illness. Recent outbreaks, involving spinach and more currently, peanut butter, has prompted the current Obama administration to address significant gaps in previous food safety initiatives to boost consumer

confidence that all food produced and imported into the U.S. is safe for consumption. The pending Food Safety Modernization act of 2009 (H.R. 875) is a wide-sweeping bill that proposes the creation of a new federal agency within the Department of Health and Human Services to consolidate and focus efforts to protect the public health by preventing foodborne illness, ensuring food safety and boosting consumer confidence. If H.R. 875 is passed by Congress, the new agency will be called the Food Safety Administration.

Compost:

Evolution from historical reference to state-of-the-art

Compost is the end result of a natural, microbiologically-dependent degradation of raw materials into a nutrient-stabilized final product that has the consistency of highly organic soil (1). Compost retains much of the nutrient properties of the initial feedstock; however the final products are be generally free of foul odors, human pathogenic microorganisms and avoid the problems associated with phosphorus and nitrogen run-off that has been contributing to environmental eutrophication. The composting process is believed to have been used since pre-historical times, as early agricultural practices recognized the benefit of converting animal manures and other biodegradable materials into fertilizer that we now call compost (66).

Historical evidence of the composting practice can be found on clay tablets dating to as early as 2300 B.C. in the Akkadian empire in Mesopotamia on the banks

of the Euphrates river (195). Ancient Chinese and Indian literature, the Bible, Talmud, 12th-Century Arab writing, medieval Church texts and Renaissance literature provide evidence that the Romans, Greeks, Israelites, India and Chinese populations also used compost (230). While the Native Americans and early European settlers on the North American continent recognized the benefits of using manure and fish as fertilizer, one of the highest-profile references to the use of compost in the United States dates to its first president. George Washington, in 1787, directed the construction of a "dung repository" adjacent to his Mount Vernon estate stables to store and later use the cured bedding/manure mixtures on his agricultural fields (96, 185, 230).

Innovation met ingenuity, when a British agronomist, Sir Albert Howard, in 1905 spent 30 years in Indore, India attempting to develop a systematic and scientific basis for the agricultural practices that had been used in China and India for centuries (15). The documented early 20th century advancements in organic farming practices began with Howard's attempts to streamline the composting process, called the *Indore method,* which recognized the need to start the process with piles containing three times more carbonaceous materials (plant waste) than nitrogenous materials (animal waste), and with turning the piles at six week intervals to generate compost in 4- 6 months (102). Howard's work stimulated much interest in the science of compost and, for his efforts, he is considered the "modern day father of organic farming" (230). Variations of the Indore method were developed by Dr. Varman Acharya in 1939 to refine previous methods that involved large amounts of labor and materials as prescribed by Howard (161). Called the *Bangalore method*, compostable

materials were layered into 1 meter deep trenches, each layer sprinkled with water and the entire trench covered in mud. This method had the benefit of composting human waste (termed night soil) with little or no need for manipulation after the piles were constructed. This process required 5-6 months to produce a finished product.

Attempts to expedite the composting process were paramount for farmers, who required the use of this compost for each season. One early such example, called the Chinese ”high temperature” composting method, modified the Indore and Bangalore methods by infusing oxygen into the piles using hollow bamboo pipes (62). The resulting aeration increased the compost temperatures to 60-70^{o}C within a few days and reduced the total composting time in half by that of the other methods. J. Rodale, the founder and proprietor of a farming research center in Kutztown Pennsylvania (Rodale Institute), continued development of the Indore method and was instrumental in introducing Americans to the value of compost for improving soil quality (195). The Twentieth Century was a boon for the composting industry, as industrialized approaches are now commonplace to control the exponentially increasing amounts of human and industrial wastes in an environmentally friendly manner (92). The ability to produce good quality compost in 2-3 months is now obtainable with the aid of modern machinery, instrumentation and rigorous process controls.

The biological principles involved in the composting process has not changed since ancient times, but Twentieth Century advances in technology now provide the tools necessary to expedite the composting process, handle an enormous amount of raw materials and ensure the quality of the finished products. Compost has

traditionally been the mainstay soil conditioner and fertilizer of organic farming practices and many conventional practices are increasingly utilizing compost as an environmentally friendly practice that also promotes sustainability.

Modern compost production methods

Composting systems can be categorically divided into two main methods: aerated (windrows, aerated piles) and non-aerated ("in-vessel" or "reactor" systems) (244). These categories can be further subdivided by management styles where the compost is either agitated (through mechanical turning to introduce oxygen and homogenize the materials) or static (where the initial compost pile structure is maintained throughout the process). Two main composting production systems that are routinely used for on-farm composting of residues are the Static Pile Method (Figure 1.1) and the Windrow Method (Figure 1.2). Static pile composting is perhaps the most widely practiced method of handling on-farm residues, because it is the simplest method requiring little, if any, management. Windrow composting is practiced by most large farming or industrial operations that generate enough waste materials to justify the expense of equipment necessary for windrow construction and maintenance. Both systems are described below.

Compost terminology, not unlike the science of composting itself, is imprecise and subject to various interpretations depending on the practitioner. For this review, "windrow" composting reflects an active process that incorporates mechanical turning and, when necessary, the addition of moisture. "Static piles" refer to the simpler method of stockpiling the on-farm residues (manure, straw, hay, grass clippings, feed, bedding materials, etc.) that may or may not incorporate active

aeration systems. Reports in the literature are often inconsistent and confusing in their use of compost terminology. Therefore, attempts to compare their methodologies are difficult. For one example, Manser and Keeling, in 1996, refer to both systems (and other designs) as "windrow composting systems" (151).

The microbiota required for the decomposition of organic matter is already contained in most farm residues and such that, when simply constructed into a pile to provide some insulation from ambient temperatures, the composting process begins immediately (as observed by microbial activity, rise in temperature and subsequent steam generation). The composting process is due to metabolic conversion of the plant and animal residues by indigenous microorganisms (bacteria and fungi) contained within the residues into a humus-like material rich in plant nutrients (15). The nutrients (carbon and nitrogen) contained within the waste residues supply the microbes with sufficient fuel to perform this task without the need for any additional additives, except for perhaps more moisture to maintain a high level of metabolic activity. The simplest on-farm practice of stock-piling residues can take a very long time to decompose (over six months), but this composting process can be expedited with a few management modifications. For example, with the proper attention to moisture and oxygen levels in the piles, the composting process time can be reduced to two to three months.

Figure 1.1 : Static compost piles constructed at the Beltsville Area Research Center composting research facility

Figure 1.2 : Windrow compost piles constructed at the Beltsville Area Research Center composting research facility

Figure 1.3 : Windrow "turner" actively homogenizing and aerating a compost windrow during the first few weeks of the compost thermophilic stage

The static-pile composting process, when constructed with residues of appropriate nutrient and bulking content, will contain the necessary microbiota to maintain the core pile temperatures for efficient thermophilic composting without any additional management. Static piles can maintain thermophilic temperatures for several weeks and even longer if they are covered with a layer of straw, hay or soil to provide an insulated layer against environmental conditions. The most efficiently composted material is that which has a carbon-to-nitrogen ratio of roughly 30-to-1 (201). Guidelines and on-line computer programs are readily available to provide operators with the necessary nutrient information for their on-farm residues. These calculators are invaluable for determining the amounts of residuals required to maintain proper C:N ratios. One such calculator can be found at the following

website:

http://www.klickitatcounty.org/SolidWaste/fileshtml/organics/compostCalc.htm.

This method can produce finished compost in 4-6 months, depending on the size of the pile, feedstock, moisture availability, insulation, ambient conditions, etc. Static pile composting can be dramatically enhanced by the addition of an active aeration system that integrates perforated pipe underneath the pile that is connected to electric blowers to constantly introduce air throughout the pile. This can speed up the composting process due to increased aerobic microbial activity and enhanced decomposition of the feedstocks.

Windrow composting is well suited for operations that produce or collect a large amount of residual waste. In this operation, the waste materials are laid in parallel rows two to three meters high and three to four meters wide and can be as long as needed. The windrows can be tailored to fit the exacting dimensions of the space and available equipment used to manufacture and maintain them. Figure 1.2 illustrates such windrows constructed using a front-end loader into 25 meter long rows of residues. After construction, 50-60% moisture content might be required to achieve optimal composting (201). The windrows should be turned several times a week during the initial thermophilic composting phase (about 55^{o}C) and then less frequently as the compost core temperatures begin to cool and eventually reach ambient. Turning the windrows actively incorporates oxygen throughout the windrow as well as homogenizes the materials which maintain high levels of microbial activity required to speed up the system. Larger composting facilities can greatly benefit from the purchase of additional equipment such as a windrow turner

(Fig 1.3) that straddles the windrow and may be self-propelled. Smaller windrow operations may simply use a front-end loader to effectively homogenize the materials. If left unturned, most of the microbiota at the windrow core will be starved of oxygen as the convection and diffusion currents of oxygen into the windrow is not sufficient to supply the aerobic demand, at least for the early thermophilic phases of the composting process. This passive aeration where ambient air enters the sides of the windrow and leaves through the top is called the "chimney effect" (93). If relying only on passive aeration, the residual decomposition will be slow and the composting process will require more than a year for completion (238). Windrow maintenance can be minimized by the addition of "bulking" feedstocks, such as wood chips, straw and leaves which can provide enough porosity in the windrows to facilitate passive oxygenation by the natural convection of oxygen currents throughout the windrow, as well as to reduce the number of anaerobic "dead" zones that may be present within the windrow. The composting process can also be expedited by simply shredding or grinding the feedstocks prior to windrow construction, which will reduce the size of each particle for more efficient microbial degradation (250).

Physico-chemical factors affecting the composting process

Carbon:Nitrogen Ratio

There are two chemical elements of importance when evaluating feedstock suitability for the composting system: carbon and nitrogen. Even more important, however, is their ratio to each other, which is termed the C:N ratio. Carbon and nitrogen serve as the primary nutrient sources for initiating and maintaining microbial populations responsible for the composting process. Carbon primarily serves as the

main energy source and nitrogen is essential for construction of essential proteins required for cellular structure and function (121). Nitrogen balance is necessary for proper composting. In nitrogen limiting environments, the microbial biomass will be limited and the composting process will be diminished, with a corresponding loss of nitrogen in form of ammonia (NH3+) or leaching from the compost which reduces its overall value as a fertilizer. In general, the best C:N ratio for composting feedstocks should be 30:1, which is optimal for the microorganisms responsible for the mineralization and humification processes (16, 76).

Moisture

One of the most important production-management variables during the composting process is the regulation of moisture. Moisture is necessary for all microbiological functions, and the need is balanced by oxygen levels and metabolic activity. Low (< 20%) moisture content in compost will prevent effective microbial activity by preventing soluble nutrients from reaching and dehydrating the microbiota (12). Conversely, a high (> 75%) moisture content in compost will create anoxic pockets in the windrow by filling the pores between feedstock particles with water, preventing oxygen from reaching the biomass thereby reducing microbial activity due to anaerobic or microaerophilic environments (35, 246, 247). Optimal moisture content of 60-70% is ideal during the initial windrow construction, and can be reduced to 50-60% after the initial peak-heating cycle has been completed (68).

pH

The optimal pH range for microorganisms is generally between 6.5-7.5 (15, 159). Bacteria are negatively affected by changes above or below these optimal levels. The pH level in compost varies during the process. The initial thermophilic phase is often characterized by a drop in pH, likely due to the initial breakdown of easily biodegradable wastes which stimulates the formation of short-chain (i.e., lactic acid) and volatile fatty acids and the evolution of carbon dioxide produced by the massive explosion of microbial activity (8, 88, 131, 168, 186). The organic acids produced during the decomposition process are transitory. The compost pH gradually increases with the microbial utilization of organic acids and evolution of volatile fatty acids and carbon dioxide (216).

The microbial population can be greatly affected by changes in the compost pH, and studies have shown that maintaining control of the pH during the composting process can dramatically accelerate the biodegradation process (167, 168, 228). In general, however, manipulation of the compost pH is not necessary to produce good quality product. In some instances, however, where the pH of compost is above 7.5, there will be much nitrogen loss in the form of ammonia. In such an instance, one study has effectively used elemental sulphur as a compost amendment to reduce the pH (152). Optimal "finished" compost pH values are usually between 5.5 and 8.0.

Temperature

There is a dynamic pattern involving the ecological succession of various microbial populations throughout the composting process, which is due, in large part, to the temperature profile achieved throughout the composting process. Careful

monitoring of the compost temperature is necessary to determine the status of the overall process. The optimal temperature for the composting process is 40-65^{o}C (37). Miller, in 1992, determined that the most efficient temperature for decomposition is between 52-60^{o}C (159). Since windrow compost can reach temperatures above 70^{o}C within a few days, the temperature should be monitored because microbiological activity is dramatically reduced at this high temperature (60). The regulation of temperature can be achieved by manipulating the compost pile through turning, by modifying its shape or the addition of moisture.

Uses and benefits

The global population has reached 6.7 billion in 2008, and is expected to reach 7 billion by 2013 (259). Traditional landfills will soon be unable to keep up with the increasing amounts of waste generated by the human population. Composting is an environmentally friendly, safe and simple method for recycling virtually all animal and human waste. It is an attractive alternative to landfills.

Composting is particularly suited to on-farm waste management, where vast quantities of organic waste materials are generated in the form of animal manures, bedding, straw, wood chips, and grass clippings among other materials. A single Holstein cow produces approximately 105 lb of manure daily (170). Most farmers use a minimal form of manure management that basically involves stockpiling their manure and/or crop residues either in a covered location or open field. This pile is usually left untouched during the winter months until spring when it is then applied to the fields. Dairy, poultry and swine manures are rich in nitrogen that is mineralized quickly and, when applied to the fields, these nutrients are almost immediately

available to the plants. The available nutrients in manures provide for an aggressive growth response in the crops similar to that after application of inorganic fertilizer. An unfortunate side effect of this practice, however, is that a large amount of these nutrients follows the rain table and is widely believed to be the main contributor to the eutrophication of streams and bays caused by the translocation of these same nutrients. The widely accepted theory is that the field application of raw manures and stockpiled manures is causing nitrogen and phosphorus runoff and groundwater leachate to enter our freshwater reservoirs, lakes and bays (220, 229).

One of the many benefits of using compost (instead of raw manure) as a soil conditioner is that the composting process transforms the nitrogen contained in the manure into a more stable organic form, which is less susceptible to leaching into the groundwater (201). Properly made and used compost virtually eliminates this problem while achieving the same end-results of applying inorganic fertilizers: i.e., larger, healthier crops. The added benefits to using composts include a much healthier soil in all of its aspects: improving microbiological diversity, physical (structure, porosity, water retention) and chemical properties (nutrients, pH, ion exchange capacity) (41).

Soil Conditioner

While compost can dramatically improve the physico-chemical and microbiological qualities of soil to make it healthy, conventional agricultural and horticultural practices result in the depletion of soil organic matter which ultimately leads to poor soil fertility and lower crop yields over time (98). Compost is one example of an organic amendment that has been used to effectively improve the

quality of low-nutrient soils by the repeated infusion of organic matter content (69, 71, 176, 177). Composts are rich in carbonaceous fractions that provide readily-available nutrients to the existing multitude of microbiota. These nutrients can dramatically enhance the physiological activity as well as increase microbial population diversity (173). The influx of organic materials also increases the aggregation, nutrient content and water holding capacity of the soils which improves irrigation efficiency (153, 196). If compost is routinely integrated into agricultural practices, soil fertility can be similarly improved (191). The Long-Term Research on Agricultural Systems project (LTRAS) at the University of California-Davis collected soils and tomato fruits over a ten year period from both conventional and organic agricultural plots and determined that organically-grown fruits contained higher levels of the flavenoids quercetin and kaempferol as compared to those grown conventionally (162). The study attributed the superior nutrient levels in the organically grown fruits to increasing amounts of soil organic matter accumulation with the additional benefit of reducing the need for manure fertilization in the organic plots each year over the ten year study. Caris-Veyrat et. al., in 2004, also determined that the fresh pulp from organically grown tomatoes contained more antioxidant "microconstituents" such as vitamin C, carotenoids and polyphenols than conventionally grown tomatoes (27). Despite these two studies, among others, that purport the nutritional superiority of organic produce, the general consensus is that there is no overall statistically significant difference in the nutritional content between the same fruit or vegetable cultivars grown either conventionally or organically (278).

Plant Disease suppression

One hypothesis for the increase in the incidence of a variety of foliar and root pathogens over the past century, is that 20th century agronomic and horticultural practices have systematically reduced soil fertility by decreasing organic matter inputs (6, 98). Because conventional methods involving soil fumigation (205), chemical fungicides (251) and solarization (181) for controlling these pathogens are currently under scrutiny for their detrimental environmental and public health effects, there is a renewed interest in using compost as an organic input to control a variety of plant diseases (140). Over the past 30 years, there have been more than 1,000 published studies to determine the effects of compost amendments on the following significant plant pathogens: *Rhizoctonia solani*, *Pythium* spp., *Fusarium* spp., *Phytophthora* spp., *Verticillium dahlia*, *Sclerotinia* spp., *Thielaviopsis basicola*, *Aphanomyces* spp., *Sclerotium* spp. and *Macrophomina phaseolina* (17).

Despite vast amounts of continued research, the mechanisms of pathogen suppression are not entirely understood, and are reflected in the lack of reliable predictors of the ability of compost to inhibit plant pathogens. Proposed factors involved in the ability of compost to reduce phytopathogens include various types of feedstock (245), compost maturities (256), application rates (214), introduction of phytopathogen inhibitors or natural pesticides (99, 284), microbial antagonism (136, 137) and induction of systemic resistance (182). Several attempts were unsuccessful in correlating pathogen suppression with various chemical, physical or microbiological constituents in compost (9, 32, 40, 61). After analyzing 36 composts from a diverse array of feedstocks, Scheuerell et. al. in 2005, determined that

suppression of disease caused by *Pythium* spp. correlated with an increase in compost microbial activity (as measured by CO^2 respiration), as compared with any other tested biological (i.e., specific microbiological populations) or physico-chemical parameters in the composts (212). Other studies have shown the potential use of another measure of microbiological activity, FDA (fluorescein diacetate hydrolysis) activity, which reflects the activity of several enzymes (i.e., non-specific esterases, proteases, lipases). FDA activity has been shown in several reports to correlate inversely with *Pythium* disease suppression (32, 242). Erhart, et. al, in 1999, however, found a positive correlation to microbial activity (as measured by FDA) and extractable phenolic compounds to the incidence of disease caused by *Pythium ultimatum* (61). Control of *Rhizoctonia solani* remains the most difficult plant pathogen to control using compost. Scheuerell et. al., in 2005, found that the ability of compost to suppress disease caused by *R. solani* did not correlate with microbiological activity or any other biological or physico-chemical parameters (212). Indeed, some studies have determined that the populations of this aggressive saprophyte actually increased after amendment with industrial papermill waste (34) or household waste compost (213).

The attractiveness of chemical fungicides to farmers who rely on immediate and proven effects is not surprising. Thus they overlook the use of compost (or other organic inputs) in their phytopathogen remediation repertoire. Most farmers rely on immediate and complete control of soil-borne and foliar pathogens to ensure a successful harvest season. However, there is little doubt that the environmental and ecological benefits for using composts far outweigh inconsistencies in their ability to

remediate phytopathogens. Much additional research is required for the development of specific composts that are able to provide consistent suppression of phytopathogen disease.

Compost as an energy source

If the old proverb "necessity is the mother of invention" holds true, then the beginning of the 21st century foretells a boon in innovative technologies to provide energy for an increasingly demanding human population. The search for alternative fuel sources to replace the rapidly diminishing supply of petroleum products has already led to innovative "green" technologies in the form of harnessing energy from the sun (solar panels), wind (turbines) and earth (geothermal coils). A lesser-known technology, pioneered by an even lesser-known Frenchman named Jean Pain, was reported by Pain in 1973, and then in 1980, in his self-published book entitled *Another Kind of Garden* (172). Pain, who experimented in harnessing thermal and chemical energy from the composting process, documented methods in his writings to ultimately eliminate energy dependence on fossil fuels. In fact, he was able to supply his house (1000 ft^2) with sufficient methane to supply cooking fuel and electricity, heat and hot water (up to 4 L/ min) for 6 months by channeling water and methane through a simple array of tubing in a 50 ton compost pile (Figure 1.4). The implications for this alternative and earth-friendly energy source are endless. Indeed, this technology has just begun to receive attention as the drive for such alternative energies is powered by the rising cost of conventional fuels. Compost is an entirely renewable, sustainable, carbon neutral and inexpensive energy source. One company

is already developing these ideas to maximize the recovery of thermal energy from food-waste compost by supplying heat and energy to greenhouses (19).

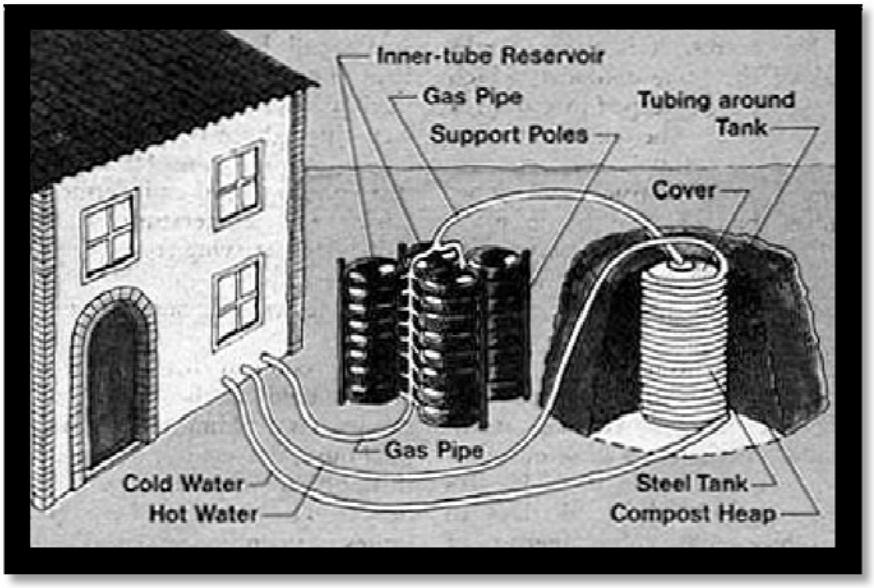

Figure 1.4 : Illustration of Jean Pain's experimental design for harnessing thermal energy from compost (172)

Human Pathogens in Compost

United States Composting Standards

Since compost is typically prepared using manure or yard-waste feedstocks that may contain enteric pathogens, one of the goals of the composting process is either to eliminate these pathogens or reduce them to below infectious levels (24, 38, 89). It is generally accepted that animal and human waste materials contain pathogenic enteric microorganisms that, if ingested, may contribute to significant human illness including gastroenteritis (Table 1.2). With the introduction and increasing use of biosolids as agricultural amendments in the last 30 years, the awareness and reduction of human pathogens in composts became paramount.

The United States government first established regulations in 1979 to ensure that all materials containing biosolids follow microbial "pathogen reduction" processes to effectively reduce human exposure (54). The 1979 USEPA regulations incorporated disinfection "**P**rocesses that are designed to **S**ignificantly **R**educe **P**athogens" (PSRP, e.g., anaerobic digestion, aerobic digestion and lime stabilization) and "**P**rocesses to **F**urther **R**educe **P**athogens" (PFRP, e.g., heat drying, composting and pasteurization). The PSRP methods were designed to reduce the number of pathogens by several logs; however, the final product may still contain high numbers of pathogens if the original source material is highly contaminated. Therefore, PFRP methods employ strategies to significantly reduce pathogens to below detection limits. The USEPA, in 1993, revised the rules to standardize the acceptable limits of pathogen content in materials containing biosolids (including compost), the details of which are located in the 40 CFR part 503 regulations referred herein as "Part 503"

(51). The 1993 Part 503 rules parsed out all biosolids-based materials into two classifications, *Class A* and *Class B*, which are defined by the concentrations of pathogen-indicator organisms in the final product. The choice of indicator organisms was based on studies by Yanko (1987) and Farrell (1993) that correlated the levels of fecal coliforms to that of *Salmonella* and other pathogens that might be detected in sewage sludge compost (63, 281). The USEPA uses the presence and quantity of both fecal coliforms and *Salmonella* to determine the classification of composted sewage sludge products.

Class A materials are defined as those that can be sold to consumers for immediate and unrestricted use and have much higher standards for pathogen content than Class B materials (i.e., Class A materials should not contain any detectable pathogens). The specific microbiological pathogen-indicator limits that are placed on Class A materials are based on the determination of the levels of *Salmonella* (less than three MPN per four grams of solids) and fecal coliforms (less than 1000 MPN per gram of solids). Class B materials refer to those that have had some PSRP processing to reduce pathogens, but the materials may still contain them in significant concentrations (i.e., fecal coliforms less than 2 $x10^6$ MPN per gram of solids). As such, Class B materials are restricted for use in areas where they would not expose the public to risk of contamination (55). The PSRP and PFRP regulations in terms of pathogen indicator reduction standards are summarized in Table 1.3, and the microbiological-quality classification system used to determine the use of biosolids and biosolids-based materials is in Table 1.4.

Table 1.2 : Potential microbiological pathogens contained in biosolids, manure and green-waste composts

Bacteria	Viruses	Parasites
Salmonella spp.	Enteroviruses	*Taenia*
Escherichia coli	Hepatitis A	*Ascaris*
Enterobacter	Coxsackieviruses	
Yersinia	Echoviruses	
Streptococcus	Reoviruses	
Proteus	Adenoviruses	
Pseudomonas	Parvoviruses	
Klebsiella	Pestiviruses	
Citrobacter		

Adapted from De Bertoldi et. al.(38); Nell, J.H et. al (169); Epstein (60); USEPA (52)

Table 1.3 : USEPA requirements for reduction of pathogens in biosolids and biosolids-based materials

PSRP[a]	PFRP[b]
> 1 log reduction of *Salmonella* spp. > 2 log reduction of fecal coliforms > 1 log reduction of enteroviruses	> 3 log reduction of enteroviruses > 2 log reduction of viable *Ascaris* ova

[a] Process for significant reduction of pathogens
[b] Processes to further reduce pathogens

Table 1.4 : USEPA classification system for determining use restrictions of biosolids and biosolids-based materials

Pathogen Indicator	Class A[a]	Class B[b]
Fecal coliforms	less than 10^3 MPN/g (dry weight)	less than $1x10^6$ MPN/g (dry weight)
Salmonella spp.	less than 3 MPN/4g (dry weight)	

[a] Biosolids-based materials that exhibit the above "Class A" microbiological characteristics have unrestricted usage and may be sold in the consumer market.
[b] Biosolids-based materials that exhibit "Class B" microbiological properties are restricted from usage that pose significant risks to humans, e.g. crop fertilization, land application or disposal to sites with immediate public access or animal grazing.

Pathogen reduction in compost

Composting results in the reduction of pathogens through several mechanisms, including: thermal destruction, competitive exclusion (competition) with indigenous microorganisms, production of inhibitory compounds (antibiotics) produced by indigenous fungi and actinomycetes, natural die-off in the non-native environment, and nutrient depletion (39, 60, 88, 97, 179). All pathogens have a specific tolerance threshold to different time/temperature combinations. Since temperature is perhaps the simplest and most appropriate parameter for the operator to measure and catalogue during the composting operation, this is the parameter that is also regulated by the USEPA for initiating pathogen reduction (88). The manufacture of "class A" compost must follow specific time/temperature guidelines *for all particles* in the windrow. For example, windrow operations must achieve a temperature of 55°C (131°F) for a minimum of 15 days, during which time the windrow must be turned (homogenized) a minimum of 5 times (53).

The time-temperature USEPA "Class A" regulations for biosolids-based windrow composting (Table 1.5) are now the accepted composting standards in the U.S. for ensuring the destruction of human pathogens in all feedstock materials. These standards were based on a large body of experimental evidence studying the effects of thermal destruction on *Ascaris*, a parasitic roundworm that is present in high numbers in fecal material from infested animals and have a very low infestation dose for both animals and humans (146). *Ascaris* ova are highly resilient to chemical and temperature disinfection strategies (88), and are extremely persistent in the environment. Infestation in animals has been shown for *Ascaris* ova in soils after 15 years (146). Brannen et. al, in 1975, determined that 99.8 % of *Ascaris* ova lose viability in 6 minutes after exposure to 55^{o}C in laboratory saline solutions (18). One study by Hays, in 1977, determined that *Ascaris* ova can be completely inactivated in compost exposed to 60^{o}C for 30 minutes (91). Another study cited by Huag, in 1993, showed a loss of *Ascaris* infectivity during windrow composting meeting class A regulations (55^{o}C) within 10 days (88). Combined with the experimental evidence that *Ascaris* is the most heat-resistant of all fecal pathogens (24, 64, 192), the loss of *Ascaris* infectivity in windrow composting should positively correlate with the destruction of other enteric pathogens (198).

Table 1.5 : "Class A" regulations for pathogen reduction in compost

Composting Method	Time/Temperature Criteria
Windrow	≥ 55^{o}C for 15 days, with 5 minimum turning events during this time
Static aerated pile or In-vessel reactor	≥ 55^{o}C for 3 days

Pathogen Indicators

Due to the high number of potential pathogens in fecal material, the expense and time required to detect and enumerate all of them is prohibitive. Thus most regulations involve the detection of indicator organisms (which are not pathogens although some may be considered to be opportunistic pathogens), to merely indicate the potential for the presence of pathogens. Fecal coliforms are used in the Part 503 regulations as a pathogen indicator, since experimental evidence correlated the reduction of fecal coliforms to *Salmonella* spp. and other pathogens (63, 281). The "class A" regulations in Part 503 suggest that if the final compost products contain less than 1000 MPN/g fecal coliforms (indicator organisms), then the levels of pathogens (e.g., *Salmonella*) will be below detectable limits and therefore pose an insignificant risk to the population (58).

Gaps in Part 503 Regulations

Pathogen reduction during the composting process depends not only on the high temperature achieved in the compost, but also on the exposure time of each microorganism to the high temperature. The Part 503 regulations stipulate that every particle in the compost must be exposed to the specific time/temperature regulations as outlined in table 1.5, and extensive research with laboratory-based composting reactors suggests that this regulation is sufficient to achieve pathogen disinfection. However, an increasing body of evidence suggests that these temperatures are difficult to achieve for all particles during on-farm composting (e.g., windrow operations). Thus lower temperatures in some windrow locations may result in residual pathogens in the final compost product. Windrow temperatures depend on

many factors, including location (core temperature is highest), C:N ratios (feedstock composition), oxygen levels (affected by turning events and feedstock porosity), wind speed, solar radiation, ambient temperature, and humidity (258).

Low temperature regions in windrow composting are a concern, not only for inconsistencies in eliminating pathogens, but also because pathogens may be able to propagate in these areas. Shuval et. al., in 1991, showed that *Salmonella* was able to re-grow in the cooler exterior areas of windrows (221) and Haug, in 1993, and Christensen et. al, in 2002, showed that *Salmonella* could re-populate in windrows as temperatures dropped after the completion of the thermophilic phase (31, 88). Turner, in 2002, further emphasized the need for careful temperature monitoring and effective homogenization (e.g., turning) in windrow operations as the levels of *E. coli* actually increased during composting at sub-optimal temperatures ($\leq 35^{o}C$) (257).

A recent regional-specific microbiological survey (Brinton et. al. 2009) on a variety of non-sludge based composts (n=94) produced with a variety of technologies (e.g., windrow, aerated static pile and turned static pile) in California, Washington and Oregon found that one compost sample contained *Salmonella* (0.45 MPN/g dry weight), 28% contained fecal coliforms above the Part 503 regulations, and 6% contained *E. coli* 0157:H7 (21). Conversely, Edrington et. al, in 2009 performed another microbiological survey of 11 dairy-manure composting operations (the location and composting methods were not reported). They found that all samples were culture negative for both *Salmonella* and *E. coli* O157:H7 (48). Another study (non-peer reviewed) included a survey at 16 biosolids-based composting facilities in Massachusetts. Soares and Cardenas, in 1995, determined that one third of finished

compost products did not meet the minimum Part 503 standards for fecal coliform content (231).

Guidelines are needed to standardize temperature monitoring in the windrow with respect to location, frequency and whether to determine if either discrete or average temperatures should be recorded. These gaps are a major limitation in ensuring that Part 503 regulations are met and are problematic when analyzing data from the literature. Similarly, Part 503 regulations do not specify where in the windrow samples should be removed for microbial compliance testing. This is disconcerting because, as research has shown, pathogens can not only survive but thrive in cooler locations within the windrows (on the surface, and ends or "toes"). These "cold" regions within the large windrow biomass may not be detected due to insufficient or infrequent temperature monitoring causing reported temperatures to inadequately reflect the entire windrow and giving a false indication intended to be met by Part 503 regulations (73, 203). Also, it is too commonly inferred that thermophilic windrow measurements of 55^{o}C taken several days apart were maintained during the interim days. This inference should not be made, since fluctuations reading as high as 15^{o}C per day have been shown in windrows (243).

Compost Tea

Definitions and Development

Compost tea, defined in the simplest terms as "watery extracts of compost," has been used in agriculture since ancient times. History is replete with references to ancient Egyptians, as well as Romans using water sprays based on composted soil, plant materials, animal manures and human wastes to enhance crop yields and reduce the incidence of foliar diseases (134). The advent of the development and use of chemical pesticides and herbicides in the 20th century has all but eliminated the incentive for using compost and compost teas for agricultural purposes; however, the pendulum is rapidly swinging back to using organic methods for crop production due to increased awareness of their benefits to human and environmental health and the need for sustainable agricultural practices. The late 20th century brought an increase in (mostly anecdotal) "evidence" about the benefits of compost teas in horticulture and agriculture. More recently, scientific evidence has been slow to confirm the benefits of compost teas (208). Superficial evidence from practical usage of compost teas includes remarkable reduction in foliar diseases, enhanced soil and rhizosphere microbial communities, improved soil structure and a reduced need for fertilizers and fungicides. Most early reports regarding CT refer to the simplest "non-aerated" form of CT production that involves placing compost in a bucket of water and allowing this mixture to "steep" for up to ten days before applying to foliage and/or soil. Modern manufacturing methods for CT emerged in the U.S. with the advent of the first commercial production system in 1997, which produced CT in 24 hours through mechanical extraction and aeration methods that usually involve addition of

supplemental nutrients (www.GrowingSolutions.com). In testament to the influence of anecdotal evidence and the power of "word of mouth" communication, American industry recognized CT as a potentially lucrative market and responded with the development of dozens of commercially available devices designed to facilitate the manufacture of ACT, all of which involve mechanical aeration and agitation to rapidly and efficiently extract the nutrients and microbiota from compost. If presence of compost tea on the Internet is any indication, a trend in growing popularity can be seen from historical "Google" searches: the term "Compost Tea" received 1900, 4000, 13,000 and 327,000 "hits" in 2001, 2003, 2005 and 2009, respectively, in the Google search engine. Information was found to involve everything from instructional manuals and commercial application services to undocumented information touting the benefits of CT.

The proliferation of CT production methods and reports in the last 20 years has resulted in some confusion over terminology (208). Many terms have been used to describe CT, including: compost tea, aerated compost tea, non-aerated compost tea, compost extract, watery fermented compost extract, amended extract, organic tea, steepages and slurries (20, 42). The term "compost tea" now universally refers to the end-product of recirculating water through compost that is contained in a porous bag while maintaining aerobic conditions in the liquid (194). More specifically, this process is now cited as aerated compost tea (ACT) and has been the focus of recent commercial manufacturing systems to produce CT more rapidly by infusing atmospheric oxygen into the CT during the brewing cycle. Aeration is accomplished by using either a vortex nozzle (111), an impeller (www.greenorganics.biz), venturi

nozzles (www.composttea.com), fine bubble diffusion mats (www.growingsolutions.com), or aquarium-style aeration stones (109). Many aerated compost tea designs involve re-circulating water through a suspended bag containing compost, and then allowing the watery extract to oxygenate as it flows back into the tank (157, 194). "Compost extracts" (273) generally refer to non-aerated CT preparations that fall into a broad category of terms including "watery fermented compost extracts" (271, 272), "steepages" (100), and "compost slurry" (33). Actually, all of these terms refer to the simplest CT production method of mixing one-part compost to five- or ten-parts water and held under static conditions (not-stirred or aerated) in a container for three-to-ten days (23, 271).

Because compost tea has become popular and routine practice among many farmers, the terminology to describe this extremely diverse process is slowly becoming standardized. There are currently two main production methods: aerated (ACT) and non-aerated compost tea (NCT). Both methods require a vessel, compost, water, incubation time and a filtration method prior to application. Both methods may (or may not) include the addition of nutrients prior to the brewing process and the addition of spray adjuvants; i.e., "spreaders" or "stickers" prior to foliar application. These two methods differ basically in the brewing process: ACT is actively aerated (or oxygenated) with the inclusion of various mechanical devices, and NCT is effectively left untouched or occasionally mixed by stirring the compost/water mixture.

There is still much debate concerning the recent popular movement toward ACT usage, as the vast majority of scientific evidence for reducing foliar and root

diseases involves the application of NCT (208). Furthermore, the production of ACT requires the additional expense of equipment and energy inputs. New studies that show positive responses to ACT on crop yields and reductions of a variety of foliar diseases are slowly emerging (Table 1.6).

Uses and benefits

Foliar Disease Suppression

Compost tea contains many of the same beneficial properties of compost from which it is prepared. However, unlike compost, CT has the ability to be applied to the phyllosphere (where many phytopathogens thrive). Interest in and the use of CT as a spray for biocontrol of foliar and fruit diseases or as a soil drench for plant-growth promotion, as well as a biocontrol of root diseases (23, 105-107, 253, 273, 274), has expanded among some growers in the U.S. and abroad. Compost teas are increasingly used in both organic and conventional farming operations for introducing the benefits of compost (e.g. microbiota, nutrients) where the broadcasting of large amounts of compost would be cost prohibitive. A review of the literature reveals studies that have used ACT and NCT to evaluate their control of various significant plant diseases including: powdery and downy mildews, fungal and bacterial blights, leaf spots, apple scab and grey mold (Table 1.6).

There are very few studies, however, that compare ACT to NCT in terms of preventing phytopathogen disease. One study used both ACT and NCT under controlled environments to study their respective phytopathogen suppressive effects on powdery mildew (*Sphaerotheca pannosa* var. *rosae*) on field-grown roses (209). , Using three different compost sources to produce ACT and NCT, Scheuerell and

Mahaffee, in 2000, found that, although all CT treatments resulted in significantly lower disease incidence than the water-spray controls, no significant differences were seen between the ACT and NCT treatments. Cronin et al, in 1996, compared the *in vitro* effects of ACT and NCT produced from manure-based spent mushroom compost on the germination of *Venturia inaequalis* conidia, a fungus that causes apple scab (33). They concluded that NCT effectively inhibited conidial germination, while ACT had no effect. A recent horticultural study comparing ACT and NCT in suppressive ability for gray mold (*Botrytis cinerea*) in Geranium plants determined that 85% of the tests produced statistically similar suppressive capabilities. However, where there were statistically significant differences, it was shown that NCT was more consistent than ACT in reducing disease severity (211). Haggag and Saber in 2007 found that both ACT and NCT, produced from either plant residues or chicken manure compost, inhibited conidia germination of both *Alternaria porri* (purple blight) and *Alternaria solani* (early blight) in *in vitro* and greenhouse experiments. However, using field trials, it was determined that NCT was more effective than ACT in suppressing early blight in tomatoes and purple blight in onions (84).

Table 1.6 : Experiments involving aerated compost tea (ACT) and non-aerated compost tea (NCT) on plant disease suppression: 1988-2009

Pathogen	*Host*	*Disease*	*Method*	*ACT/ NCT*	*Control*[a]	*Pathogen Inoculum*	*Compost Type*	*Source*
Alternaria alternata	tomato	blight	Field	ACT	-	Natural	Not reported	Granatstein 1999
Alternaria panax	ginseng	blight	In vitro Seed assay	NCT	- -	5 x 10^5 spores/ml	Spent Mushroom	Yohalem et al 1994
Alternaria porri	onion	Purple blight	In vitro Greenhouse Field	ACT	+ + -	Conidia, not stated Natural	Plant residues and chicken manure	Haggag and Saber 2007
Alternaria septoria	tomato	blight	Field	ACT	-	natural	Vermicompost	Barker-Plotkin 2000
Alternaria solani	tomato	early blight	Field	NCT	+	conidia, not stated	cattle manure	Tsror, 1999
	tomato		In vitro Greenhouse Field	NCT	+ + +	Conidia, not stated Natural	Plant residues and chicken manure	Haggag and Saber 2007
	Potato		Field	ACT	-	Natural	Vermicompost, wood chips, thermal compost	Al-Mughrabi 2006
Blumeriella jaapii	Cherry leaf	leaf spot	Field	ACT	-	Natural	Not reported	Pscheidt and Wittig 1996
Botrytis cinerea	Geranium	gray mold	Greenhouse	ACT NCT	+/- +/-	1 x 10^5 spores/ml	29 different sources	Scheuerell and Mahaffee 2006
	Tomato		field	NCT	+	natural	horse, sheep, cattle	Hmouni et. al. 2006
	Strawberry		Field	ACT NCT	+ +	Natural	Cattle and chicken manure	Welke 2004
	bean		In vitro Leaf assay	NCT	+ +	2 x 10^6 spores/ml	Wheat straw, horse, cattle and chicken manure	McQuilken et al 1994
	bean		Leaf assay	NCT	+	2 x 10^6 spores/ml	cattle manure	Urban and Trankner 1993
	grape		Leaf assay berries	NCT	+ +	2 x 10^6 spores/ml	horse-straw-soil	Ketterer et al 1992
	grape berries		Field	NCT	+	2 x 10^6 spores/ml	horse-straw-soil	Ketterer et al 1992
	lettuce		Greenhouse	NCT	+	2 x 10^6 spores/ml	horse bedding, chicken litter	McQuilken et al 1994

[a] '+' indicates statistically significant decrease in disease (P = 0.05); '-' indicates no significant disease suppression
Adapted from Scheurell and Mahaffee 2002 (208) and Litterick et. al. 2004 (145)

Table 1.6 continued

Pathogen	Host	Disease	Method	ACT/ NCT	Control[a]	Pathogen Inoculum	Compost Type	Source
Botrytis cinerea	strawberry strawberry		Field Field	NCT	- -	natural natural	Cattle manure Chicken manure	Welke 1999
	strawberry strawberry		Field Field	NCT	+ +	natural natural	Cattle manure Horse manure	Stindt 1990
	strawberry		Field	NCT	+ early - late season	2×10^6 spores/ml	horse-straw-soil	Urban and Trankner 1993
	tomato pepper grape		Leaf assay Leaf assay berries	NCT	+ + +	2×10^5 spores/ml	Cattle manure	Elad and Shtienberg 1994
	tomato pepper grape		Leaf assay Leaf assay berries	NCT	+ + +	2×10^5 spores/ml	horse manure	Elad and Shtienberg 1994
	tomato pepper grape		Leaf assay Leaf assay berries	NCT	- + +	2×10^5 spores/ml	Grape marc	Elad and Shtienberg 1994
Choanephora cucurbitarum	ochra	Wet rot	In-Vitro Greenhouse	ACT	+ -	5×10^4 spores/ml	Chicken manure, rice straw and oil palm fruit	Siddiqui et. al. 2009
Clavibacter michiganensis	tomato	canker	Greenhouse	ACT	+	1×10^9 cfu/ml	Vermicompost	Utkhede and Koch 2004
Cochliobolus carbonum	maize	leaf spot	In Vitro Seed Assay	NCT	+ +	5×10^5 spores/ml	spent mushroom	Yohalem et. al. 1994
Diplocarpon rosae	rose	black spot	field	ACT	+	natural	fruit	Anon, 2001
(pathogen not reported)	lettuce	drop rot	field	ACT	- spring + summer	natural	not reported	Granatstein 1999
Erysiphe betae	sugar beet	powdery mildew	In vitro	NCT	+	not determined	organic waste	Samerski and Weltzien 1988
Erysiphe graminis sp. Hordei	winter barley	powdery mildew	leaf assay	NCT	+	not determined	horse manure, straw, soil	Weltzein 1989
Erysiphe polygoni	bean	powdery mildew	greenhouse	NCT	+	not stated	not stated	Ketterer and Schwager 1992
Erwinia carotovora sp. Carotovora	potato	soft rot	field	ACT	-	natural	Vermicastings, wood chips, thermal compost	Al-Mughrabi 2006

[a] '+' indicates statistically significant decrease in disease (P = 0.05); '-' indicates no significant disease suppression
Adapted from Scheurell and Mahaffee 2002 (208) and Litterick et. al. 2004 (145)

Table 1.6 continued

Pathogen	Host	Disease	Method	ACT/ NCT	Control[a]	Pathogen Inoculum	Compost Type	Source
Fusarium sp.	dry rot	potato	field	ACT	-	natural	Vermicastings, wood chips, thermal compost	Al-Mughrabi 2006
Fusarium oxysporum sp. cucumerinum	none	wilt	In-Vitro	NCT	+	5 x 10^7 spores/ml	hemlock bark	Kai et al. 1990
Helminthosporium solani	potato	silver scurf	field	ACT	+	natural	Vermicastings, wood chips, thermal compost	Al-Mughrabi 2006
Monilinia fructicola	Peach	brown rot	field	ACT	-	natural	not reported	Pseidt and Wittig 1996
Monolinia taxa	cherry	brown rot	field	ACT	+	natural	not reported	Pseidt and Wittig 1996
Phytophthora infestans	potato	late blight	field field	NCT NCT	- +	natural	horse, straw, soil	Ketterer 1990
	potato		Seedling field	NCT NCT	+ -	not reported	not reported	Jongebloed et. al. 1993
	tomato		greenhouse	NCT	+	not reported	not reported	Ketterer and Schwager 1992
	tomato		leaf assay	NCT	+	8 x 10^4 spores/ml	horse, straw, soil	Ketterer 1990
	potato tomato		leaf assay leaf assay	NCT NCT	+ +	not reported	horse, goat, hog, straw, soil	Weltzien 1989
	potato		leaf assay In Vitro	ACT ACT	- -	not reported	commercial compost tea product	Sturz et. al. 2006
	Potato		Field	ACT	-	Natural	Vermicompost, wood chips, thermal compost	Al-Mughrabi 2006
	potato		In Vitro Greenhouse	ACT ACT	- -	1 x 10^4 spores/ml	Vermicompost	Olanya and Larkin 2006
Plasmopara viticola	grape	downy mildew	leaf assay	NCT	+	8 x 10^4 spores/ml	horse, straw, soil	Weltzien and Ketterer 1986
	grape		field	NCT	+	8 x 10^4 spores/ml	horse, straw, soil	Ketterer 1990
	grape		leaf assay greenhouse	NCT NCT	+ +	1 x 10^4 spores/ml	cow dung, soil	Achimu and Schlosser 1991

[a] '+' indicates statistically significant decrease in disease (P = 0.05); '-' indicates no significant disease suppression
Adapted from Scheurell and Mahaffee 2002 (208) and Litterick et. al. 2004 (145)

Table 1.6 continued

Pathogen	Host	Disease	Method	ACT/ NCT	Control[a]	Pathogen Inoculum	Compost Type	Source
Pseudomonas syringae	Arabidopsis	bacterial speck	seedling	NCT	+	1 x 10^8 cfu/ml	pine bark	Zhang et al. 1998
Pseudopepiza tracheiphila	leaf blight	grape	field	NCT	+	natural	horse, straw, soil, spent mushroom	Weltzien 1989
Podosphaera leucotricha	apple	powdery mildew	field	ACT	-	natural	not reported	Pscheidt and Wittig 1996
Post Harvest Loss	Blueberry		field	ACT	+	natural	not reported	Granatstein 1999
Rhizoctonia solani	potato	black scurf	field	ACT	+	Natural	Vermicompost, wood chips, thermal compost	Al-Mughrabi 2006
Sphaerotheca pannosa	rose	powdery mildew	field field field field field field	ACT ACT ACT NCT NCT NCT	+ + + + + +	natural natural natural natural natural natural	Chicken manure Yard debris Mixed source Chicken manure Yard debris Mixed source	Scheuerell and Mahaffee 2000
Sphaeropsis sapinea	red pine	shoot blight	In Vitro Seedling	NCT NCT	+ +	5 x 10^5 spores	Spent mushroom	Yohalem et. al. 1994
Sphaerotheca fuliginea	cucumber	powdery mildew	leaf assay	NCT	+	not reported	various	Samerski and Weltzien 1998
Streptomyces scabei	potato	common scab	field	ACT	-	Natural	Vermicompost, wood chips, thermal compost	Al-Mughrabi 2006
	potato		field	ACT	+	natural	not reported	Al-Mughrabi 2008
Taphrina deformans	peach	leaf curl	field	ACT	-	natural	not reported	Pscheidt and Wittig 1996
Uncinula necator	grape leaf grape cluster	powdery mildew	field field	ACT ACT	- +	natural	not reported	Pscheidt and Wittig 1996
	grape		field	NCT	+ +	natural	cattle horse	Sackenheim 1993
	grape		greenhouse	NCT	+	natural	horse, straw, soil	Weltzien 1989
Venturia inaequalis	apple leaf apple fruit	apple scab	field	ACT	- -	natural	not reported	Pscheidt and Wittig 1996

[a] '+' indicates statistically significant decrease in disease (P = 0.05); '-' indicates no significant disease suppression
Adapted from Scheurell and Mahaffee 2002 (208) and Litterick et. al. 2004 (145)

Table 1.6 continued

Pathogen	Host	Disease	Method	ACT/ NCT	Control[a]	Pathogen Inoculum	Compost Type	Source
Venturia inaequalis	not reported		In-Vitro In-Vitro	ACT NCT	- +	5×10^4 spores/ml	spent mushroom	Cronin et. al. 1996
	apple		In-Vitro seedling	NCT NCT	+ +	5×10^5 spores/ml	spent mushroom	Yohalem et. al. 1994
	apple		field field	NCT NCT	- -	natural natural	spent mushroom cattle manure	Andrews 1993
	apple		field	NCT	+	natural	spent mushroom	Yohalem et. al. 1996
	apple		field	NCT	+	not reported	manure, straw, soil	Trankner and Kirchner-Bierschenk 1988
Venturia pirina	pear	pear scab	field	ACT	-	natural	not reported	Pscheidt and Wittig 1996
Xanthomonas vesicatoria	tomato	bacterial spot	greenhouse field	NCT NCT	+ +	1×10^8 cfu/ml	cow manure	Al-Dahmani et. al. 2003

[a] '+' indicates statistically significant decrease in disease (P = 0.05); '-' indicates no significant disease suppression
Adapted from Scheurell and Mahaffee 2002 (208) and Litterick et. al. 2004 (145)

Mechanisms of disease suppression

There are many theories regarding the ability of CT to suppress foliar pathogens, most of which involve the direct interactions of the CT microbiota applied to the phyllosphere. Theories include: competitive exclusion, antagonism, predation, antibiotic production, and induced systemic resistance in the host. It is possible, given the diverse array of the microbial community found in CT, that most or all of these factors are involved. It is clear, however, that the ability to inhibit phytopathogens is dependent on the microbiota contained within the CT. There is evidence that CT microbiota are able to produce metabolic products (i.e., antibiosis) and/or effectively compete for phyllosphere surfaces (i.e., exclusion) and nutrients (i.e., competition) to the detriment of phytopathogen development.

Several studies suggest that microbiological competition for nutrients and phylloplane surface areas may be the main mode of phytopathogen suppression by CT (260, 276, 277). Studies have shown that when the microbiota are effectively eliminated from CT, either by filtration or autoclaving, the suppressive properties are also eliminated (273). Ketterer, in 1992, correlated the total culturable biomass in NCT to suppression of *Botrytis cinerea* on grape leaves, by showing that heat sterilizing the NCT nearly eliminated disease suppression (128). Siddiqui, in 2009, found that heat and filter sterilized ACT prepared from agro-waste compost was not effective in suppressing wet rot (Choanephora cucurbitarum) of okra, while the non-sterilized ACT was effective in preventing disease (222).

The extent to which attachment and survival of CT microbiota to phylloplane surfaces after application is dependent on many variables including available

attachment sites, atmospheric conditions, available nutrients and the fitness of individual microbes. Trankner, in 1992, showed that NCT application can increase the phylloplane populations of total heterotrophic bacterial populations by 10^3 cfu/cm^2 on bean leaves; these populations can be maintained for at least five days under moist conditions, but reduced by 3 Logs under dry atmospheric conditions (253). Siddiqui, in 2009, finding that the environmental conditions could be detrimental for the ACT microbiota, thereby suggested that frequent application of ACT was necessary to maintain disease suppression of wet rot in okra (222). While it is apparent that the microbiota in CT is a necessary component for foliar disease suppression, it is still unclear whether specific populations are responsible, and whether the suppression is a result of direct competition or antibiosis.

Some evidence for the production of antibiotic metabolites in CT comes from studies by Al-Dahmani et al, in 1998, that suggested suppression of tomato bacterial leaf spot (*Xanthamonas campestris* pv. *Vesicatoria*) was due to an extractable, heat-stable metabolite contained within the compost feedstock used to prepare the CT (2). Cronin et. al., in 1996, determined that a heat-stable, non-protein antibiotic compound was necessary for inhibiting germination of *Venturia inaequalis* conidia (causes apple scab). In their study, when NCT was prepared using sterilized spent mushroom compost, there was no suppression. However, when NCT was prepared from the same (non-sterilized) compost, then filter-sterilized through a 0.1µm filter, suppression was achieved and equal suppression was seen even after autoclaving the filtrates (33).

Induction of plant "immuno-defense" systems is another theory for CT's ability to suppress phytopathogens. Several studies suggest that plants may respond to ACT and NCT application events systemically by inducing defense mechanisms. Early work by Samerski and Weltzien, in 1988, show NCT-treated sugar beets responded to *Sphaerotheca fuliginea* conidia (powdery mildew) through morphological changes in host cell walls, characterized by increased papillae formation, lignification, necrotic reactions and deformation of hyphae ends (204). Zhang et. al., in 1998, observed an increase in beta-1,3-glucuronidase (GUS) activity in response to NCT application events to Arabidopsis (285). GUS is a marker for the plant defense gene induction system. Siddiqui, in 2009, determined that ACT applied to okra plants stimulated the induction of host resistance genes, as measured by the increase in peroxidase, polyphenol oxidase and phenylalanine ammonia lyase enzymes, all of which have a role in resistance to plant diseases (222).

Enhanced soil microbial community structure

One of the benefits of the application of compost as a soil amendment is that the microbiota of the soil is enhanced. It is widely purported that compost tea also enhances the quantity and diversity of soil microbiota after application, although research is limited in providing conclusive evidence to this effect. One study investigated ACT alone and in conjunction with various other biological treatments and crop rotations to study effects on the soil microbial community in relation to potato disease (138). Larkin determined that soil-applied ACT was able to alter the soil microbial community structure, producing significantly less soil-borne potato

disease and increased harvest yields. However, this effect was only seen during a particular crop rotation (barley/clover) treatment. The ACT treatments were not able to suppress soil-borne disease on potatoes when the crops were not rotated with another planting in subsequent years. But Knewston, in 2009 did not find any significant differences in soil microbiological respiration or biomass after as much as three CT applications (133).

CT as fertilizers

Recent reports suggest that compost teas have the ability to supply plants with nutrients, thereby reducing the added cost of supplying plants with fertilizers. Hargreaves et. al, found that NCT prepared from municipal solid waste compost and ruminant manure compost was able to supply similar amounts of nutrients to strawberry (87) and raspberry (85) plants as the respective compost (and fertilizer) treatments. Knewston, however, in 2009, could not conclude that CT applications had improved plant nutrient uptake after two years of ACT applications to collard greens grown either organically or conventionally (133).

Evolution of U.S. Compost Tea Production Methods

Unfortunately, current trends in compost tea manufacturing in both industry and small farm practitioners in the U.S. have not evolved from science-based information. Somewhere along the CT evolutionary line, there has been a leap from the sound practices based on scientific research to the current practices seen throughout the United States based largely on anecdotal evidence and reports outside of peer-reviewed journals. A large contribution to our knowledge concerning the

efficacious usage of non-aerated compost teas (NCT) emanates from controlled studies by Trankner (253-255) and Weltzien (272-277). These and a large number of other studies since have shown that NCT has the ability to suppress a variety of foliar diseases (Table 1.6). It is likely that once news of this technology hit the American "organic movement" in the early 1990's, entrepreneuring agricultural engineers recognized the potential of CT manufacturing as a potentially lucrative new industry. Thus began the quest for producing the perfect CT, which interestingly is not supported by scientific study and literature.

The current trend in U.S. ACT production suggests that the older, more established European methods of NCT manufacture are inefficient (requires three to ten days), ineffective (as compared to ACT) and perhaps even dangerous (i.e. may contain human pathogens). Proponents of current ACT manufacturing methods maintain that modern technology can dramatically reduce the time needed for producing effective CT to 24 hours by using machinery to facilitate the extraction of nutrients, organic matter and microbiota of the compost feedstock. The reduction of CT production time to 24 hours is very attractive for farmers that require multiple applications and they do not have to gamble on weather conditions prior to producing and applying the tea. Furthermore, in attempts to generate ACT with the maximum quantities of bacteria and fungi within 24 hours, a common trend has been to supplement the ACT with a variety of nutritional supplements (including molasses) to stimulate microbial growth. Again, there are many unsubstantiated reports that suggest adding nutritional supplements to ACT improves both the microbiological content and diversity necessary for improving phytopathogen suppression.

Food Safety Concerns

The first report that enteric bacteria may be present in NCT produced from a variety of compost sources was from Stindt, in his 1990 doctoral dissertation (Table 1.7) (239) and reported in a review of biological control using NCT by Weltzien (271). Urban and Trankner reported that the enteric populations of NCT could also be increased with the addition of yeast extract (10g / L) (260). While it is well known that most members of the Family *Enterobacteriaceae* are not considered true human pathogens, Yohalem et. al., in 1994, suggested that enteric pathogens may also be propagated during the production of NCT (283). Indeed, if the conditions are conducive to the propagation of enteric bacteria during the CT brewing process, then pathogens such as *E. coli* O157:H7 and *Salmonella* would also be able to survive and perhaps even increase to infectious levels.

Table 1.7 : Counts of *Enterobacteriaceae* for Non-Aerated Compost Teas (NCT) after 8 days of extraction

Compost Type	Cfu/ml
Cattle Manure	2×10^6
Grape Marc	3×10^5
Horse Manure	5×10^6

Adapted from Stindt 1990 (239)

The introduction of human pathogens into the food supply using contaminated CT on fresh fruit and vegetable crops is a concern. Only one researcher, Sylvia Welke in Montreal, has provided scientific evidence to suggest that fecal coliforms may be transferred to produce exposed to foliar applications of NCT. In a series of

field and greenhouse studies using leek, broccoli and strawberries as a model, Welke applied ACT and NCT containing low concentrations of fecal coliforms and determined persistence on harvested broccoli and leek tissue (Tables 1.8 and 1.9) (268-270). It should be noted that Welke produced both the ACT and NCT without nutrient additives, and in 1999, used chicken manure-based compost that was determined to contain below detectable levels of fecal coliforms (< 3 MPN/g). In all studies, the CT was applied twice weekly to all crops; however, it is not clear how many fecal coliforms might have been applied to the produce. Also the amount of time that had elapsed between CT applications and the harvest for microbiological analyses was not reported. If perhaps Welke had produced CT using nutrient additives, as is standard practice among farmers, then the levels of fecal coliforms might have propagated to much higher concentrations in the CT. And if the CT was applied up until the day before harvest, the produce samples might also have contained higher levels of surviving fecal coliforms. Furthermore, it is hard to infer the epiphytic fitness of *E. coli* or *Salmonella* from the data presented only for fecal coliforms. The survival of fecal coliforms on the produce is an important indication for the potential for contamination of human pathogens, but studies are needed using *E. coli* and/or *Salmonella* as a model. The current issues and concerns involving the potential for human pathogens to propagate during ACT/NCT production methods has eclipsed any beneficial attributes attributed to CT and has stimulated the formation of a national Task Force in 2003 which reviewed and provided CT recommendations to the National Organic Standards Board (NOSB).

Table 1.8 : Summary of compost tea trials adapted from Welke 1999 (270)

	chicken manure compost		*cattle manure compost*	
	Fecal coliforms	***Salmonella***	**Fecal coliforms**	***Salmonella***
compost	ND[1]	ND	930	ND
NCT[2]	0.8	ND	35	ND
Broccoli	3.0	ND	ND	ND
Leek	43	ND	ND	ND

[1] Not Detected (below detection limits)
[2] NCT (8 day extraction) produced without nutrient additives
[3] All microbiological counts are reported as MPN/ml (for CT) and MPN/g (for compost and produce samples)

Table 1.9 : Summary of compost tea trials adapted from Welke 2001 (269) and 2004 (268)

	Chicken compost	***Cattle manure compost***					
	NCT (8:1)[a]	**ACT (8:1)[a]**	**ACT (4:1)**	**NCT (8:1)**	**NCT (4:1)**	**Strawberry**	**Broccoli**
Salmonella	ND[b]	ND	ND	ND	ND	ND	ND
Total Coliforms[c]	40.0	2.3	15.0	23.0	93.0	ND	15.0
Fecal Coliforms	ND	0.4	ND	1.5	0.9	ND	ND

[a] ACT and NCT (8 day extraction) produced from cattle manure compost without nutrient additives. Dilution of water: compost (vol:vol) in parenthesis.
[b] Not Detectable (below detection limits)
[c] All microbiological counts are reported as MPN/ml (for CT) and MPN/g (for produce samples)

Human Pathogens in Compost Tea

It is a common misconception that compost is a pathogen-free product. As previously noted, *E. coli* and *Salmonella* have the potential to survive and even thrive in thermophilically prepared composts that do not follow strict time temperature guidelines as outlined in the Part 503 regulations. While this may not be a concern for composts that are land applied, as low levels of soil-inoculated *E. coli* and *Salmonella* are rapidly degraded in this environment, there is a concern that human pathogens may propagate in current CT production systems. Even more disconcerting is the fact that these CT products are often applied to fruits and vegetable commodities that may be consumed raw. Only three peer-reviewed publications were found to study the ability of *E. coli* to propagate in CT as prepared by the current CT manufacturing recommendations (22, 44, 123), and each has been discounted by current practitioners and CT proponents as "unrepresentative of real-world scenarios."

The available microbiological research has illuminated two critical components of modern ACT manufacture: nutrient amendments and aeration. There appears to be a misconception among CT proponents that *E. coli* and *Salmonella* grow better under anaerobic conditions. Indeed, this may be one reason for the movement away from European NCT methods and why the current focus in modern CT brewing systems pays careful attention to the amount of oxygen that is "infused" during the 24 hr brewing cycle. This is obviously a gross misconception, however, as it is common for enteric bacteria such as *E. coli* and *Salmonella* (and other facultatively anaerobic bacteria) to be cultured typically under aerobic conditions in

the laboratory (166). Regardless, statements are still being made that, if ACT is maintained at oxygen concentrations above 6 ppm (mg/L), then enteric bacteria will not be able to compete with the multitude of indigenous flora that is also extracted from the compost (108, 110).

There has been limited research on the ability of *E. coli* and *Salmonella* to survive and/or propagate during the production of compost tea. The current strategy of modern ACT manufacturing methods is to produce the largest and most diverse bacterial and fungal populations possible in the shortest amount of time. This objective is achieved by the addition of one or more of several nutrient additives (i.e., molasses, yeast extract, kelp, humic acid and fish hydrolysates), which may provide an immediate "bloom" of the indigenous microbial populations extracted from the compost. The obvious concern with this practice is that the additives may also provide any indigenous enteric pathogens with enough nutrients to propogate well above their infectious dose concentrations.

Duffy et. al., in 2004, studied the effect of *E. coli* O157:H7 and *Salmonella* Thompson in response to two types of compost and molasses in CT bioassays (44). They placed 20 g of either chicken manure or dairy manure compost into 500 ml Erlenmeyer flasks containing 180 ml of sterile water, which was then incubated on a rotary incubator/shaker (100 rpm) at 20^{o}C for three days. Each flask was prepared with either *Salmonella* or *E. coli* O157:H7 at a concentration of 1.0 cfu/ml to which molasses was also added at concentrations ranging from 0-1 % (vol/vol.) At the end of three days, microbial concentrations were determined. When molasses concentrations were below 0.2%, no re-growth of any pathogen was found. Above

0.2% molasses concentration, however, there was a positive correlation between the growth of both pathogens and molasses concentration. Duffy concluded that the common practice of adding molasses-based nutrient amendments to the production of ACT should be avoided to prevent the potential propagation of residual human pathogens that may be present in compost. While these results are informative, this study has been criticized by practitioners and CT proponents because the CT brewing method used in this study does not accurately reflect methods currently used by industry. Also, Duffy et. al. did not report the concentration of oxygen in their experiments that some proponents claim is necessary for the proper production of ACT (and reduction of human pathogen populations).

Brinton et. al., in 2004, studied the growth response of *E. coli* using two commercial ACT brewing methods that were commonly used as "best practice" methods at the time of their study and included nutrient amendments and maintained oxygen levels above 6 ppm (22). They also included a traditional European NCT brewing method (without nutrient amendments) in their experimental design to compare any differences that *E. coli* populations may have as a result of the amount of aeration and added nutrients. It was determined that when no *E. coli* were introduced into the CT brewing system then *E. coli* was not detected in the final ACT or NCT products. When high levels of laboratory broth-cultured *E. coli* ($> 10^4$ cfu/ml) were inoculated at Time Zero into each system, there were high levels of recoverable *E. coli* in each 24 hr ACT (> 11,000 MPN/ml) and much fewer recovered *E. coli* in the NCT (4 MPN/ml). The researchers concluded that despite the high levels of competing heterotrophic populations and aeration (6 ppm oxygen) in the 24

hr ACT, *E. coli* was able to survive and maintain concentrations at or above the inoculum levels when nutrient amendments were used. However, the inoculated *E. coli* populations tended to immediately decline in the European NCT, even though the concentrations of competing heterotrophic bacteria were approximately half that contained in the corresponding ACT. Results suggest that the use of nutrient-based amendments during the production of ACT is conducive to the propagation of *E. coli* and therefore should be avoided. Critics point out that, in the field, thermophilically processed compost will never have the high levels of *E. coli* ($>10^5$ cfu/ml) that was used in this study. The conclusions have been criticized as not appropriate for an industry that still maintains that, when ACT tea is properly aerated, the small amounts of *E. coli* that may be present in compost will not be able to effectively compete with the indigenous microbial populations even when nutrient amendments are added. Further study is necessary to determine what effects the indigenous populations of total heterotrophic bacteria, as extracted at several time points throughout the brewing cycle, have on the growth and/or survival of *E. coli*.

A third peer-reviewed publication concerning the effects of nutrient amendments on foodborne pathogens in ACT observed the growth responses of a non-pathogenic *E. coli* strain to different amounts of molasses and kelp in both ACT and NCT. Kannangara et. al., in 2006, produced laboratory compost teas in 2 L jars by suspending 26 g compost in 1 L water, inoculating with $1x10^6 – 1x10^7$ cfu/ml *E. coli* and continuously aerating the jars at 0.8L/min for 48 hr at room temperature (123). Various concentrations (0, 0.1, 1.0, 2.0, 4.0, 8.0 g/L) of either molasses or kelp were used to determine the effects of nutrients on *E. coli* populations during ACT

production. Kannangara also studied the effects of various compost sources (e.g., dairy, swine, horse manure compost and vermicompost) on the *E. coli* population. They confirmed the findings of Duffy et. al (2004) that a positive correlation exists between the *E. coli* population and concentrations of molasses and kelp ranging from 0.1 to 8.0 g / L in ACT. Swine manure compost provided the highest sustained population of *E. coli* while vermicompost produced the lowest. One important contribution that was underplayed in this paper was the fact that the addition of carrot juice in both ACT and NCT was able to dramatically reduce the concentration of inoculated *E. coli*. While this research provides excellent information regarding the ability of different compost sources to influence the growth of *E. coli,* and one additive that may inhibit or reduce *E. coli* populations, it has also been criticized for its inability to make "real world" comparisons to current ACT production practices. It is difficult for CT practitioners to glean useful information from studies using laboratory flasks and equipment that bear no resemblance to what is being used in the field, as well as from studies using unrealistic *E. coli* "contamination" events and at microbial concentrations largely above what would normally occur in thermophilically treated compost.

A fourth study, presented by Bess et. al. at the 2002 International Symposium Composting and Compost Utilization (13), analyzed the ability of *E. coli* as contained in naturally contaminated (immature) compost (10-100 MPN/g) to propagate when producing ACT using a commercialized brewing system. Bess investigated the effects of molasses, yeast extract, kelp, barley malt and fish emulsion (2 g/L each) on the levels of *E. coli* after individual 24 hour ACT brewing cycles. The results were

consistent with the other reports that showed increasing *E. coli* levels in response to the nutrient amendments. Bess also recorded the dissolved oxygen levels to ensure that the ACT was "properly aerated" above 6 ppm oxygen levels, and reported that the elimination of nutrient amendments could reduce or even eliminate the *E. coli* levels from the initially low concentrations.

It is clear from the present research that if enteric pathogens (e.g. *Salmonella*) are not present in the compost, nutrients, or water used to prepare ACT, then the final product will not contain pathogens. However, it is unclear what effects the various modern ACT and NCT practices have on foodborne pathogen populations when naturally contaminated and inoculated composts are used to prepare the CT. Current research has focused on using immature compost containing residual *E. coli* or broth-cultures to inoculate the ACT or NCT with *E. coli* to determine effects on the inoculated bacterium. Also, much of the current data evolved from studies on ACT where non-standard brewing methods were used to determine the effects of nutrient amendments on *E. coli* and *Salmonella* populations. More study and data are urgently needed that use actual ACT and NCT methods currently practiced by farmers. These studies also should use finished "class A" composts that are either naturally or artificially inoculated with levels of *E. coli* and *Salmonella* that are typically found in real world scenarios (i.e. 10-100 cfu/g).

Compost Tea Task Force Recommendations

Based on the available literature concerning human pathogen survival and potential for propagation in both ACT and NCT, and in response to the pressure from the NOSB to set guidelines for safe compost tea manufacture and usage, its Compost

Tea Task Force has generated a set of recommendations to reduce the potential for all compost teas to introduce foodborne pathogens into the food supply (171):

1) Potable water must be used to make compost tea
2) Compost tea brewing equipment must be sanitized before use
3) The compost feedstocks must be compliant with the NOSB Compost Task Force Guidelines set on April 18, 2002. This applies to both thermally produced manure-based compost as well as 100% plant-based compost and vermicompost.
4) Compost tea prepared without nutrient additives may be applied without restriction.
5) Compost tea made with nutrient additives may be applied without restriction provided the tea has been microbiologically tested to meet the EPA recommended guidelines for recreational water quality (i.e., *E. coli* < 126 cfu/100ml and enterococci < 33 cfu/ml). CT prepared with nutrients that has not been tested falls under the same 90/120 day application restrictions used for raw manure: CT may be applied 90 days prior to harvesting crops that do not contact the soil (e.g., tomatoes) and up to 120 days prior to harvesting ground-lying crops (e.g., strawberries).
6) Compost extracts: any mixture of compost, water, nutrient additives and adjuvants that is not held for more than one hour after preparation, may be applied without restriction
7) Raw manure extracts and compost leachates may not be applied to foliage, and is restricted to the 90/120 day rule for soil application

Scope of Dissertation

The objective of this research was to enlarge our capability to respond to several pre-harvest food safety issues by considering three hypotheses:

1) Current time/temperature regulations (40CFR Part 503) for windrow composting do not consistently produce a human pathogen-free compost product (with respect to *E. coli* and *Salmonella*).
2) Current methods for compost tea manufacture are insufficient for guaranteeing a pathogen-free product (with respect to *E. coli* O157:H7 and *Salmonella*). .
3) Compost tea, when contaminated with *E. coli*, may introduce and increase the epiphytic fitness of this microbe on the surfaces of strawberries during application events.

This dissertation supports the needs for more stringent guidelines to prepare pathogen free compost and provides further guidelines for the preparation of compost teas that may be applied to fresh produce without restriction. Contrary to common perception, this research showed that a variety of commercial composts prepared according to existing guidelines can be potentially contaminated with foodborne pathogens, thus presenting a hazard for application and use. In the laboratory, it was shown that these pathogens can be amplified through the manufacture of compost tea. When applied to strawberries in the field, however, results indicate that the current NOSB guidelines may be too stringent even for CT that is contaminated with levels of *E. coli* above the recommended EPA guidelines for recreational water usage. The

immediate outcome of this research is a better understanding of the risks of in-the-field microbial contamination of produce via compost and compost tea utilization, and consequently, the level of monitoring and intervention required to ensure that only safe produce reaches consumer markets.

CHAPTER 2

FECAL BACTERIAL PATHOGENS AND INDICATORS IN COMMERCIALLY AVAILABLE COMPOST

Abstract

Compost is a valuable soil amendment used by many organic and conventional growers to improve the physical, chemical, and biological properties of soil. In the United States, compost results from the treatment of a variety of feedstocks that are potential sources of pathogenic microbes, e.g., landscape trimmings animal or poultry manure, food residuals, and biosolids from municipal or industrial wastewater treatment facilities. Aerobic, thermophilic compost production processes are designed to achieve significant reductions in fecal coliforms and salmonellae through timed-temperature exposures. Currently, only biosolids-based compost is required to meet time and temperature process standards according to federal statute (40 CFR Part 503). Few states have pathogen or pathogen indicator standards for marketable compost. Thus, compost product quality could vary widely, and, if inadequately treated, compost product could introduce pathogens into facilities producing fresh fruits, vegetables, and herbs that might be consumed raw.

A study was conducted of the microbial quality (total bacterial heterotrophs, total and fecal coliforms, *E. coli*, *Salmonella*, and *Enterococcus*) and seasonal variability of finished, marketable compost from 15 facilities across the United States. Samples (n=105) of mature compost were collected in March, August, and November 2000 and enumerated by either MPN or spread-plating techniques. Similarly, the physico-chemical parameters (temperature, moisture, pH, electrical conductivity,

soluble carbon, and carbon:nitrogen ratio) were analyzed for each compost sample. Community structures of *E. coli* and *Salmonella* populations were determined, based on cluster and dendrogram analysis of FAME principal components profiles of 182 *E. coli* and 74 *Salmonella* isolates. Multiplex PCR assays targeting Stx1, Stx2 and eae genes of *E. coli* O157:H7 were conducted on all of the *E. coli* isolates. Feedstock compositions included a wide range of materials: leaves, grass and woodchips, animal manure, biosolids, food processing sludge, and agricultural residuals. Results showed that 20% and 3% of all compost facilities had products that exceeded the USEPA 503 limit for Class A product; i.e., fecal coliforms <1000 MPN/g and *Salmonella* <3 MPN/4g, respectively. One *E. coli* isolate (0.55% of the total) was determined to be positive for Stx2 and was recovered from sewage sludge compost that met the EPA 503 standards. In total, 69% of the samples were positive for *E. coli* and 81% of these same samples met the fecal coliform standards. Prevalence of toxigenic *E. coli* in commercial composts was very low despite the relatively high number of samples that contained *E. coli*. The concentrations of fecal coliforms were at their highest levels in samples collected during the Summer (July) and Winter (November) months. All of the facilities in this study used outdoor compost systems in which most, but not necessarily all, particles were subjected to pathogen destructive thermal process time-temperatures. Data in this study showed that commercial composts that met the fecal coliform and *Salmonella* standards could still contain low levels of pathogenic *E. coli* and *Salmonella*. Circumstantial evidence is presented that associates the *Salmonella* serogroups obtained in these compost samples to human illness in the states from which the samples were tested. A regimen for routine

pathogen testing is needed to help assure the selection and use of quality and safe composts by fresh produce growers. Based on evidence presented in this study, modification of the current standards for biological safety is warranted.

Introduction

Compost usage has become an integral part of many conventional and organic farming practices to reduce, reuse and recycle biological waste products in an ecologically and environmentally friendly manner (72, 77). The composting process biologically transforms on-farm residuals and industrial by-products into an organic, stable product that can be easily stored, handled, and used to enhance soil quality while providing slow-release nutrients for crops. In addition to on-farm composting, which then re-uses compost in the fields, numerous centralized composting facilities located throughout the United States generate products from municipal, industrial and agricultural by-products that are sold to the public in bulk or by bag. Recent survey information counted fourteen municipal solid waste composting facilities, 250 biosolids, 3,260 yard trimmings and over 175 food residual composting sites across the United States (75, 235). Current federal regulations (EPA 40 CFR Part 503) require that only biosolids-based compost must be tested to meet specific levels of fecal coliform (< 1000 MPN/g) and *Salmonella* (< 3 MPN/ 4g) content prior to application, usage or distribution to consumers (57). When appropriate procedures are implemented, composting operations should be able to produce high quality, stable composts containing undetectable concentrations of *E. coli* and *Salmonella* spp. (78, 79, 95, 116, 117). Skavanis, in 1994, presented evidence to suggest that biosolids-based composts may indeed be introducing *Salmonella* into the human population (227).

The purpose of this study was to conduct a cross-sectional evaluation of the microbial quality of commercially available compost in the United States, with

particular attention to fecal coliform, *E. coli*, *Salmonella* and enterococci content. The analyzed materials represent "point-of-sale" products. In view of a need for rapid and reliable tests to determine compost maturity and microbial pathogen content, correlations between pathogens, indicators of pathogen presence, and physico-chemical parameters (e.g., moisture, electrical conductivity, soluble carbon, pH and carbon:nitrogen ratios) were investigated. Some of these parameters have been previously associated with pathogen re-growth potential in biosolids-based compost (199, 231). Further investigation is needed, however, to determine which parameters are important for pathogen survival during the composting process.

The U.S. Composting Council has devised protocols for the composting industry to determine the physical, chemical, and biological conditions of feedstocks, material-in-process and finished compost products. These tests are termed TMECC (Test methods for the examination of Composting and Compost). The microbiological testing methods used in this study are described in detail in Appendix A.

In this study, the data suggest that many of the outbreaks attributed to *Salmonella*, *E. coli* and other pathogens associated with raw fruit and produce could be the result of using pathogen-contaminated compost on production farms. In such cases, the adulterated composts were likely inadequately treated as required for thermal killing of pathogenic bacteria. However, one study postulated that adequate thermal processing alone may not be adequate to ensure a pathogen-free compost (79). Gong, et. al. determined that *E. coli* O157:H7 and K12 strains were able to survive the thermophilic properties during composting when moisture content of the

compost was below 40%, suggesting a higher tolerance to heat when the moisture content was reduced.

This study investigated the complex relationships between pathogen content in various composts, collected seasonally, from a cross-section of compost sources across the nation and analyzed within a period of only a few months, (November, July and November) in relation to several physical, chemical and seasonal parameters. The primary purpose of this study was to resolve two main questions: (1) Are foodborne pathogens able to withstand optimal composting conditions (as determined by time/temperature metrics), and (2) do physico-chemical parameters effect the survival and growth of foodborne pathogens in compost.

Materials and Methods

Compost Sampling and Processing

Fifteen commercial composting facilities across the U.S. (Figure 2.1) were invited to collect samples over a nine month period (March, July and November) of their best quality compost, i.e. 'Class A' products. Each facility that used biosolids or industrial waste was required to test their compost for pathogens (Table 2.1). The sampling plan outlined in this study was adapted from the U.S. EPA's Test Methods for Evaluating Solid Waste, Physical/Chemical Methods, SW-846, Third Edition, September, 1986 (59). Three locations in the center (or 'hot zone') of each windrow were selected for sampling: one in the middle, and two from either end. From each of these locations, three "sub samples" (approximately 1000g each) were aseptically removed in a cross-sectional pattern to represent as much of the final product as possible (fig 2.2). These individual samples were placed into individual zip-lock™ bags (1.0 gal), labeled and layered in between frozen ice packs in a bucket (5.0 gal). Three buckets were shipped overnight from each facility. Each bucket was stored in a large 4°C cold room in the laboratory. Each of the nine samples (one facility, one sampling month) was processed within 3 days of receipt. The 1000g sub-samples (1a, 1b, 1c), (2a, 2b, 2c), and (3a, 3b, 3c) from each location (1, 2, 3) were homogenized in a large, sterile, plastic bin and composited (3000g) before further processing for chemical, physical and microbiological parameters (Fig 2.3 and 2.4). Although not all facilities provided compost samples for all three months (Table 2.3), all but one facility (facility #3) provided samples in at least two of the three months of the study. A total of 105 compost samples were collected for analysis.

Figure 2.1 : Geographical locations of composting facilities

Table 2.1 : Composting facility information[a]

#	Sample Code	State	Feedstocks	Annual Volume (yds^3)	Pathogen Testing
1	101	MD	Biosolids	70,000	Yes
2	111	FL	Biosolids, Yard Waste	175,000	Yes
3	121	ME	Biosolids	50,000	Yes
4	131	CA	Yard Waste / Wood	50,000	No
5	141	CT	Coffee grounds, tea leaves, yard waste, coconut by-product, food processing sludge	50,000 - 60,000	Yes
6	151	IA	Industrial and Ag. By- products	35,000	Yes
7	161	WA	Yard Waste	60,000	No
8	171	OH	Yard Waste	20,000	No
9	181	GA	Biosolids / Peanut Hulls	75,000	Yes
10	191	NY	Yard Waste	N/A[b]	No
11	201	NC	Yard Waste, Manure, Industrial sludge/ wood waste	N/A	Yes
12	211	MD	Yard Waste	N/A	No
13	221	IA	greenwaste, woodpallets, carboard, drywall	N/A	No
14	241	CA	Biosolids / Greenwaste	N/A	Yes
15	251	IA	Biosolids / Greenwaste	N/A	Yes

[a] Three different types of samples were from IA and two each were from MD and CA.

[b] N/A = Information not available

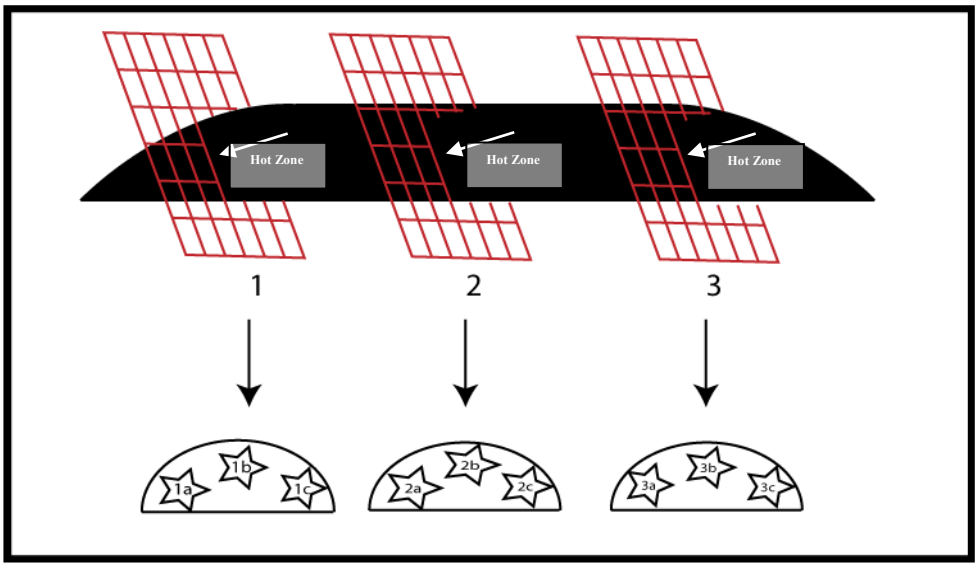

Figure 2.2 : Cross-sectional sampling schematic for compost windrows. 3x1000g samples from each cross section were placed in individual Zip-Lock™ bags and transported overnight in 5-gal buckets layered in-between frozen ice packs.

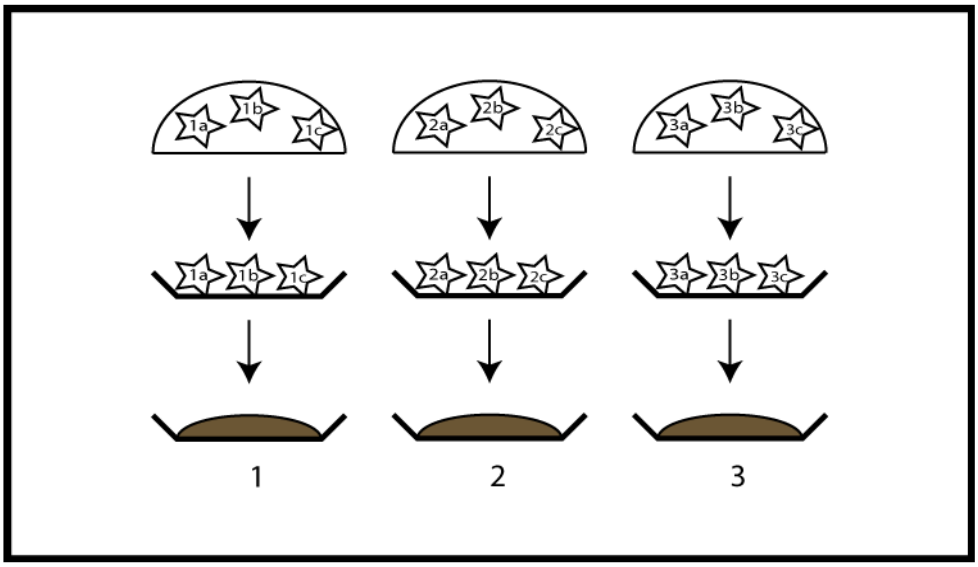

Figure 2.3 : Compost sample homogenization (under aseptic conditions in the laboratory). 3 x 3000 composite samples from each windrow were used for all microbiological, chemical and physical analyses.

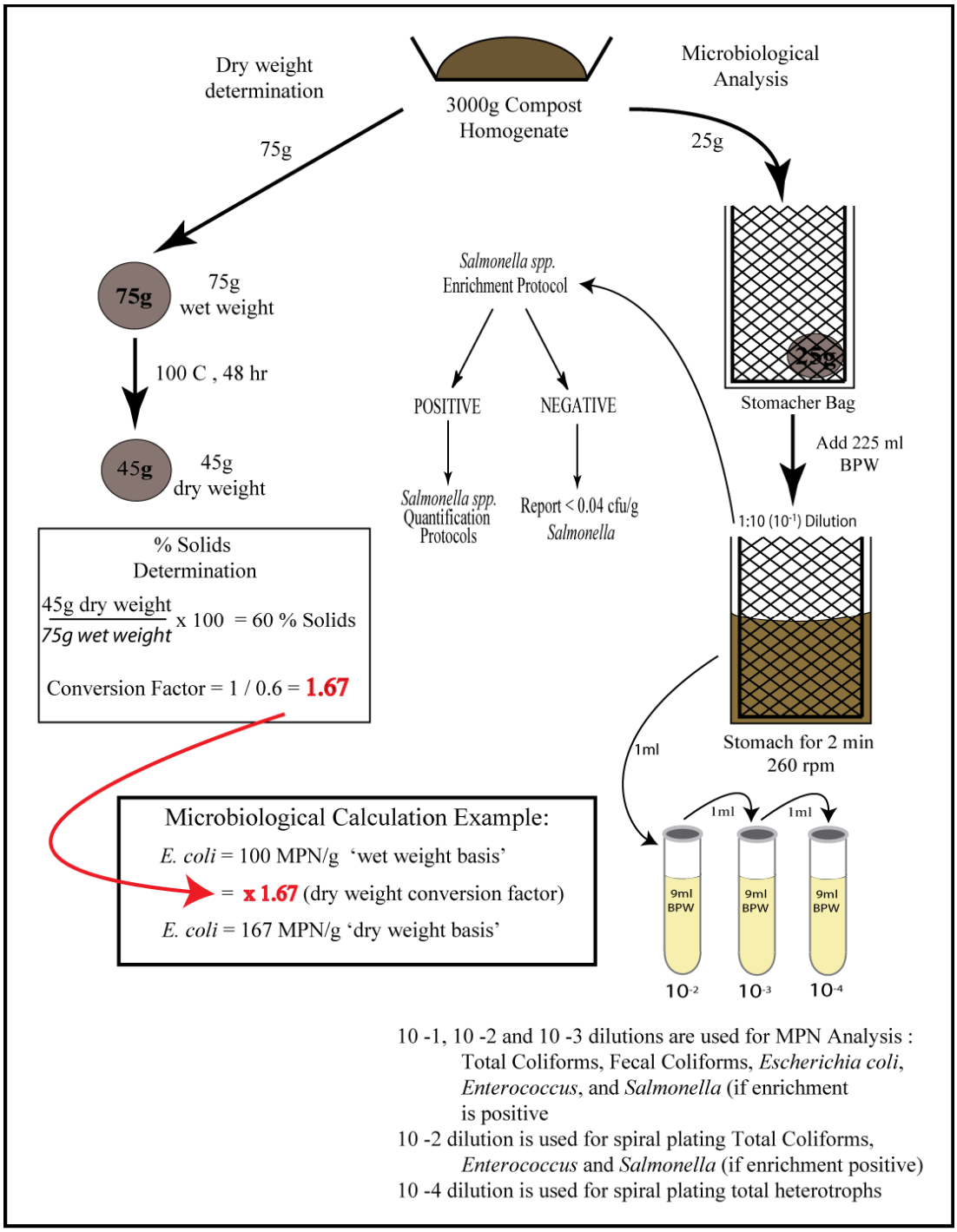

Figure 2.4 : Schematic for the microbiological processing of compost samples

Dry Weight Determination

Microbiological analysis of each compost sample, containing varying amounts of moisture, was performed on the samples "as received." Since the microbiota can vary widely with the moisture content in compost samples as reported by others, drying each sample was necessary to conduct accurate statistical analyses. The microbiological data were always obtained using samples on a "cfu/g wet weight" basis and then the data were transformed into "cfu/g dry weight" using a conversion factor. Dry weights of each compost sample were determined on duplicate samples, for which microbiological data were obtained. Dry weights were determined by aliquotting approximately 75g of each compost sample into a tin dish and drying in an oven (Lab-line model PR305145G, Thermo Scientific, Waltham, MA) at 100^{o}C for two days. The "dry weight" conversion factor was determined by dividing the (g) dry weight by the (g) wet weight and multiplying by 100 to determine the % solids and then multiplying the cfu/g (wet weight basis) by 1 / % Solids (g) to determine the cfu/g on a dry weight basis. For example, if the compost sample contained 60% solids, then the cfu/g would be multiplied by a correction factor of 1.67: (1 / 0.6) = 1.67. In this example, if the wet-weight "as received" microbiological determination was 100 cfu/g, then the reported "dry weight" value for this sample was 167 cfu/g (i.e., 100 cfu/g x [*1.67 conversion factor*] (Figure 2.4)

Microbiological Analyses

Three standard microbiological techniques were used to analyze the compost: Spiral Plating, the Most Probable Number (MPN) method, and Enrichments. This enabled the efficient quantification of specific microbes over a broad range of concentrations. Microbial analyses were performed on aliquots of each of the three (3000g) consolidated samples per compost site. Each sample consisted of a composite of three individual compost samples (1.0 gal each) from the same location (Fig 2.3) to help with homogeneity and accurate representation of the microbiology from each compost windrow. A schematic for the microbiological processing is shown in Fig 2.4. Protocols are briefly described below and a detailed protocol and description of equipment is located in Appendix A.

Sample Preparation

Each compost sample (25g) taken from each 3000 g composite sample (Fig 2.4) was aseptically placed into a stomacher bag (400c, Seward Laboratory Systems inc., New York) to which 225ml Buffered Peptone Water (Difco, Becton Dickinson, New Jersey) was added for an initial 1:10 (w:v) dilution. The samples were then stomached for two min at 160 rpm (Model 400c, Seward Laboratory Systems Inc., New York).

Spiral Plating

The concentrations of total heterotrophic bacteria, coliforms, gram negatives, fecal coliforms, *E. coli*, *Salmonella* spp. and enterococci were determined by serial dilution and spiral plating (WASP2, Microbiology International, Frederick, MD). From each of three sample dilutions (10^{-2}, 10^{-3}, 10^{-4}), predetermined volumes (100µl) were plated in duplicate on the following media (Difco, Becton Dickinson, Franklin Lakes, NewJersey): MacConkey's agar (with and without MUG), XLT4 agar, Modified Enterococcus agar, and Trypticase Soy Agar. Serial dilution was used also, because spiral plating became inefficient when plating dilutions lower than 10^{-2} of the original sample. Due to the increased concentration of particulate matter in the lower dilutions, the spiral plating instrument would frequently become clogged or jammed with the debris. All agar plates were incubated at 37^{o}C for 24 hr, except for the modified *Enterococcus* agar, which were incubated for at least 48 hours. All plates were counted manually according to manufacturer's instructions (Microbiology International, Frederick, Maryland). Presumptive *E. coli* and *Salmonella* isolates were confirmed biochemically and identified using gas chromatography to analyze their fatty acid methyl ester components (Sherlock® Microbial Identification System, MIDI Inc., Newark, Delaware). Confirmed *Salmonella* isolates were further characterized serologically with slide agglutination techniques using poly-O antiserum (DIFCO). Confirmed *E. coli* isolates were further characterized for the presence of virulence genes associated with Enteropathogenic (EPEC), Enterohemorrhagic (EHEC) and Shiga-Toxigenic (STEC) *E. coli.*

Most Probable Number (MPN)

The Most Probable Number (MPN) technique involved a three-tube system to determine the concentrations of total coliform, fecal coliform, *E. coli*, *Enterococcus* and *Salmonella* content. The MPN system was used to complement the agar plating techniques by providing a much lower detection threshold for all tested microbes. The basic format involved making two dilutions (1:100 and 1:1000) of the primary 1:10 sample using BPW as a diluent and aseptically pipetting 1 ml of each dilution into each of three tubes containing 9.0 ml of either primary enrichment or selective enrichment broths targeting specific organisms. The tubes were then incubated for at least 24 hr before observing and/or culturing appropriately to determine the presence (or absence) of each target organism. Biochemical confirmation of each presumptive *E. coli*, *Salmonella* spp. and enterococci was performed. *Salmonella* spp. isolates were also confirmed and sero-grouped using poly-O antiserum (DIFCO, Becton Dickinson). Fatty acid methyl-ester (FAME) profiles of all *E. coli* (n=182) and *Salmonella* (n=74) isolates were analyzed using gas chromatography to confirm identification and to determine community structure using Sherlock® Microbial Identification System (MIDI, Inc., Newark, Delaware) The number of positive tubes from each dilution set was then recorded to determine the most probable number per gram (or milliliter) of sample. The freeware MPN calculator (VB6 version; Michael Curiale) (www.i2workout.com/mcuriale/mpn/index.html) was used to calculate the final MPN/g per each sample.

Total Coliforms, Fecal Coliforms and E. coli MPN

The total coliform, fecal coliform and *E. coli* MPNs were coordinated to build on the results of the previous MPN. For example, after the total coliform MPN was performed, each "positive" tube was carried through the subsequent fecal coliform (FC) MPN, and then each "positive" FC tube was analyzed for the presence of *E. coli*. A detailed protocol is located in Appendix A: section 1A (total coliforms), 1B (fecal coliforms) and 1C (*E. coli*).

Total Coliforms. One ml from the 1:10 (10^{-1}), 1:100 (10^{-2}) and 1:1000 (10^{-3}) sample dilutions was transferred into each of three tubes, respectively, containing 9 ml Lauryl Tryptose (LT broth, DIFCO) and inverted Durham tubes. All nine tubes were incubated at 37^{o}C for 24 hours and observed for gas bubble formation inside the Durham tubes. The presence of gas bubble formation indicated the presence of coliforms. The number of positive tubes in each dilution set was used to calculate the MPN/g with the MPN calculator.

Fecal Coliforms. Thirty µl from each "positive" LT tube was transferred into a sterile tube containing 9.0 ml of *E. coli* broth amended with MUG (EC broth with MUG, DIFCO) and inverted Durham tubes. All of the tubes were incubated at 44.5^{o}C for 24 hours and observed for turbidity and gas formation. The presence of turbidity at 44.5^{o}C with gas formation in the Durham tubes indicated the presence of fecal coliforms. The number of positive tubes in each dilution set was used to calculate the fecal coliform MPN/g with the MPN calculator.

E. coli. Each "positive" EC-MUG tube was placed under a long wave ultraviolet light source (~465 nm). Any tube that fluoresced and contained gas in the

Durham tube was considered positive for *E. coli,* and was used to calculate the MPN g^{-1} for presumptive *Escherichia* with the MPN calculator. Each presumptive *E. coli* was confirmed biochemically and characterized using the Sherlock Microbial Identification System (MIDI).

Salmonella MPN

The *Salmonella* MPN was performed on a duplicate sample only after an initial enrichment of each sample was performed and confirmed for the presence of *Salmonella* spp. The detailed *Salmonella* MPN methodology is provided in Appendix A. The enrichment technique was performed by incubating the original 1:10 dilution (Compost:BPW w:v) at 35^{o}C for 24 hr, aseptically transferring 20 ml of this primary enrichment into 180 ml of Tetrathionate Broth (Hajna) formulation and incubating for an additional 24 hr at 35^{o}C. Two loopfuls of this selective enrichment were streaked for isolation onto XLT4 agar and incubated for 48 hours. Three presumptive positive colonies (Black or Red colony coloration) were confirmed biochemically and serogrouped using slide agglutination techniques and poly-O antiserum (Difco, Becton Dickinson). Each of the nine tubes in the MPN method were also streaked onto XLT4 and incubated at 35^{o}C for 24-48 hours to confirm the presence of *Salmonella* spp. Presumptive biochemical identification of the *Salmonella* isolates was performed as described below.

Enterococcus MPN

The *Enterococcus* MPN was performed using the same 9-tube format as in the *Salmonella* and Coliform MPN methods. A detailed method of the Enterococcus MPN is outlined in Appendix A. Azide Dextrose Broth (Difco) was used as the selective enrichment broth in each MPN tube to culture *Enterococcus* from the compost, which was then streaked on modified *Enterococcus* agar (Difco). The isolates were further characterized for temperature (45^{o}C) and salt tolerance (6.5% NaCl) in Brain Heart infusion broth (BHI, Difco) for confirmation as enterococci. Only confimed isolates were used to determine the concentrations of enterococci (MPN/g or CFU/g) in the compost samples.

Biochemical and Serological Confirmations for E. coli and Salmonella spp.

***E. coli*:** Each "positive" EC-MUG tube and three colonies from each MAC-MUG plate containing presumptive *E. coli* were confirmed biochemically. For each EC-MUG tube, a loopful (~20µl) of growth was transferred onto both MacConkey's Agar and Eosin-Methylene Blue Agar (DIFCO), followed by incubation for 24 hours at 44.5^{o}C and 37^{o}C, respectively. *E. coli* colonies on EMB had a metallic green appearance within 18-24 hr, but at times appeared dark purple (Fig. 2.5). *E. coli* produced a deep pink coloration on MAC plates, and the medium surrounding this culture had a "fuzzy" pink halo appearance around the colony due to the precipitation of bile salts and low pH resulting from acid by-products from the metabolism of lactose (Fig. 2.6).

Figure 2.5 : *E. coli* on EMB Agar

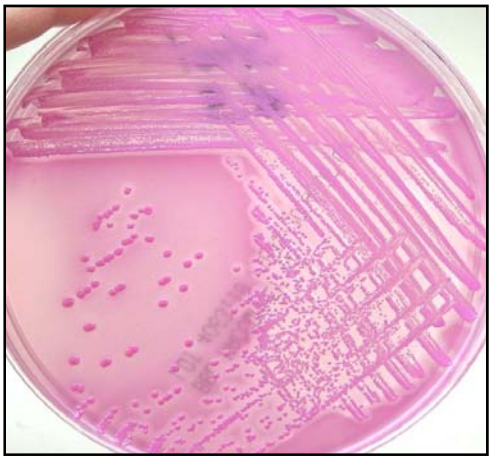

Figure 2.6 : *E. coli* on MacConkey's Agar

Using a sterile inoculation needle, a cell mass that exhibited a positive reaction on both EMB and MAC was transferred onto both Triple Sugar Iron (TSI, Difco) Agar slants and Motility Indole Lysine (MIL, Difco) tubes. TSI and MIL tubes were incubated at 37^{o}C for 24 hr. Two drops of Kovac's reagent were added to the MIL tubes. The combination of positive TSI and MIL tubes provided an efficient and powerful tool for differentiating among members of the Family *Enterobacteriaceae* (46, 193). *E. coli* exhibited the following biochemical reactions:

TSI —Acid Slant (A/Yellow), Acid Butt (A/Yellow), Gas production (bubbles) throughout the medium (Fig 2.7)

MIL—Basic Slant (K/Purple), Basic Butt (K/Purple), Motility (medium was cloudy), and Indole detection (Red band at the top of the tube, after the addition of Kovac's Reagent). (Figure 2.8)

Figure 2.7 : Triple Sugar Iron (TSI) tube containing *E. coli* and incubated for 24 hr at 37^{o}C

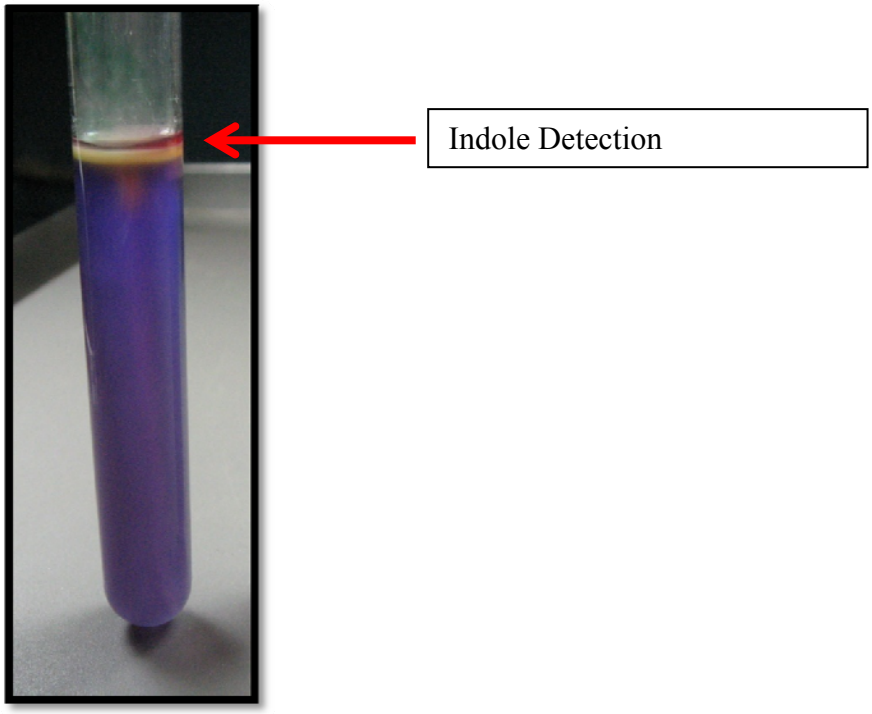

Figure 2.8 : Motility Indole Lysine (MIL) tube containing *E. coli* with added Kovac's Reagent to detect Indole presence (noted by red coloration on surface of medium)

***Salmonella* spp. :** After 24-48 hours of growth on XLT4 agar, colonies of *Salmonella spp.* appeared either red, red with black centers or entirely black (Fig. 2.9). Cells from each of three presumptive colonies from the XLT4 plates were aseptically transferred to individual MIL and TSI media for 24 hours at 35°C. *Salmonella* exhibited the following reactions:

TSI — Basic slant (K/Red) and Acid butt (A/yellow). Most isolates produced hydrogen sulfide (H_2S) revealed by the reduction of ferric citrate to ferrous sulfate, and a resulting black coloration in the tube. At times the black precipitate masked the yellow coloration in the butt.

MIL — *Salmonella* exhibited a purple coloration (K/basic) on both the top and the bottom of the tube, and exhibited swarming throughout the medium (the stab-line was not visible). Salmonellae did not produce Indole in the MIL medium as demonstrated

by the absence of a red-band formation after adding two drops of Kovac's reagent on the surface of the medium.

Figure 2.9 : *Salmonella* spp. on XLT4 Agar after 48 hr incubation at 35^{o}C

Each *Salmonella* isolate, confirmed biochemically via the MIL and TSI, were further characterized using the slide-agglutination technique (Appendix A). Using antibodies specific for the *Salmonella* O-antigen (Poly-O Antiserum, DIFCO), each isolate was first confirmed as *Salmonella* spp. and then further serotyped using group-specific antiserum (*Salmonella* Antiserum groups A-E, DIFCO). The slide-agglutination technique was performed according to the manufacturer's instructions. It should be noted that all salmonellae remained *presumptive positive* until serologically confirmed.

MIDI Protocol

Technology for fatty acid analysis is based on gas chromatographic (GC) analysis of fatty acid methyl esters (FAME) of isolated colonies. The GC profiles were compared to a standard database that provided identification of bacterial isolates. All biochemically and/or serologically confirmed *E. coli* (n=182) and *Salmonella* (n=74) isolates were analyzed using modified MIDI (Microbial Identification Inc., Newark Delaware protocols developed by Buyer, 2003 and 2006 (25, 26) that dramatically increased the GC efficiency (Fig. 2.10). Using a Hewlett-Packard 5890 GC Series II instrument and Sherlock® software (version 6.1, MIDI, Inc., Newark Delaware), FAME profiles of unique *E. coli* were identified and these isolates were further characterized for virulence factors using a real-time PCR technique (see below).

Reagent Preparation:

Reagent #1 Saponification Reagent
(NOTE:Add NaOH pellets to water/methanol while stirring)
45g NaOH pellets (certified ACS)
150ml Methanol (HPLC grade)
150ml deionized distilled water

Reagent #2 Methylation Reagent
(NOTE: Add Acid to methanol while stirring)
325ml 6.00N Hydrochloric acid
275ml Methanol (HPLC grade)

Reagent #3 Extraction Solvent
(NOTE: Add MTBE to hexane and stir)
200ml Hexane
200ml Methyl-tert Butyl Ether

Reagent #4 Base Wash (dilute NaOH)
(NOTE: Add pellets to water while stirring)

10.8g Sodium hydroxide (certified ACS)
900ml deionized distilled water
Saturated Sodium Chloride (for breaking emulsion)
100g Sodium Chloride (certified ACS)
deionized distilled water in plastic "squirt" bottle

It should be noted that reagents #1 and #4 are caustic and reagent #2 is acidic. Safety glasses and gloves are required during preparation and usage of these reagents at all times. Reagent #3 is flammable (extinguish all flames and heat sources before using).

Growth and Harvesting Procedure

1) All isolates were streaked onto TSBA plates in a quadrant-streak format, to allow for ample growth of the target organisms as well as a check for purity via visualization of individual colonies.

2) The TSBA plates were incubated at 28^{o}C +/- 1^{o}C for 24 +/- 2 hours, which were critical time and temperature profiles to ensure consistent fatty acid production.

3) Using a sterile loop (metal or disposable plastic), enough cell mass from the TSBA plate was removed to coat the bottom of a 100x13mm culture tube, capped with a teflon-lined screwcap.

It should be noted that the culture tube may be stored at this stage in a -20^{o}C freezer for over 1 year without loss of identification accuracy.

Saponification Procedure

4) One ml of reagent #1 was added to the tube and capped tightly and vortexed for 5-10 seconds.

5) The tubes were boiled in 100^{o}C water bath for 5 min.; (Pressure will develop in the tubes, so inspect each for volume loss and replace any caps that appear to be leaking).

6) The tubes were vortexed for 5-10 seconds and returned to the 100^{o}C waterbath for an additional 25 minutes. The tubes were cooled.

Methylation Procedure

7) Two ml of reagent #2 was added, capped tightly, and vortexed for 5-10 sec.

8) The tubes were placed in an 80^{o}C +/- 1^{o}C water bath for 10 minutes, then *cooled rapidly* by immersion in a cold water bath for a few minutes.

Extraction and Base Wash Procedure

9) Reagent#3 (1.25 ml) was added to the tubes which were then tightly capped, and rotated for 10 minutes using a hematological/chemical rotator.

10) The bottom phase was removed and discarded using a Pasteur pipet and the top phase was saved in the culture tube.

11) Three ml of reagent #4 was added, the tubes tightly capped and rotated for 5 minutes using a hematological/chemical rotator

12) If required, several drops of saturated NaCl solution were added to break the emulsion formed inside the top layer. Two thirds of the top layer was removed and placed into a GC vial, capped securely and placed in the refrigerator (4^{o}C) until ready to analyze.

It should be noted that 20-50 samples could be extracted in a single "batch." A known control isolate (*Xanthomonas maltophilia*) and negative control (reagent blank) were included in each batch to verify each Fatty Acid Methyl Ester (FAME) extraction protocol.

13) Each GC vial was loaded into the autosampler and run through the GC system (Hewlett-Packard 5890 GC Series II instrument) and the FAME *profiles* were compared to the database using Sherlock software (MIDI) which provided identification of isolates

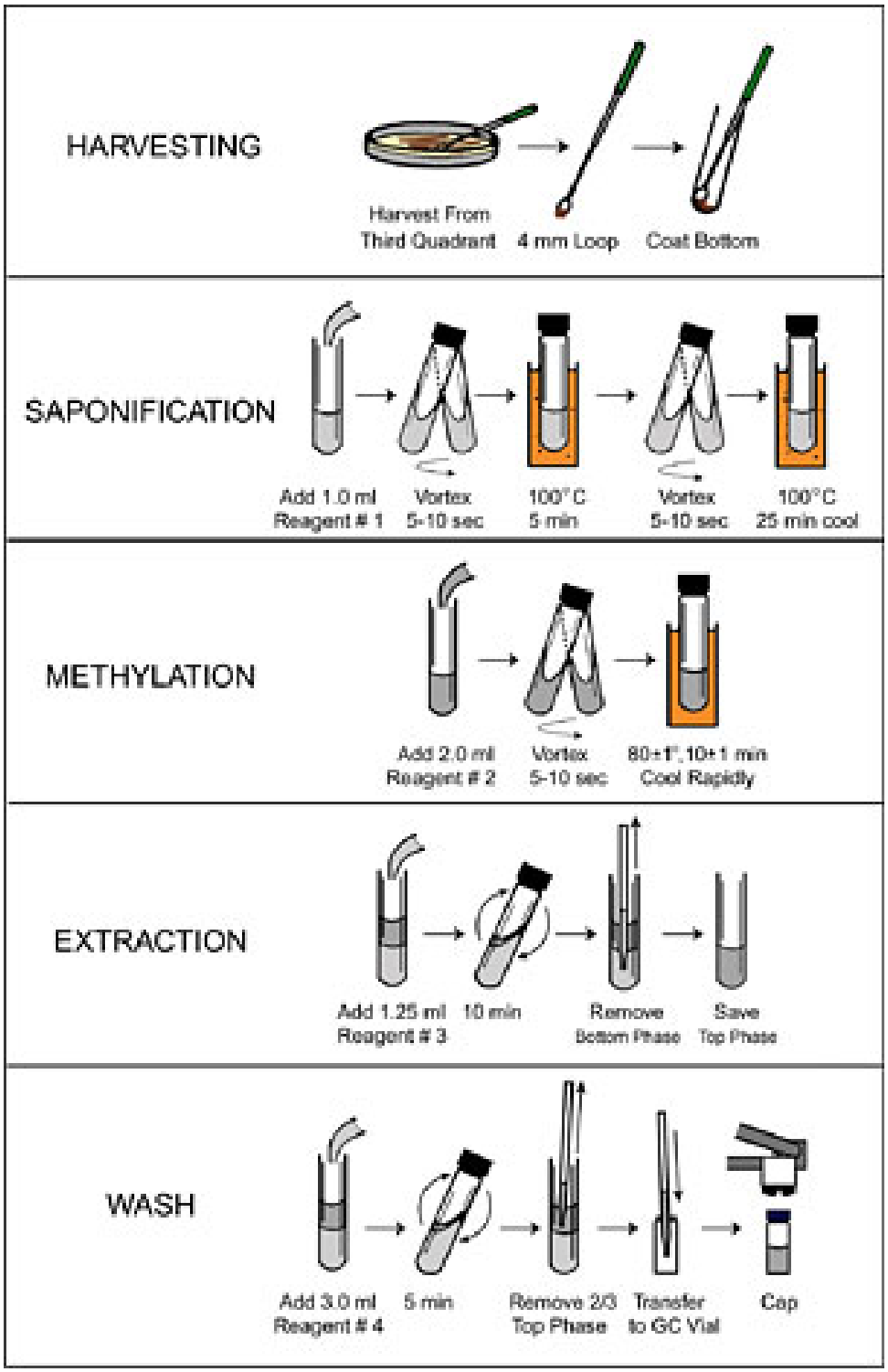

Figure 2.10 : Protocol for extraction of whole cell Fatty Acid Methyl Esters (FAME) for isolate identification using Sherlock Microbial Identification System. (Picture provided by scanned image of instructional manual, courtesy of MIDI, Inc., Newark Delaware)

Real-Time PCR Detection of Virulence Genes in *E. coli* isolates

DNA isolation

One ml of each actively growing bacterial isolate in Tryptic Soy broth (DIFCO, Becton Dickinson) was centrifuged at 12,000 rpm (18,500*g*) for 5 min. After discarding the supernatant, the pellet was suspended in 200 µl of Instagene lysis buffer (Bio-Rad Laboratories, Hercules, California and vortexed for 10 sec. Lysis was carried out by incubating the cell-lysis suspension at 100ºC for 15 min in a dry-block heater (VWR International Inc., Bridgeport, New Jersey..

Primer and Probe design

Primers and probes were chosen from previously published literature and examined for their ability to detect *E. coli* virulence genes when used as a "cocktail" within a single PCR reaction (Table 2.2) (104, 124, 217). The reporter dyes HEX, FAM, and CY5 were conjugated at the 5' end for stx1, stx2, and eae probes, respectively. The quencher dye BHQ was used over other quencher dyes because of its preferred, signal-to-noise ratio. The reaction mixture (50µL) contained all primers to 300 nM final concentration, probes to 250 nM, qPCR mastermix (25µL, Eurogentec North America Inc., San Diago, California), and 2 µL DNA sample.

Multiplex PCR conditions

Multiplex PCR was performed in a 50µl volume containing 2µl of DNA, 0.3 µM of each primer, 0.25 µM of each probe, and 25µl of mastermix plus low ROX (Eurogentec, San Deigo, California). PCR conditions consisted of 50°C for 2 min, 95°C for 10 min to denature the DNA, 40 cycles (95°C for 15 s, 60°C for 1 min). The plate setup contained a positive DNA template purified from *E. coli* O157:H7

strain, and two negative *E. coli* controls. PCR was performed with the real time iCycler iQ PCR (BioRad Laboratories, Hercules, California).

Statistical Analysis

Data were analyzed as a mixed effects model using PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). For the analysis of both chemical and microbial parameters, the model included sampling month and biosolids content as fixed effects. Microbial concentrations were transformed to Log10 values prior to analyses. All data were analyzed for normality and homogeneity of variance prior to ANOVA. Significant variation among chemical parameters across each month required variance grouping by monthly results. Means separations were carried out using the SAS macro PDMIX800 (206) and the least significant difference method (α=.05). Thus, the experimental design adequately accounted for inherent monthly variations among feedstocks. Pearson correlation coefficients were determined between each physico-chemical parameter and microbial populations using the PROC CORR procedure.

Table 2.2 : Primer and Probe Information used for real-time PCR detection of virulence genes in *E. coli* obtained from compost samples

Target	Name (Primer/Probe)	5'-3' Sequence	5'	3'	Target (bp)
stx1[a]	MI3 – Probe	TGAATGTCATTCGCTCTGCAATAGGTACTC	HEX	BHQ	150
	MI1 - Upstream	GACTGCAAAGACGTATGTAGATTCG			
	MI2 - Downstream	ATCTATCCCTCTGACATCAACTGC			
stx2[b]	VS6 – Probe	CTATCAGGCGCGTTTTGACCATCTTCG	FAM	BHQ	120
	VS4 - Upstream	GGGCAGTTATTTTGCTGTGGA			
	VS5 - Downstream	TGTTGCCGTATTAACGAACCC			
Eae[c]	JKTM10 - Probe	CAGGCTTCGTCACAGTTGCAGGC	CY5	BHQ	80
	JKP11 - Upstream	GGCGATTACGCGAAAGATACC			
	JKP12 - Downstream	CCAGTGAACTACCGTCAAAGTTATTACC			

[a] A. Mark Ibekwe, Pamela M. Watt, Catherine M. Grieve, Vijay K. Sharma, and Steven R. Lyons. Multiplex Fluorogenic Real-Time PCR for Detection and Quantification of *Escherichia coli* O157:H7 in Dairy Wastewater Wetlands. (Appl.Environ.Microbiol. 68 (10):4853-4862, 2002.)

[b] Sharma, V. K., E. A. Dean-Nystrom, and T. A. Casey. 1999. Semi-automated fluorogenic PCR assays (TaqMan) for rapid detection of *Escherichia coli* O157:H7 and other shiga toxigenic *E. coli*. (Mol. Cell Probes 13:291-302.)

[c] Karns, J. S., Van Kessel, J. S., McClusky, B. J., & Perdue, M. L. (2007). Incidence of *Escherichia coli* 0157:H7 and E. *coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. (J. Dairy Science, 90(7), 3212-3219.)

Results

Composting Facilities

The composting facilities involved in this study were all commercial suppliers to the general public that produced at least 20,000 cubic yards of compost annually. All facilities participated for the month of July, however not all facilities provided samples for both March and November (Table 2.3).

Table 2.3 : Participation of composting facilities for each month

Facility Number	Location	March	July	November
1	MD	Yes	Yes	Yes
2	FL	Yes	Yes	N/A
3	ME	N/A[a]	Yes	N/A
4	CA	Yes	Yes	Yes
5	CT	N/A	Yes	Yes
6	IA	N/A	Yes	N/A
7	WA	Yes	Yes	N/A
8	OH	Yes	Yes	Yes
9	GA	Yes	Yes	Yes
10	NY	N/A	Yes	Yes
11	NC	Yes	Yes	N/A
12	MD	Yes	Yes	N/A
13	IA	Yes	Yes	Yes
14	CA	Yes	Yes	Yes
15	IA	Yes	Yes	Yes
Total per month		**11**	**15**	**9**

[a] N/A = Not Available

The geographical distribution of the participating compost facilities was more concentrated on the East Coast (eight locations): MD (2), FL (1), ME (1), CT (1), GA (1), NY (1) and NC (1); Four sites in the Central U.S.: IA (3) and OH (1); Three sites on the West Coast: CA(2) and WA(1). Eight of the facilities (Numbers 1,2,3,6,9,11,14 and 15) that collected biosolids and/or industrial waste materials for their starting materials were required to test their "finished" materials for pathogen content and comply with federal regulations to meet "class A" standards (Fig 2.11). Although it was not determined whether the products were tested in-house or sent to third-party testing facilities, all final compost products were tested in this study to determine compliance with federal regulations.

Criteria for meeting Class "A" product (40 CFR Part 503)

Parameter	Unit	Limit
Fecal Coliform	MPN/g	1000
Salmonella	MPN/4g	3

Figure 2.11 : "Class A" standards for *Salmonella* and fecal coliform content (40 CFR Part 503)

Physical and Chemical Parameters of Compost Samples

The moisture content (% Moisture), pH, soluble carbon (ppM), electron conductivity (EC, 1:5 w/w basis, mmhos/cm) and Carbon/Nitrogen ratios (C:N) were obtained for each compost sample. These parameters were chosen based on their impacts on biological and chemical stability of the finished compost samples (36,

190, 201, 286). Good quality, "finished" compost should have the chemical parameters that fall within the following ranges: pH (6.5-8.0), Moisture (35-60%), C:N ratio (10-25), EC (1:5 dilution; 1-2 mmhos/cm). Soluble carbon is the amount of organic carbon immediately available to the microbial community. Although there are no recommendations for the soluble carbon content in compost products, it has been shown to be strongly correlated with compost maturity (70). The concentration of soluble salts in the compost samples (e.g. Ca, Mg, Cl, Na) were measured indirectly by measuring electron conductivity (EC), i.e., in millimhos/cm^{-1} or deciSiemens/cm^{-1}. EC is a typical measure of compost maturity used to evaluate suitability for planting crops. Some vegetable plants are highly susceptible to salt concentrations above the maximum recommended 2 mmhos/cm range for planting in 100% compost (241). Where compost is diluted or tilled into the soils, the high EC content of some compost may not be an issue due to the large dilution effect and buffering capacity of the soils. In 100% compost environments, however, where EC values are much higher than 2 mmhos/cm, the salt concentrations can have a negative impact on plant vigor due largely to the limitation of free water access to the roots. High EC values effectively lower the water potential in the planting medium.

The effect of salt concentrations on microbial populations has been exploited in clinical labs to help isolate certain genera, such as *Enterococcus spp*. which are highly resistant to soluble salt concentrations above 6.5 % (29, 188, 189). Gram positive organisms, in general, are either much more resistant or tolerant to desiccation and survival in low moisture environments than are gram negative bacteria. In this study, an attempt was made to determine the physical and chemical

parameters of compost that may contribute to the survival of specific microbial populations. Pearson correlation coefficients were correlated to determine the ultimate effect of the physico-chemical properties (percent moisture, pH, EC, soluble carbon and C:N ratio) to the total heterotrophic, gram negatives, fecal coliforms, *E. coli* and *Enterococcus* bacterial populations of each individual compost sample.

The mean values of the monthly-gathered compost samples were within the recommended guidelines for "finished" class A products: (Moisture-Fig. 2.12; pH-Fig. 2.13; Electron Conductivity-Fig. 2.14; Soluble Carbon-Fig. 2.15; C:N Ratio-Fig. 2.16). However, in each month, there were a few samples (outliers) that were outside the recommended physical and chemical parameters. Monthly variances for each parameter showed significant differences within the pooled samples for each month as indicated in the figures cited above.

Observing data from all compost samples, there were no significant interaction effects (P=0.05) between any physical or chemical parameters on the specific microbial populations as determined in this study. These results were consistent with Lemuneir et. al., in 2005, where populations of inoculated *Salmonella*, *E. coli* and *Listeria monocytogenes* were not determined to correlate with any of the same physico-chemical parameters in another system, i.e., experimental biowaste composts (142). Also, no significant interaction effects were seen in this study when the physical and chemical parameter data were compared with microbial populations only from compost samples that failed the EPA Class A standards based on fecal coliform and *Salmonella* content. However, when parsing-out the observed data from biosolid and non-biosolid based compost, significant differences (P<.001) were found

for all parameters except for pH (Table 2.4). All biosolids-based composts had significantly lower moisture content, electrical conductivity, soluble carbon and C:N ratios than non-biosolids based composts.

Based on prior reports, positive correlations for all microbe classes were expected in response to moisture content, soluble carbon and maturity (C:N) ratios, (79, 199, 231). However these findings were at variance with those in this study. The physico-chemical effects on specific microbial populations between biosolid and non-biosolid based composts are shown in table 2.4. Pearson correlation coefficients between overall microbial content and the physical and chemical parameters are shown in Table 2.5. Looking at specific microbial populations, the fecal coliform concentrations failed to show significant correlation with any of the chemical parameters. *E. coli* concentrations, however, showed a significant inverse correlation (r^2 = -0.245; P<.01) with soluble carbon content, which differed from the expected results. Conversely, El Sabaie, et. al found a positive correlation between fecal coliforms and total carbon content during the manufacture of sludge compost(49). Enterococci concentrations showed a significant positive correlation (r^2 = 0.257; P<0.01), while gram negative bacteria showed a significant inverse correlation (r^2 = -0.321; P<.001) to electrical conductivity content in the same compost samples. This was consistent with the knowledge that gram positive microbiota generally are tolerant to higher salt concentrations than gram negatives.

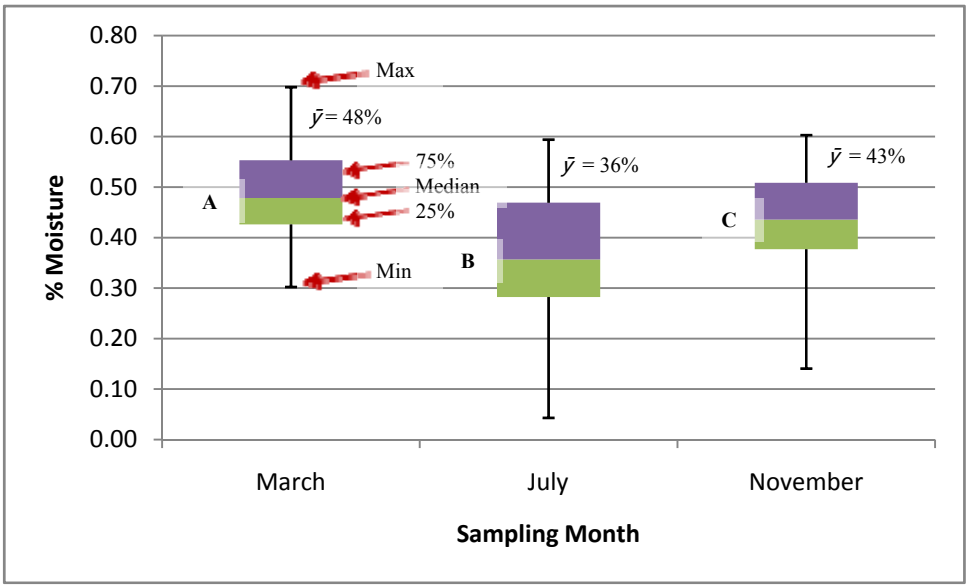

Figure 2.12 : Moisture data for compost samples. Box and Whisker plots include max, min, median, 25% and 75% confidence intervals for each month. '$\bar{y}$ ' = Grand means for each month. Months with different letters represent statistically significant differences (P<0.05)

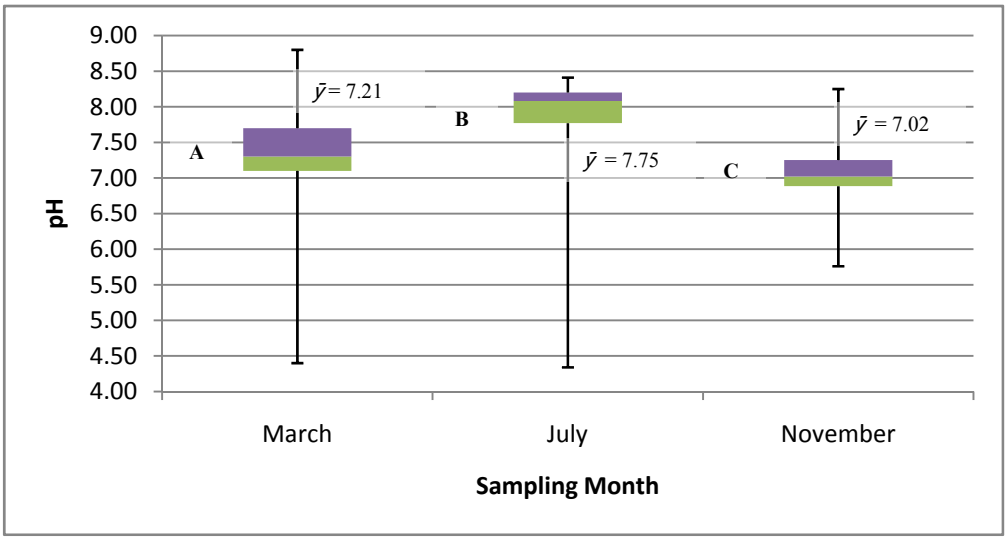

Figure 2.13 : pH data for compost samples. Box and Whisker plots include max, min, median, 25% and 75% confidence intervals for each month. '$\bar{y}$ ' = Grand means for each month. (See Fig 2.12) Months with different letters represent statistically significant differences (P<0.05)

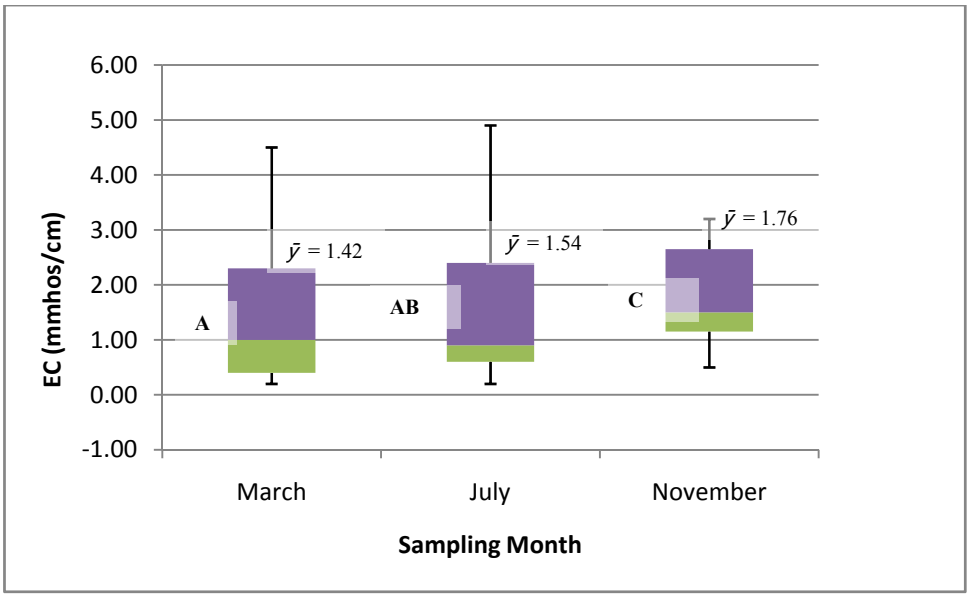

Figure 2.14 : Electrical conductivity data for compost samples. Box and Whisker plots include max, min, median, 25% and 75% confidence intervals for each month. 'ȳ ' = Grand means for each month. (See Fig 2.12) Months with different letters represent statistically significant differences (P<0.05)

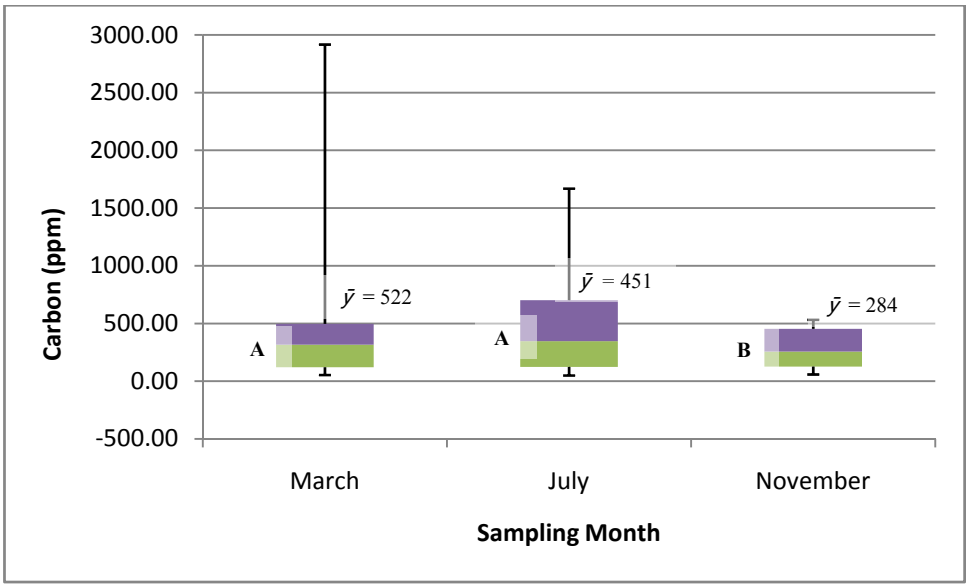

Figure 2.15 : Soluble carbon data for compost samples. Box and Whisker plots include max, min, median, 25% and 75% confidence intervals for each month. 'ȳ ' = Grand means for each month. (See Fig 2.12) Months with different letters represent statistically significant differences (P<0.05)

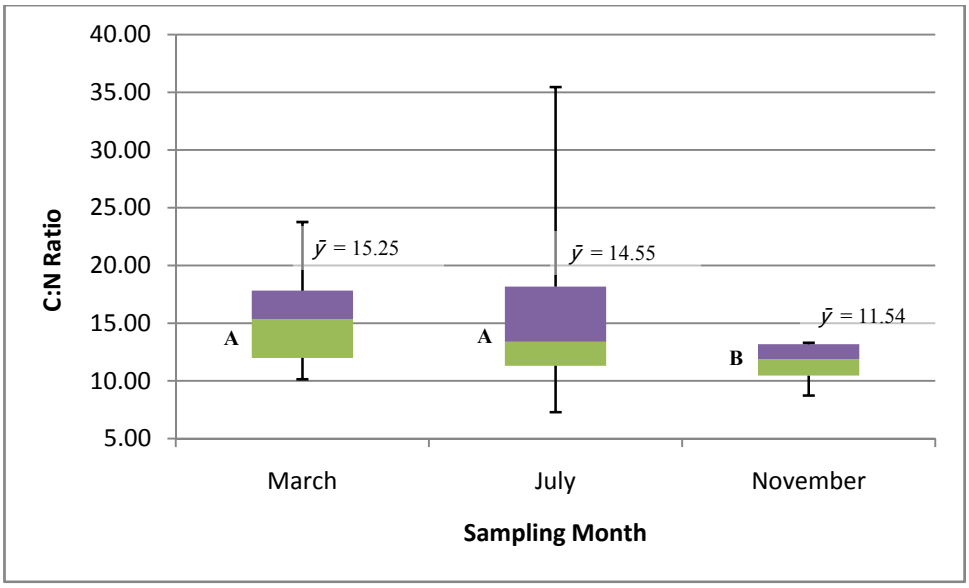

Figure 2.16 : Carbon:Nitrogen ratios for compost samples. Box and Whisker plots include max, min, median, 25% and 75% confidence intervals for each month. 'ȳ ' = Grand means for each month. (See Fig 2.12) Months with different letters represent significant differences (P<0.05)

Table 2.4 : Analysis of Variance for chemical parameters across compost samples containing biosolids or non biosolid-based feedstocks. Tables produced using SAS version 9.2 using Proc Mixed and multiple pairwise means comparison method using least significant difference set at α=.05.

Chemical Parameter	Biosolids[a]	Grand Mean (Log Cfu/g)	Biosolids[a]	Grand Mean (Log Cfu/g)	P value
% Moisture	No	45.50	Yes	39.56	<.0001
pH	No	7.38	Yes	7.28	NS[b]
Electrical Conductivity (mmhos/cm)	No	2.20	Yes	0.71	<.0001
Soluble Carbon (ppm)	No	598	Yes	184	<.0001
C:N Ratio	No	15.58	Yes	12.52	<.0001

[a] Biosolids = indicates compost sample where the feedstock contained biosolids or similar industrial waste products.
[b] NS = Not Significant (P > 0.05).

Table 2.5 : Pearson correlation coefficients between chemical parameters and microbial content of all compost samples

	Moisture (%)	*pH*	*Electron Conductivity (mmhos/cm)*	*Soluble Carbon (ppm)*	*C:N Ratio*
Heterotrophs	NS[d]	NS	NS	NS	NS
Gram Negative	0.404[c]	NS	-0.321[c]	0.247[b]	NS
Total Coliforms	0.201[a]	NS	NS	0.209[a]	NS
Fecal Coliforms	NS	NS	NS	NS	NS
E. coli	NS	NS	NS	0.245[b]	NS
Enterococci	NS	NS	0.257[b]	NS	NS

[a] $P<0.05$; [b] $P<0.01$; [c] $P<.001$. [d] NS = Not Significant ($P>0.05$)

Coliform and *Salmonella* Concentrations in Compost Samples

Fecal coliform and *E. coli* concentrations that were determined by spiral plating and MPN tube assays were similar but not always in agreement. Some samples *failed* the fecal coliform "Class A" limit (MPN/g <1000) by one method and *passed* by the other. The results presented in this study are always the larger number (either Cfu/g using spiral plating or MPN/g using the MPN system) as obtained by the respective method. Results presented for each facility (1-15) represent compost samples collected for each month (n=3). Observing the physical and chemical parameters of the compost samples, significant differences (monthly variations) were seen when the data were pooled for each month (Fig 2.12-2.16). Therefore, the microbial content, for each month was analyzed independently to contain variances within each month, as well as determine any effects that the seasons may have on the microbial populations.

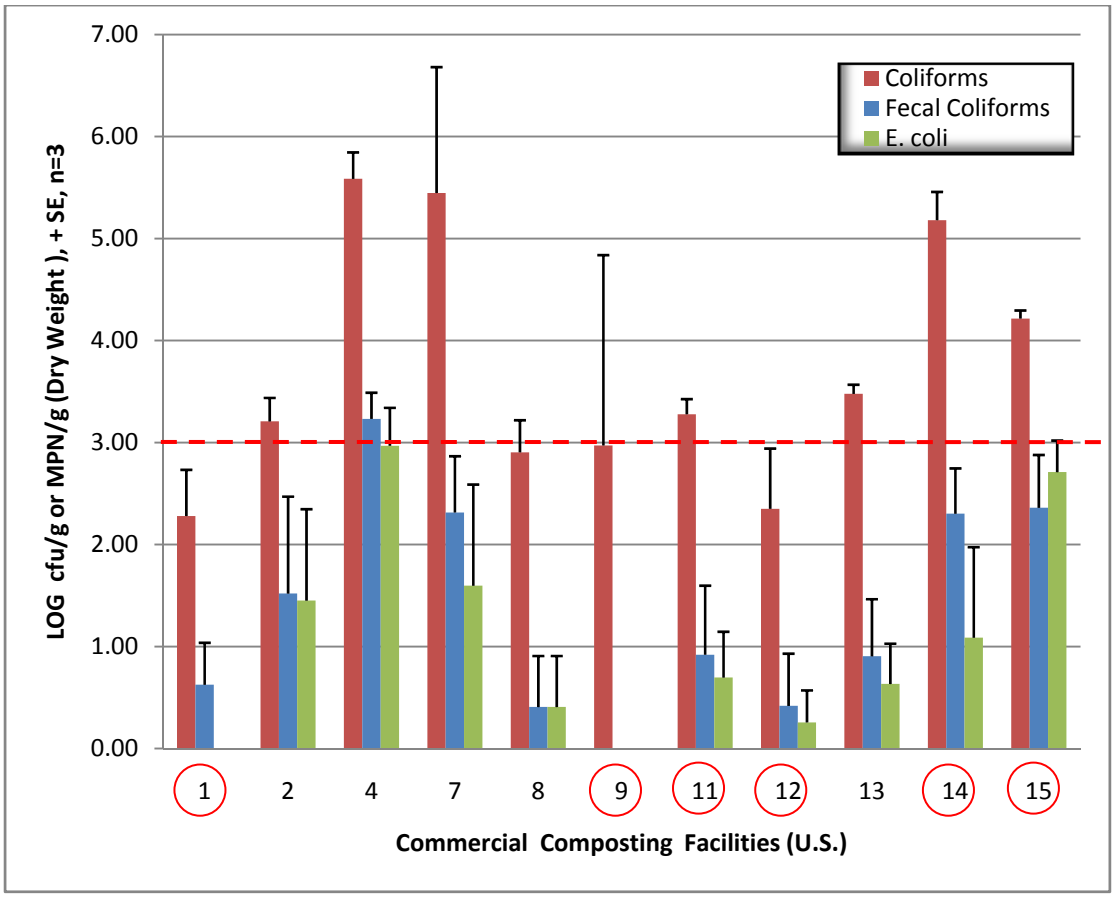

Figure 2.17 : March sampling results for coliform content of compost samples. The dotted red line indicates the acceptable fecal coliform levels for EPA 503 "Class A" product. Facilities with a circle "○" incorporate biosolids or industrial waste products in their composting feedstocks. The higher value of the LOG cfu/g or MPN/g (dry weight) was used.

One compost facility (# 4) in the month of March failed to meet EPA "Class A" standards set for fecal coliform content (Fig. 2.17). This facility was not required to follow routine pathogen testing on the finished products as no biosolids were used. Further, this facility restricted the feedstocks to municipal yardwaste and woodchips. No *Salmonella* spp. was recovered from any of the facilities during the March sampling period.

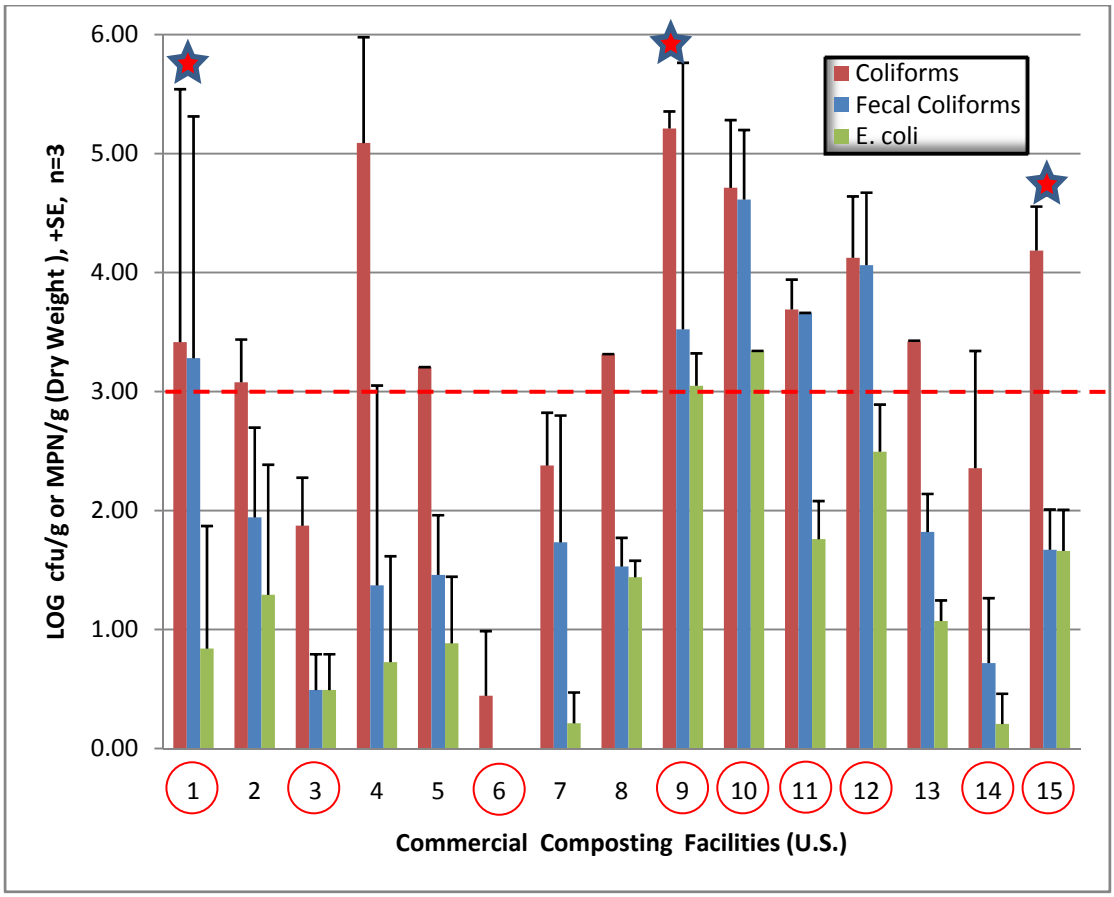

Figure 2.18 : July sampling results for coliform content of compost samples. Samples positive for *Salmonella* spp. are indicated with a "★". The dotted red line indicates the acceptable fecal coliform levels for EPA 503 "Class A" product. Facilities with a circle "○" incorporate biosolids or industrial waste products in their composting feedstocks.

In the month of July (Figure 2.18), five composting facilities (1, 9, 10, 11, 12) failed to meet the "Class A" standards for fecal coliform content. All of the facilities that did not meet this standard were also constructed from feedstocks that contained biosolids. Samples from facilities 1, 9 and 15 were positive for *Salmonella* spp., (20 MPN/ 4 g, < 1 MPN/ 4g, and < 1 MPN/4g total solids, respectively). It should be noted that even though *Salmonella* was recovered from facility #15, the compost samples from this facility were in compliance with EPA 503 "Class A" pathogen

limits for fecal coliforms and *Salmonella* content. Facility #1 failed to meet the "Class A" limits for both fecal coliforms (<1000 MPN/g) as well as the limits for *Salmonella* (<3 MPN/4 g total solids). Facility #9 failed to meet the "Class A" limits only for fecal coliforms.

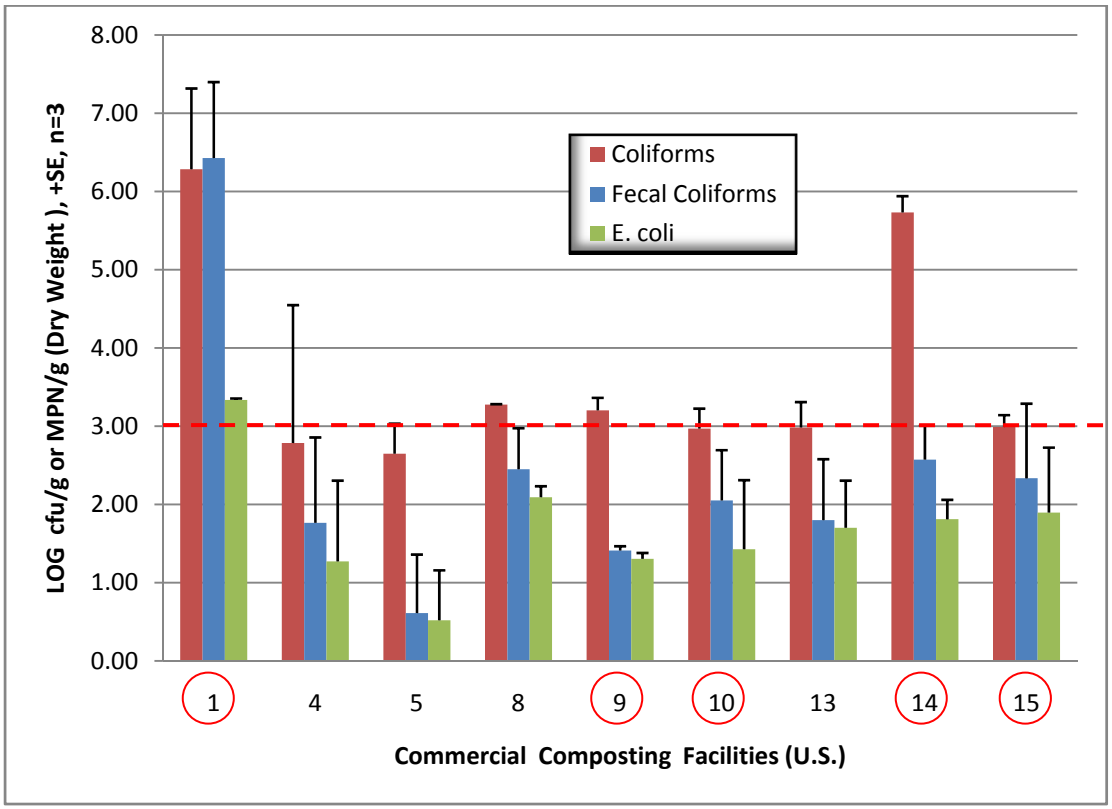

Figure 2.19 : November sampling results for coliform content of compost samples. The dotted red line indicates the acceptable fecal coliform levels for EPA 503 "Class A" product. Facilities with a circle "◯" incorporate biosolids or industrial waste products in their composting feedstocks.

One composting facility (#1) failed to meet the EPA 503 "Class A" requirements for fecal coliform content during the November sampling event (Fig. 2.19). No *Salmonella* spp. was detected from any of the samples in November.

Heterotrophic, Gram Negative and Enterococci populations in Compost Samples.

Total heterotrophic and total gram negative plate counts were determined for all compost samples to ensure that the samples were collected, transported and appropriately stored prior to microbial analysis (Figures 2.20, 2.21 and 2.22). The relatively high heterotrophic counts (as compared to coliforms) assured that the samples were not abused by heat or otherwise decontaminated prior to analysis. This is the first report of a quantitative survey of enterococcal content in finished compost samples from multiple facilities across the United States.

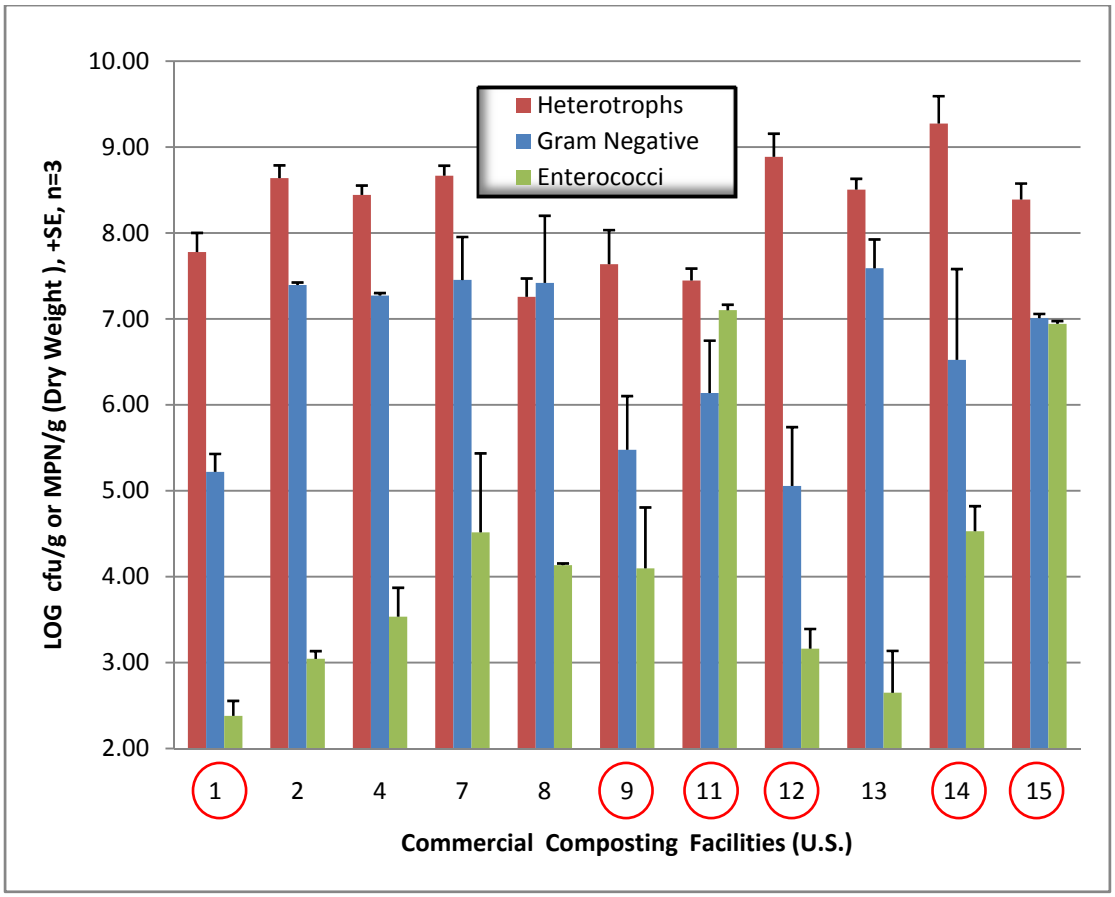

Figure 2.20 : March sampling results for total heterotrophs, gram negative and enterococci content of compost samples. Facilities with a circle "◯" incorporate biosolids or industrial waste products in their composting feedstocks

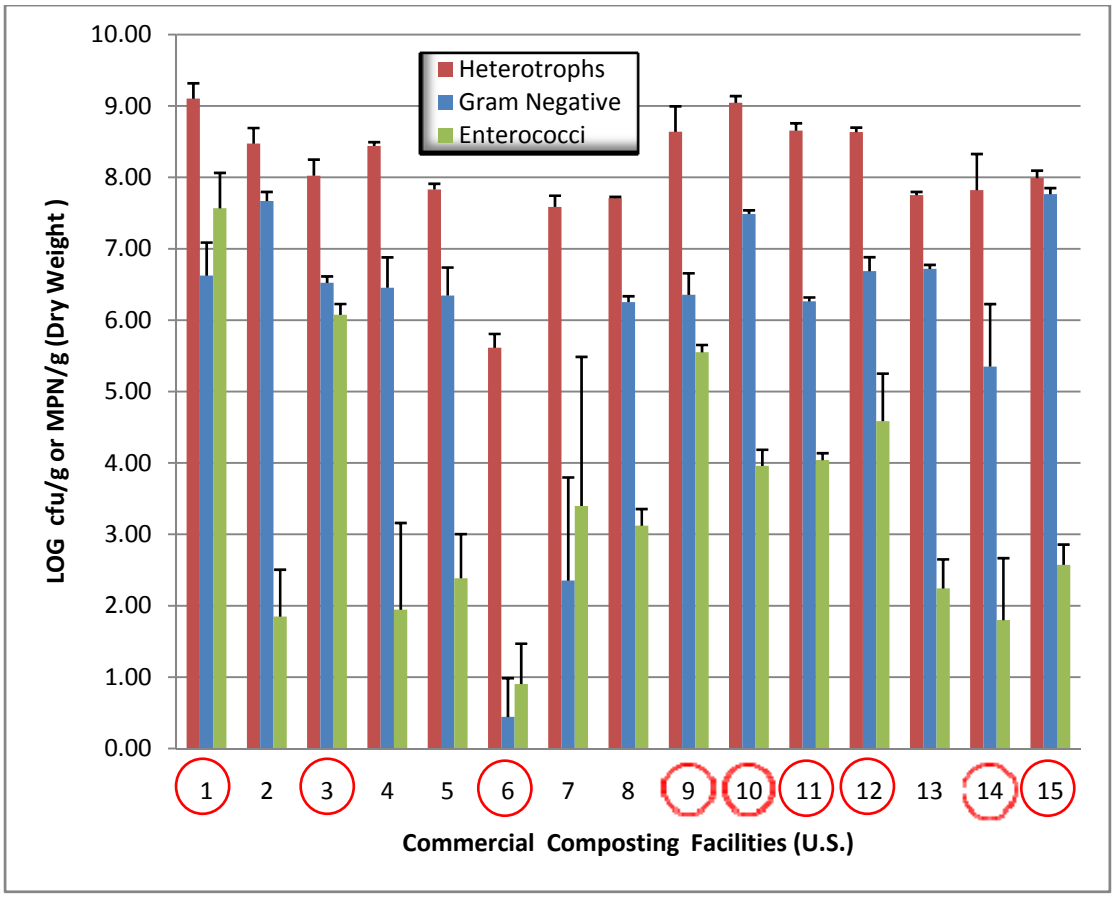

Figure 2.21 : July sampling results for total heterotrophs, gram negative and enterococci content of compost samples. Facilities with a circle "○" incorporate biosolids or industrial waste products in their composting feedstocks

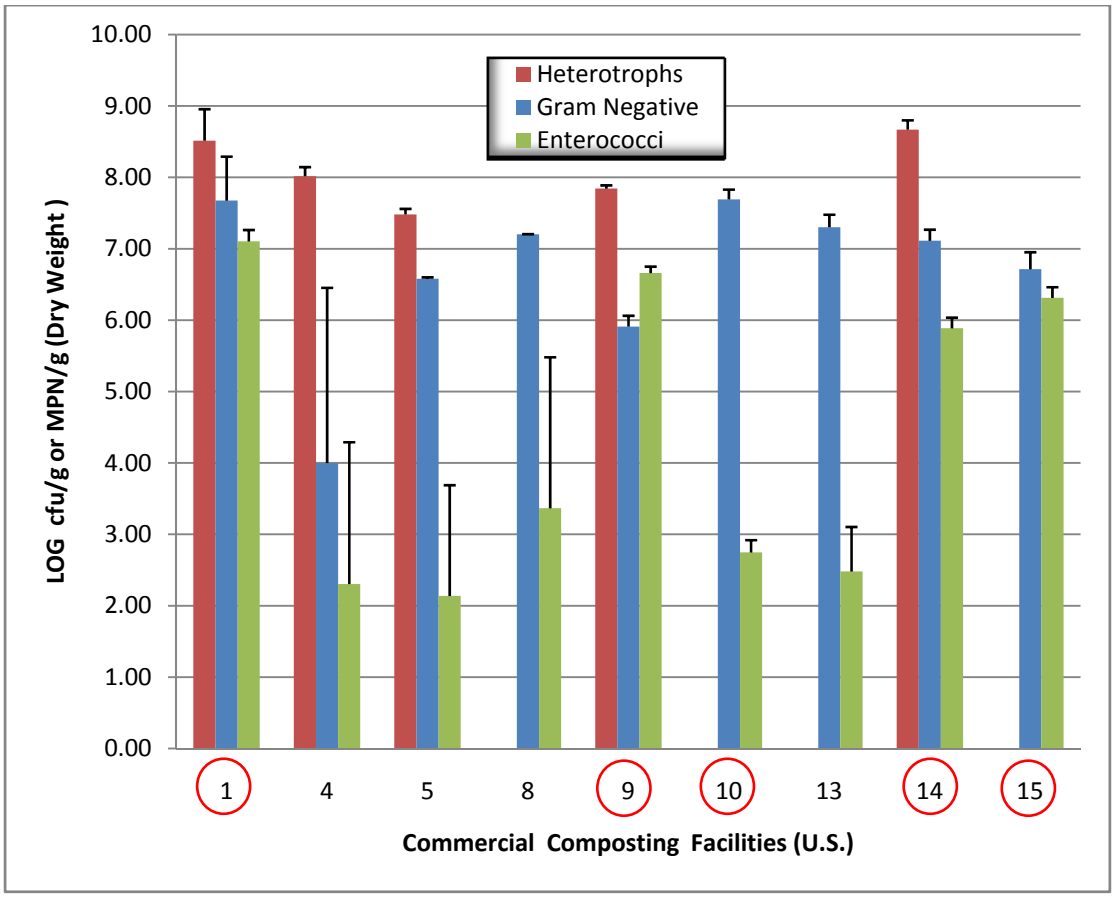

Figure 2.22 : November sampling results for total heterotrophs, gram negative and enterococci content of compost samples. Facilities with a circle "◯" incorporate biosolids or industrial waste products in their composting feedstocks

Monthly Analysis for Fecal Coliform, *E. coli* and *Salmonella* content

Monthly analysis (Tables 2.6 and 2.7) of the individual compost samples and facilities provided some insight concerning the seasonality of the populations of fecal coliforms, *Salmonella* spp. and *E. coli.* Fecal coliform contamination was at the highest frequency of detection in the month of November, in which 89% of all samples were positive, followed by July (82% positive) and March (67% positive). Similarly, *E. coli* was recovered with the highest frequency occurring in November, in which 85% of the samples were positive, followed by 67% of the samples in July

and 58% of the samples containing *E. coli* in March (Table 2.6). While the frequency of fecal coliform and *E. coli* detection in the compost samples was always greater in November than in the preceding months, more facilities failed to meet the EPA regulations for fecal coliform content in July (n=5) than in both March and November combined (n=2) (Table 2.7). Of the seven composting facilities that failed to meet the EPA 503 regulations for fecal coliforms, five were sampled in July. Since all of the composting facilities operated by using similar materials (feedstocks) machinery and personnel for all of the months tested, the higher concentrations of fecal coliforms, *E. coli* and *Salmonella* in certain samples collected in July were likely the result of either some biological, environmental or physico-chemical influence on the microbial population. All of the composting facilities were operated outdoors with the windrows subject to ambient weather conditions. The average temperature and total rainfall data (Table 2.8) showed that the average daily temperatures for all of the facilities in July were at least 20^{o}F higher and had an average of over one inch more rain than in the months of March and November. The warm and moist ambient conditions in July could have provided appropriate conditions, in certain compost samples, to boost the fecal coliform and *Salmonella* populations above the EPA Part 503 regulated levels.

Salmonella spp. was recovered from 11% of the samples in the month of July, while March and November samples were negative. The *Salmonella* isolates (n=74) recovered from facilities #1 (MD), #9 (GA) and #15 (IA) in the month of July were serogrouped into group C (MD) and group E (GA, IA) based on slide agglutination techniques using poly-O antiserum. Clinically significant serotypes pertaining to

Salmonella serogroup C1 and group E that have been isolated from human cases in Maryland, Georgia and Iowa in the year 2000 are depicted in Table 2.10. These data provided circumstantial epidemiological evidence linking the *Salmonella* serotypes found in compost samples to human illness possibly through contaminated fruit and vegetable primarily in these three states during the year 2000.

The fecal coliform and *E. coli* contamination levels in compost were expected to follow a seasonal trend that has been reported for the incidence of foodborne illness, which has been reported to be usually higher in the summer months (June-August) (158, 180). This observation corresponded with findings of increased shedding of enteric pathogens (e.g. *E. coli* O157:H7) in animal feces over the summer months (3, 30, 47, 130). These results were compatible with the findings of this study, considering the particular time-frames, i.e., when the feedstocks were collected for subsequent windrow production at the beginning of the composting process. The "finished" compost samples in November were constructed from feedstocks that were most probably collected during the summer months, and therefore these feedstocks (e.g. manure, biosolids, yardwaste) likely contained more fecal coliforms and *E. coli* than feedstocks collected in other seasons.

Table 2.6 : Monthly percentages of individual compost samples (n=105) that contained detectable numbers of fecal coliforms, *E. coli* and *Salmonella* spp.

	March (n=33)	July (n=45)	November (n=27)
Fecal Coliforms	67%	82%	89%
E. coli	58%	67%	85%
***Salmonella* spp.**	0%	11%	0%

Table 2.7 : Monthly percentages and location of composting facilities that failed to meet EPA guidelines for fecal coliform and/or *Salmonella* content

	March (n=11)	July (n=15)	November (n=9)
***Fecal Coliforms* (>1000 MPN/g)**	9% [a]	33% [b]	11% [c]
***Salmonella* spp. (>3MPN/4g)**	0%	7% [c]	0%

[a] = One composting facility: (#4: CA)
[b] = Five composting facilities: (#1: MD*; #9: GA*; #10: NY; #11: NC*; #12: MD).
[c] = One composting facility: (#1: MD*)
* = Facilities with an asterisk contained biosolids-based compost.

Analysis of all microbial data pooled for each month showed no statistically significant differences ($P>0.05$) among total heterotrophs, total coliform or *E. coli* populations (Figure 2.23). For all of the combined or pooled samples for each month, however, fecal coliform concentrations were at their highest in November and July and at their lowest concentration during the March sampling period. The July sampling produced the lowest concentrations significantly of both gram negative bacteria as well as enterococci populations ($P<0.05$), which was interesting considering that the July sampling month had the highest percentage of facilities 33%) that failed to meet the fecal coliform and *Salmonella* standards.

Table 2.8 : Average temperature (^{o}F) and total precipitation (inches) for all composting facilities in March, July and November for the year 2000

		Temperature Data (^{o}F)			Total Precipitation (inches)		
Facility	Location	March	July	November	March	July	November
1	MD	48.5	72.7	44.2	4.35	5.64	1.73
2	FL	72.9	82.7	70.5	2.00	6.43	5.28
3	ME	33.8	63.6	35.9	5.11	4.27	3.47
4	CA	58.7	76.5	57.2	1.52	0	0.10
5	CT	44.0	69.6	41	3.82	6.77	3.36
6	IA	44.2	72.6	33.9	0.44	3.54	1.52
7	WA	43.0	62.8	40	4.67	1.17	3.97
8	OH	44.4	70.4	41.5	2.26	5.31	1.23
9	GA	55.5	79	49.6	3.41	1.44	5.13
10	NY	43.2	70.7	43.6	4.67	4.1	4.22
11	NC	58.2	79.8	52.7	5.16	10.49	3.34
12	MD	47.6	69.8	42.5	4.72	4.66	2.06
13	IA	45.4	73.0	35.6	0.78	3.77	2.17
14	CA	55.9	79.2	49.1	1.30	0	0
15	IA	46.4	74.4	37.8	1.27	3.35	2.53
MEANS		49.45	73.12	45.01	3.03	4.06	2.67

Data compiled from the Northeast Regional Climate Center (NRCC), Midwestern Regional Climate Center (MRCC) and the National Climatic Data Center (NCDC), all operating under authority of the National Oceanic and Atmospheric Administration (NOAA).

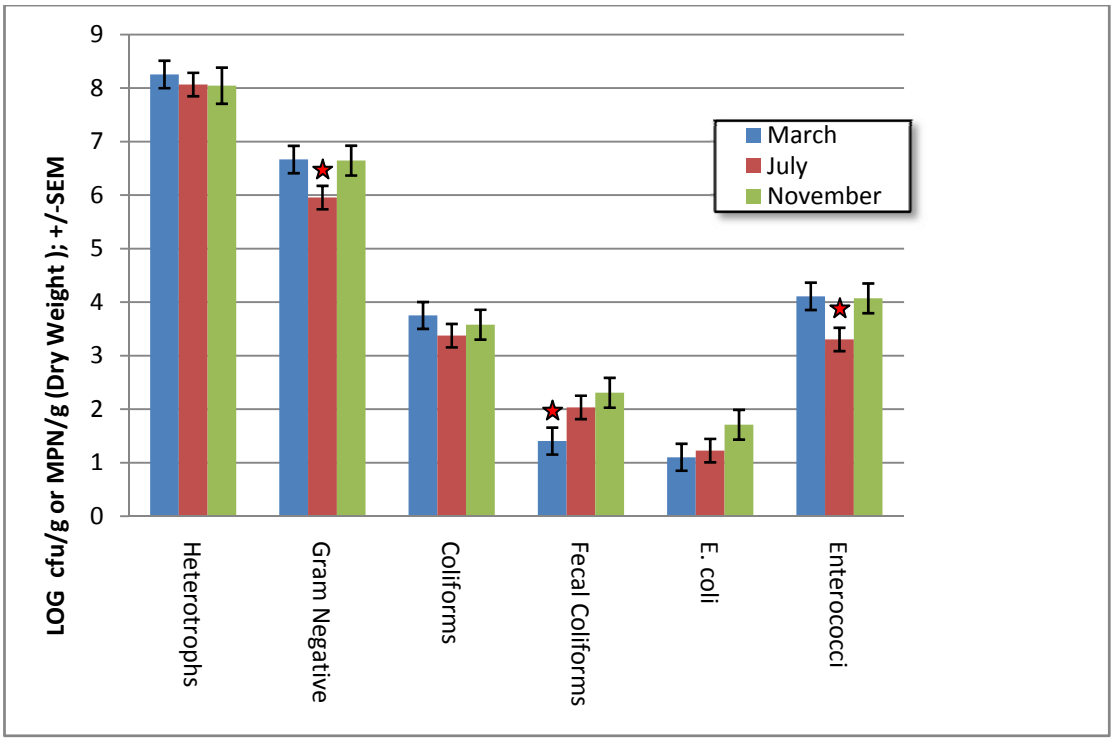

Figure 2.23 : Microbiological content of all compost samples pooled for each month. Bars with a ✭ indicates statistically significant differences (P<0.05) for microbial species between months.

Analysis of Compost Samples Based on Biosolids Content

One-way analysis of variance of compost samples using biosolids or industrial waste products as fixed effects to discern microbial population differences between biosolids-based and non-biosolids-based compost (as collected in this study) are shown in Table 2.9. Using multiple pairwise means comparison techniques and the least significant difference method of separation showed no statistically significant differences (P<0.05) between total heterotrophic, gram negative, coliform, fecal coliform or *E. coli* content. There was, however, a large difference (P<0.0001) in enterococci content between samples containing biosolids in the feedstocks versus those without any biosolids in the feedstocks. This finding suggested that compost

constructed from biosolids as a feedstock might either ordinarily contain a significantly larger population of enterococci in the finished material compared with other composts, or might reflect a regrowth during storage.

Table 2.9 : Analysis of variance of all compost samples across all sampling months comparing microbial content of samples containing biosolids with those containing no biosolids or other industrial waste products

Microbial Species	Biosolids[a]	Grand Mean (Log Cfu/g)	Biosolids	Grand Mean (Log Cfu/g)	P value[b]
Heterotrophs	No	8.0465	Yes	8.2202	NS[c]
Gram Negative	No	6.5144	Yes	6.2015	NS
Total Coliforms	No	3.5203	Yes	3.5733	NS
Fecal Coliforms	No	1.6574	Yes	2.1701	NS
E. coli	No	1.2180	Yes	1.4255	NS
Enterococci	No	2.8235	Yes	4.6701	<.0001

[a] indicates compost sample where the feedstock contained biosolids or similar industrial waste products.
[b] Data generated using SAS version 9.2 using Proc Mixed and multiple pairwise means comparison method using least significant difference set at α=0.05.
[c] NS indicates not significant (P > 0.05).

Characterization of *E. coli* and *Salmonella* isolates

The November sampling month produced the highest frequency of detection of *E. coli*. During this sampling period, 182 *E. coli* and 74 *Salmonella* isolates were harvested from positive samples, and confirmed using biochemical methods previously described. The Fatty Acid Methyl Ester (FAME) components of all isolates were extracted and analyzed using Sherlock software (MIDI, Inc.). The

FAME profiles of all isolates were matched to a database (standard/rapid aerobic bacterial database version 6.0, MIDI, Inc.) and all isolates were positively identified as *E. coli.* Cluster analysis of the FAME principle components (PC1 and PC2) provided a profile of the different communities of *E. coli* from each compost site (Fig. 2.24).

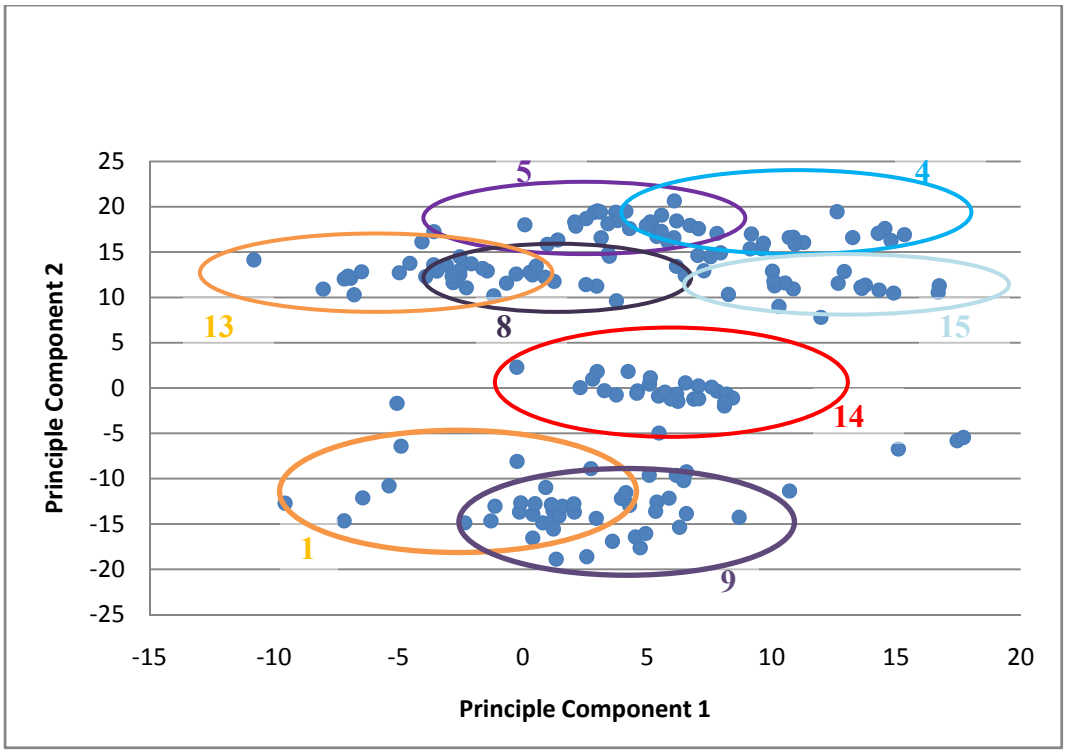

Figure 2.24 : Cluster Analysis of Principle Components (PC1 and PC2) from Fatty Acid Methyl Ester (FAME) profiles for all *E. coli* isolates obtained from November. Circled clusters indicate populations of *E. coli* from compost sites 1,4,5,8,9,13,14,15. Cluster analysis was performed using Sherlock Software v.5 (MIDI, Inc.)

Eight clusters could be identified to differentiate the composting facilities. Many clusters were overlapping, suggesting that several compost sites included feedstocks containing *E. coli* from similar sources (animal manure, biosolids, yardwaste, etc.). The population of *E. coli* from site #14 produced unique FAME

profiles from those of other composting facilities (no overlapping clusters), and also contained the only *E. coli* isolate (#3456) that was positive for the Stx2 gene (Fig. 2.24). The real-time PCR reaction showed a clear amplification of the DNA target for the stx2 gene in isolate #3456 (Fig. 2.25). The PCR reaction was performed twice to confirm the findings.

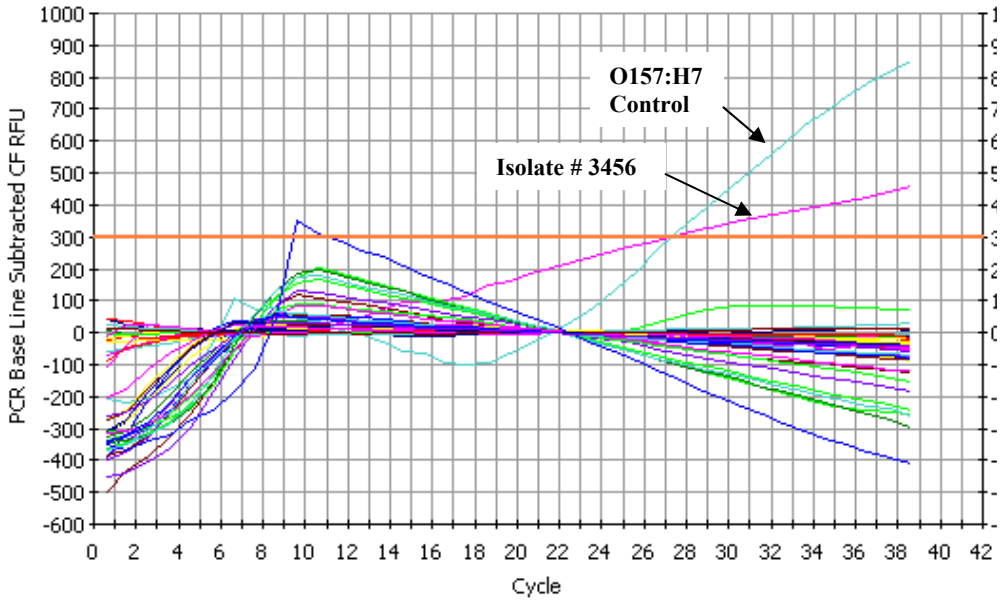

Figure 2.25 : Example of results from a Real-Time PCR reaction for detection of the stx2 gene in selected *E. coli* isolates from compost samples. Curves above the orange baseline indicate a positive amplification of the target gene

Cluster analysis of FAME principle components (PC1 and PC2) for *E. coli* isolates pooled from site #14 (Fig. 2.26) provided a picture of the community relatedness of all isolates within this compost.

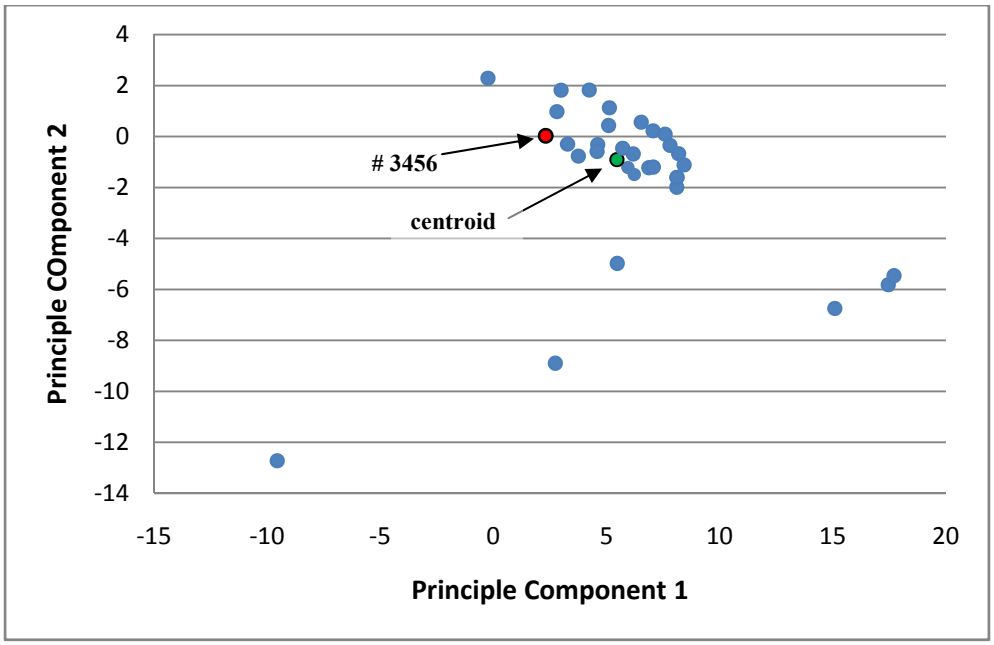

Figure 2.26 : Cluster Analysis of Principle Components (PC1 and PC2) from Fatty Acid Methyl Ester (FAME) profiles for *E. coli* isolates obtained from the November sampling of compost site #14. Isolate indicated in RED (# 3456) was positive for the stx2 gene. The Cluster Centroid is shown in GREEN. Cluster analysis performed using Sherlock Software v.5 (MIDI, Inc.)

Dendrogram analysis of all *E. coli* from site #14 (Figure 2.27) showed that strain #3456 was unique among the other *E. coli* isolates analyzed in this compost sample. The dendrogram analysis contained in the Sherlock software program compared the fatty acid components of all isolates by producing pair-grouping that correlates with genetic similarities. Isolates that are within the Euclidian Distance (E.D.) of '2' are considered the same strain (i.e. clones) and isolates within 6 E.D. are considered the same subspecies. Isolate #3456 was recovered from sewage sludge compost that met the "Class A" classification for both fecal coliforms and *Salmonella* content. In conclusion, 0.55% (n=182) of the confirmed isolates were found to be positive for any of the *E. coli* virulence factors included in our PCR assay.

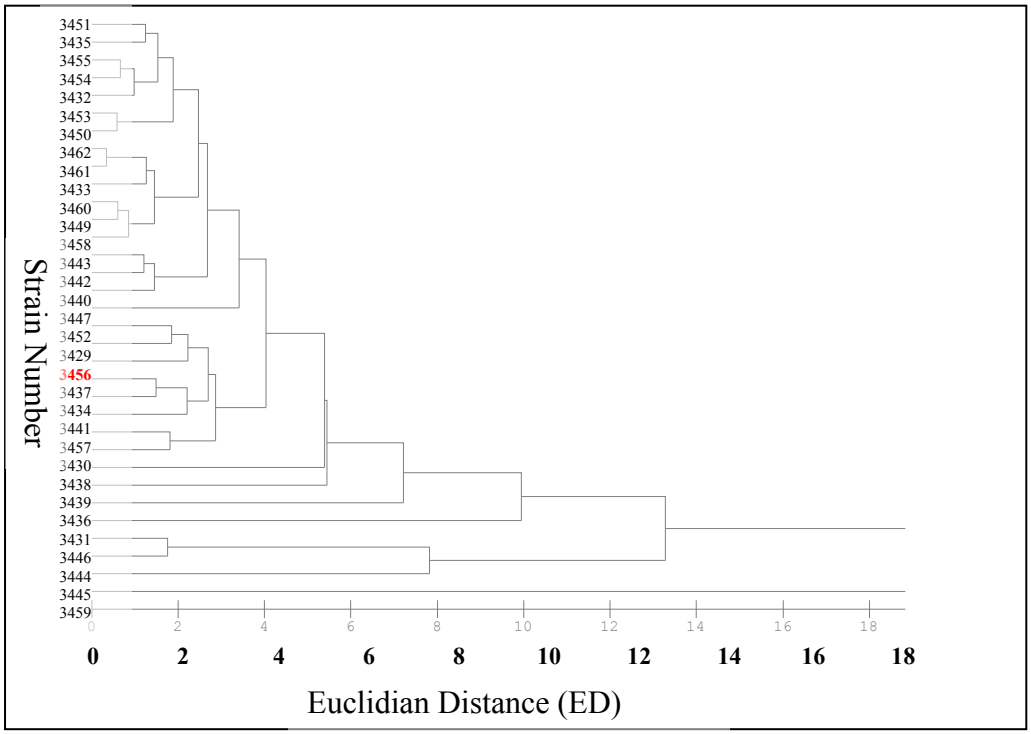

Figure 2.27 : Dendrogram from fatty acid methyl ester (FAME) compositions of *E. coli* isolates from the November sampling from compost site #14. Isolate in RED (#3456) was positive for the stx2 gene. The dendrogram was created using Sherlock Software v.5 (MIDI, Inc)

Salmonella isolates (n=74) were obtained from three composting facilities (#1, #9 and #14) during the July sampling month. All isolates were confirmed biochemically and community analysis was performed using a gas chromatograph equipped with FAME profiles similar to the procedure used with the *E. coli* isolates. Cluster analysis of the principle components illustrates the *Salmonella* community from each composting facility (Figure 2.28). Isolates from facility #1 were serogrouped into *Salmonella enterica* Group C1 while isolates from both facility #9 and #15 were in Group E.

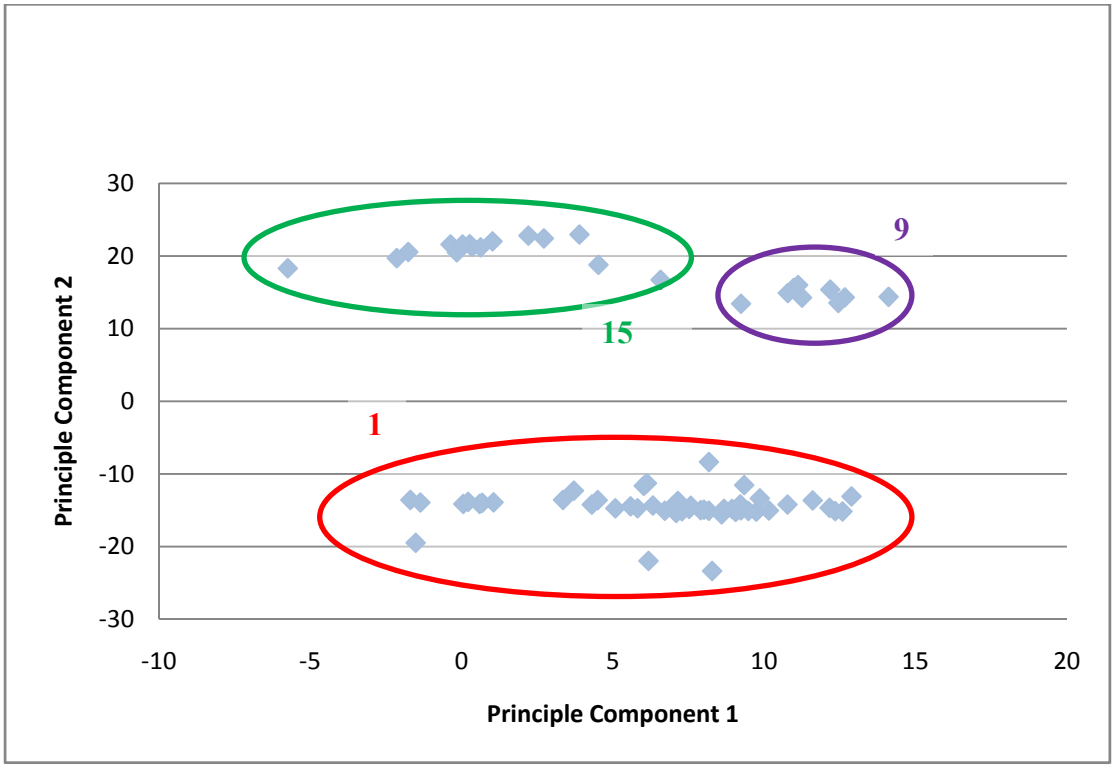

Figure 2.28 : Cluster Analysis of Principle Components (PC1 and PC2) from Fatty Acid Methyl Ester (FAME) profiles for all *Salmonella* isolates (n=72) obtained in July. Circled clusters indicate populations of *Salmonella* from each compost site (1,9,15). Cluster analysis was performed using Sherlock Software v.5 (MIDI, Inc.)

Table 2.10 : A partial listing of *Salmonella* serogroups C1 and E isolations from human clinical sources by serotype, serogroup and states related to this study for the year 2000 (28)

Serotype	Serogrouping[a]	Georgia[b]		Iowa[b]		Maryland[c]	
		# isolates	% of total	# isolates	# isolates	% of total	% of total
Anatum	E	**2**	**0.12**	**2**	**0.57**	4	
Muenster	E	**2**	**0.12**	0		3	
Uganda	E	**1**	**0.06**	0		5	
Bareilly	C1	0		2		**16**	**2.1**
Braenderup	C1	3		11		**25**	**3.4**
Hartford	C1	13		3		**2**	**0.27**
Infantis	C1	41		2		**14**	**1.9**
Mbandaka	C1	5		2		**1**	**0.14**
Montevideo	C1	47		16		**9**	**1.23**
Ohio	C1	0		1		**2**	**0.27**
Oranienburg	C1	17		2		**4**	**0.55**
Tennessee	C1	1		1		**1**	**0.14**
Thompson	C1	20		13		**10**	**1.36**
Virchow	C1	0		0		**2**	**0.27**

[a] Serogrouping as determined by the slide agglutination method using Poly-O antiserum (Difco).

[b] Georgia and Iowa human isolates that are shaded correspond to *Salmonella enterica* serogroup E that were isolated from composting facilities #9 and #15. The numbers in parentheses represent the percentage of the total *Salmonella* human isolates reported from Georgia (n=1726) and Iowa (n=351) for the year 2000. The concentration of *Salmonella* determined in the compost samples for Georgia and Iowa was < 1 MPN/4g.

[c] Maryland isolates that are shaded correspond to *Salmonella enterica* serogroup C1 that were isolated from composting facility #1. The numbers in parentheses represent the percentage of the total human isolates reported from MD (n=733) for the year 2000. The concentration of *Salmonella* determined in the compost samples from Maryland was 20 MPN/4 g.

Summary for Fecal Coliform, *E. coli* and *Salmonella* Content

In summary, a total of 105 compost samples from 15 facilities were collected, during a nine month period: 11 facilities participating in March, 15 facilities participating in July and 9 facilities participating in November. The pathogen content of each sample was determined independently, and the means of three samples were calculated to determine whether each facility was in compliance with the EPA 503 regulations. Of all the facilities, 80% were in compliance for the fecal coliform standards (< 1000 MPN/g) and 97% met the *Salmonella* standards (<3 MPN/4g). Most samples were in compliance with the "class A" requirements; however most of the samples tested also contained countable populations of fecal coliforms and *E. coli*. When determined by the MPN system (lower detection limit than the spiral plating methods), 79% (n=83) of all the samples contained fecal coliforms; 69% (n=72) contained *E. coli*; and 6% (n=6) contained *Salmonella* spp. It should be noted that while most of the populations of these potential pathogens were within the EPA acceptable standards, the compost samples could not be considered completely pathogen-free with any assuredness.

Discussion

The majority of the 105 compost samples collected in this study contained detectable numbers of fecal coliforms (79%) and *E. coli* (68%), with the highest frequency of detection found in the month of November. The greater numbers of composting facilities that failed the "Class A" pathogen equivalency limit occurred in July as compared with March and November. Of the facilities in this study, 20% failed to meet the fecal coliform "Class A" standard, which was consistent with a 1995 survey of 16 biosolids-only composting facilities in Massachusetts where 30% of the facilities failed to meet this standard (231). In this study, four of the seven composting facilities that failed to meet the "Class A" standard had used biosolids or industrial sludge as a feedstock. One compost facility (#1: MD) produced 'finished' products that exceeded the "Class A" pathogen equivalency limit across two months of sampling (July and November).

All compost samples contained enterococci, which were determined to be similar in concentration to the amounts of total coliforms across all samples. Due to this high prevalence, the use of enterococci as an indicator organism for the presence of fecal contamination does not appear appropriate based on the results obtained in this study. Furthermore, Tonner-Klank et. al in 2007 also presented evidence against using enterococci as an indicator organism due to the potential for rapid regrowth after proper disinfection of the compost had been accomplished (252). It is possible that the enterococci populations in the finished compost samples, in this study, were a result of re-colonization during the curing phase of the compost manufacturing process. However, the sampling scheme should have precluded the detection of

environmental contaminants, because only products from the centers of each windrow sampling location were analyzed.

It might seem that the sampling scheme would have benefitted this study by including analysis of all nine individual samples from each windrow (Fig 2.2), rather than consolidating the samples for analysis (Fig. 2.3). While this would have provided more information concerning pathogen content in different locations of each windrow, the scope of this study did not involve analysis of within-windrow sampling locations. The primary focus was to provide accurate microbiological content from a representative sample from each composting facility and to determine patterns in seasonal data and possible correlations with pathogens and pathogen-indicators to the physico-chemical parameters as determined in this study. This was achieved by combining the samples from each of three locations (two ends and one from the middle) within the windrows. Population variances within each windrow sampling-location were minimized by consolidating and homogenizing three 'grab samples' from each location. Even though each of the three samples was collected at the same sampling depth (e.g., the 'hot zone') at each of the three sampling locations within each windrow, the microbial population variances between each consolidated sample remained quite large. The physico-chemical (moisture, soluble carbon, EC, pH, and C:N) profiles of the materials were similarly different at each windrow location. Although the dependence of the bacterial populations on the physico-chemical variances appeared to be based on sampling location within each windrow, the approach for this study by consolidating samples obviated dependence upon individual sample characteristics. If this information is provided in similar studies in

the future, it could produce sampling protocols to ensure homogeneity and elucidate locations within the windrows where favorable conditions for pathogen presence and homogeneity might be less variable.

Temperature gradients within the compost windrow can be quite large. Temperatures vary not only along the length of the windrow (the ends of the rows are cooler than the middle) but the temperature also fluctuates from the surface of the windrow towards the center. That is, the middle and center of each windrow will generally contain the highest temperatures perhaps because these areas typically contain the most bulk and are somewhat better insulated from the environmental elements. Indeed, Shepherd et. al. in 2007, reported that *E. coli* O157:H7 was able to survive on the surfaces of on-farm, minimally managed compost piles that had lower temperatures than the middle, center or bottom of the pile locations, where the pathogen, inoculated at 10^5 cfu/g, did not survive after 5 days in ovine manure (218). The types of materials used to construct compost windrows are usually non-uniform and bulky. These materials are difficult to homogenize, and as such are prone to produce a variety of areas containing differing levels of microbial activity. Windrow composting, therefore, involves an active process of mechanical homogenization, or "turning", of the piles at least five times during the peak heating cycles, which is required to reach a minimum temperature of at least 131^{o}F or 55^{o}C for 15 days. This is the minimum recommendation set by the United States Composting Council. This process, after several months, effectively homogenizes the feedstocks into a uniformly biodegraded and soil-like material.

The sampling scheme, in this study, was chosen to represent three locations within each windrow "hot zone," which is an area located in the center portion of the windrow. These areas were regions most likely to contain well homogenized material that should have been relatively protected from pathogen re-contamination either from the environment or wildlife. All composting facilities in this study produced compost as outlined by the United States Composting Council and, where required, complied with the EPA 503 regulations for fecal coliform and *Salmonella* levels before samples were supplied for this study.

The chemical and physical parameters used in this study (pH, Moisture, EC, Soluble Carbon and C:N ratios) were good indicators of compost quality, and indicated that all compost facilities in this study followed good manufacturing guidelines to produce excellent quality "class A" compost. While the analysis of variance did not result in significant interactive effects between any physico-chemical parameter and specific microbiological populations, significant Pearson correlation coefficients were determined when correlating microbiological data with moisture content, electrical conductivity and soluble carbon. All significant correlations can be seen in Table 2.5. Of particular interest for this discussion, *E. coli* populations were positively correlated with soluble carbon content in the compost samples and enterococci populations positively correlated with electrical conductivity values. The fact that more correlations were not observed was surprising, considering that the literature is replete with examples of such correlations in smaller, laboratory-based experiments. Gong, et. al, in 2005, (79) determined a correlation between moisture content and survival of *E. coli* in compost. Oddly, they determined that *E. coli* was

more prevalent in compost samples containing low moisture content (< 40% moisture). This finding suggested that the temperature susceptibility of gram negative enterics (i.e. *Salmonella* and *E. coli*) was more pronounced when the microbes were actively growing, or in log-phase, which, conversely, occurs more often in high moisture samples. Under low moisture conditions (low water activity), these microbes are not as metabolically active (i.e., stationary phase) as in log phase and may be more resilient to the physico-chemical stressors contained in the compost. El-sabaie et. al (2002) determined a positive correlation between total coliforms and fecal coliforms (including *E. coli*) and soluble carbon content in compost samples (49), suggesting that a few cells that may survive the temperature cycling during the composting process would be able to rapidly repopulate in composts when carbon was readily available.

The results of this study do not provide evidence to suggest that either low moisture or soluble carbon content of compost samples are indicators of the prevalence of either *Salmonella* or *E. coli* in the final compost products. Therefore, in the absence of their ability to predict the presence of these organisms, physico-chemical parameters do not appear to be applicable as *reliable testing standards* to indicate the potential for pathogen content in mature compost samples. Although this study concludes that microbial pathogens in final compost products may be due to factors in the compost other than the physical and chemical parameters, it is also important to consider that favorable conditions present for pathogen growth or re-growth is only likely to be significant if the pathogen is introduced at some point during composting, e.g., through the original compost material or through wildlife

excrement. For example, as shown by Hayes, et. al in 2000, optimal moisture levels are not necessarily an indicator of the presence of *Salmonella* but do provide favorable conditions, when and if, *Salmonella* is introduced into a particular ecological niche (90). Further, routine pathogen testing is necessary to provide assurance, but by no means guarantees, that microbial pathogens are absent in final compost products.

Rather than contributing to the already overflowing landfills, the production of compost is rapidly becoming an accepted and universal method for reducing the amount of organic waste that can be re-used in an environmentally acceptable fashion. As such, there has been much research, however unsuccessful, into the development of a universal biological additive or amendment that would consistently and efficiently expedite the composting process (264, 279) Investigations into the complex and fascinating world of microbiological community succession throughout the composting process has determined that, indeed, the thermophilic composting process involves many population stages that can be identified by tracking microbiological communities based on lipid analysis of the microbes contained in the compost (94, 125, 237). Steger in 2003 (237) and Herrmann in 1997 (94) have definitively shown that although gas chromatograph analysis of fatty acid methyl ester (FAME) profiles may be used to determine that the compost has completed the thermophilic stage, reached maturation, and has been properly cured, it does not reflect the potential for microbial pathogen content or potential for pathogen re-growth.

In this study, gas chromatography analysis of Fatty Acid Methyl Esters (FAME) from individual microbial isolates has been shown to be an effective method for identifying specific genera and species, as well as identifying clonality in a population based on closely paralleling DNA and ribosomal RNA homologies (135, 144, 261). Using the Sherlock™ Microbial Identification System software (MIDI, Inc., Newark Delaware), FAME profiles from multiple isolates were compared using cluster analysis to illustrate strain similarities. The dendrogram and 2-D cluster analysis capability of the FAME principle components system was used to graphically illustrate the populations of *E. coli* collected from eight compost sites in November, and *Salmonella* isolates collected from three compost sites in July. Due to the inherent variability of microbial fatty acid production based on availability of nutrients, temperature and moisture, careful attention was given to the growth of each isolate using the same medium and atmospheric conditions for all isolates from which the fatty acids were extracted by following the strict protocols as described in the materials and methods section of this study. Cluster analysis of all *E. coli* isolates (Fig. 2.24) showed unique populations from each of the compost facilities. Cluster analysis of *Salmonella* isolates produced a similar differentiation based on compost facility (Fig. 2.28). The 2-D analysis of FAME principle components is a powerful tool used to visualize relationships of distantly related organisms, and we expected to see similar clusters of *E. coli* and *Salmonella* isolates based on compost facility location and types of feedstocks (e.g., biosolids or otherwise) used at each facility.

Whole-cell Fatty Acid Methyl Ester (FAME) profiles have been used with mixed success in attempts at microbial source-tracking to identify *E.coli* isolates from

human and non-human sources. For example, Parveen et. al., in 2001 was not able to identify human from non-human *E. coli* isolates based on FAME profiles (175), while Seurinck et. al, 2006, provided evidence to suggest that FAME profiles may be used to differentiate *E. coli* isolates between human and non-human sources(215). Indeed, in the study presented here, FAME principle component clusters of *E. coli* from biosolids-based compost (Fig.2.24, Site#1,9,14,15) tended to group separately from yard and foodwaste composts (Fig. 2.24, Sites# 4,5,8,13). This observation suggested that *E. coli* populations in compost constructed from mainly biosolids-based feedstocks (e.g., containing *E. coli* isolates from human origin) might provide unique FAME profiles as compared to *E. coli* isolates from compost constructed from other sources (e.g. animal and environmental origin). The cluster analysis for the *Salmonella* isolates (Fig. 2.28) did not show any 'overlapping' clusters, as all isolates were well defined within the cluster for each composting facility. This was surprising considering that all compost samples that contained *Salmonella* were constructed from biosolids. Based on the hypothesis, if all isolates were indeed from the same feedstock material, there should have been some overlapping isolates between the composting facilities. However, this was not observed, perhaps because the *Salmonella* isolates were indeed from wildlife or other environmental contamination, or that there are sufficient differences among the biosolids sources to manifest different populations of *Salmonella*.

Real-time PCR analysis was used to determine the prevalence of Shiga-toxigenic *E. coli* among 182 isolates obtained from eight facilities in November. One *E. coli* isolate (0.55%) was positive for the Stx2 gene that was recovered from sewage

sludge compost (site #14) that met the EPA 503 standards for *fecal coliforms* and *Salmonella*. The dendrogram analysis, which uses cluster analysis techniques to provide pair-matching of isolates based on fatty acid compositions to illustrate these differences using Euclidian Distance as a measure, provided a closer inspection of thirty three *E. coli* isolates obtained from facility #14 (from which only sixteen unique profiles were found). Using this scale to analyze two isolates, a Euclidian Distance of less than "2" suggests clonality (i.e., the strain is being compared to itself). The isolate that was positive for Stx2 (#3456, Fig 2.26 and 2.27) was unique among the sixteen individual strains identified in the compost sample. While the low prevalence of toxigenic *E. coli* that was determined in the compost samples may not be alarming, one must consider that the encoding and subsequent expression of Shiga toxin by *E. coli* requires merely the infection by a shiga-toxin gene encoding coliphage. The possibility remains that since one *E. coli* isolate was indeed found to contain the Shiga toxin virulence factor, the other closely resembling *E.coli* isolates in the same cluster might also be at risk for phage transformation into shiga-toxigenic *E. coli* (STEC).

Although the determination of the levels of coliphage in compost samples was not within the scope of this study, future studies are warranted to determine the prevalence and infectivity of coliphage both during the composting and curing processes of commercial composting facilities.

The National *Salmonella* Surveillance System (NSSS), since 1968, has collected surveillance data regarding clinical and non-clinical *Salmonella* isolations from all 50 state public health laboratories, which now electronically reports real-time

information through the Public Health Laboratory Information System (PHLIS). The NSSS is maintained and operated by the Centers for Disease Control and Prevention (CDC). All clinical *Salmonella* isolates from clinical diagnostic laboratories are submitted to each state public health agency for biochemical testing, serotyping and further characterization of these pathogens. The information reported by the NSSS for the year 2000 was analyzed to generate circumstantial evidence to suggest that the *Salmonella* isolates obtained from three of the composting facilities (#1: Maryland; #9: Georgia; #15:Iowa) could possibly be linked to clinical cases through the dissemination and usage of the compost as soil conditioners, land application, and organic fertilizers in agriculture in these states.

All *Salmonella* isolates in this study were grouped according to their "O" polysaccharide antigens, which is the first step in the further identification of the isolate to species. Pulse-field electrophoresis (PFGE), as used by PulseNet, is a powerful classification system that is used to differentiate and track individual subspecies through epidemiological studies. Serotyping, however, remains the most reliable and widely used (worldwide) tool available for epidemiologically tracking these pathogens and the National *Salmonella* Surveillance System uses serotyping to track *Salmonella* isolates. All *Salmonella* isolates from the Maryland facility were typed to group C1, and all isolates from Georgia and Iowa were typed to group E. Summary information for the clinically significant isolates within each of these serogroups is provided in Table 2.10 (including some of the prevalence data of each serogroup by human clinical illness occurring in the year 2000). Table 2.10 contains the serotypes (and associated serogroup as determined in our study) that were isolated

from human illness in Georgia, Iowa and Maryland for the year 2000. Indeed, *Salmonella* from serogroups C1 and E have been associated with human clinical illness in Maryland, Georgia and Iowa. Furthermore, five of the top twenty *Salmonella* serotypes involved in human clinical illness in the United States in the year 2000 were from serogroup C, which was a characteristic of some of the isolates in this study.

The concentrations of *Salmonella* that were recovered from the compost samples were very low. A total of 74 *Salmonella* isolates were recovered for analysis from facilities #1 (MD), #9 (GA) and #15 (IA) which were at concentrations of 20 MPN/ 4 g, < 1 MPN/ 4g, and < 1 MPN/4g total solids, respectively. While these concentrations do not appear to be considered a hazard, especially if the compost is land-applied and the *Salmonella* populations would most likely be diluted with soil microbiota as well as exposed to environmental conditions to further reduce the population, re-colonization on fruit and vegetables under favorable conditions could allow *Salmonella* to grow to critical numbers. This could become a danger for human ingestion. Also, the potential for *Salmonella* re-growth in compost has been well established, therefore posing the possibility of an increase in the bacterial populations under improper storage conditions of the final compost product (43, 199, 223).

Commercially available compost, whether produced from animal manure, sewage, or other materials should not be considered "pathogen free" at present. The results of this study show the need and value of periodic quality control analyses on compost processes that claim to meet the product decontamination standard via a time-temperature exposure protocol. The presence of <10^3 MPN fecal coliforms/g

in compost was generally typical of the count of *E. coli.* The presence of biosolids as composting feedstocks did not produce final products with significantly different amounts of heterotrophs, gram negatives, coliforms, fecal coliforms or *E. coli* bacteria than other composts. Enterococci counts, however, were significantly higher among compost samples containing biosolids as a feedstock. This was surprising as we expected most enterococci to be killed during the composting process. This finding may be attributed to potentially higher populations of indigenous enterococci in biosolid-based feedstocks. The use of enterococci as a "process control" indicator for the production of high quality composts has been suggested by Larson et. al., 1994 (139). However, due to the presence of this microbe in all final products in this study, it is difficult to accept Larson's suggestion. Because enterococci are typically more resilient to temperature and physico-chemical stressors during the composting process (as are most gram positive organisms compared to gram negatives), recognition of a significant reduction of this organism from feedstock to final compost product may provide a conservative indicator of reduction or destruction of pathogenic gram negative bacteria (i.e., *Salmonella* and *E. coli*).

If "Class A" compost with low concentrations of *E. coli* or *Salmonella* were to be used for the production of alternative fertilizers, e.g., nutrient supplemented compost tea, re-growth of these bacteria could achieve levels exceeding the limit for potable water used for irrigating or spraying crops. Routine testing of final compost products is critical for assuring the microbiological quality and safety of these products. If only annual testing is prescribed, then these results suggest testing during either the summer or winter months (e.g., in July or November) would be more

beneficial to detecting the fecal coliforms, *E. coli* and *Salmonella*. This research shows that some compost can contain Shiga-toxigenic *E. coli* and *Salmonella* even when the fecal coliform concentrations are within "Class A" pathogen limit standards. This suggests that the current EPA criteria for producing "Class A" compost should be revised.

CHAPTER 3

POTENTIAL MULTIPLIER FACTORS EFFECTING COMPOST TEA AS A SOURCE OF *ESCHERICHIA COLI* AND *SALMONELLA*

Abstract

Compost tea (CT) is an unheated, on-farm infusion of compost used as a spray or soil drench to promote plant-growth and control foliar/root diseases. Food safety protection guidelines from farm-to-fork suggest that CT should meet basic microbiological criteria for water quality. Therefore, this research describes the effects of two CT production processes, aerated (ACT) and non-aerated (NCT), on growth and survival of foodborne pathogens and fecal coliforms under various conditions. Seven commercially available nutrients used to supplement CT were tested individually and in combination for their effects on the growth of *E. coli* and *Salmonella*. Composts containing 10^1-10^3 CFU/g initial concentrations of *E. coli* O157:H7 and *Salmonella enterica sv* Enteritidis (hereafter termed *S. enteriditis*) were used to assess growth and survival responses to ACT (36 hr) and NCT (8.5 days). Pathogen and fecal coliform populations were undetectable by 8.5 days in NCT without nutrient supplements. *E. coli* O157:H7 decreased to below detection levels in ACT at 36 hr without the use of supplements. In contrast, the addition of commercially formulated mixtures or combinations of nutrient supplements resulted in growth of *E. coli* O157:H7, *Salmonella* and fecal coliforms by 1-4 logs in both ACT and NCT. When nutrient supplements were added, ACT revealed higher concentrations of *E. coli* O157:H7, *S. enteritidis* and fecal coliforms than NCT.

Results showed that addition of supplements stimulated growth of human pathogens inoculated at very low initial concentrations in ACT. Based on the findings of this study, the addition of nutrients to CT should be avoided when CT is used on fresh produce.

Introduction

Interest in and use of compost extracts as crop protection sprays originated with reports documenting control of plant and fruit diseases on grapes, beans, and tomatoes (20, 50, 128, 129, 202, 276). Earlier evidence showed that motility of sting nematodes, *Belonolaimus longicaudatus Rau*, a root pathogen, was impeded by refuse compost extracts (103). Current interest by organic growers and others in non-chemical control of plant disease has encouraged the development of a wide variety of commercially available devices and products for on-farm production of watery extracts of compost, now currently referred to as compost tea (CT). Plant protection and growth promotion efficacy data with several variations of currently available CT production devices and products is slowly emerging, but shows a high degree of variability (105, 108, 200, 207, 208, 212). Interest in and the use of CT as a spray for biocontrol of foliar and fruit diseases or as a soil drench for plant-growth promotion, as well as a biocontrol of root diseases (23, 105-107, 253, 273, 274), have expanded among growers in the U.S. and abroad.

This trend is particularly the case as more equipment and information about this on-farm practice has become available through the internet. In the United States, the National Organic Standards Board, which advises the Secretary of Agriculture on various aspects of implementing the USDA National Organic Program, has

recognized that, in addition to the widespread use of compost, there is an increasing use of compost tea among growers. The NOSB Compost Tea Task Force reviewed the known practices and scientific information on the subject to develop and report their recommendations: which can be found at (http://www.ams.usda.gov/nosb/meetings/CompostTeaTaskForceFinalReport.pdf).

In contrast to initial reports on the crop protection benefits of CT, either prepared without aeration or incorporated with supplemental nutrients (4, 33, 208, 210, 253), recent commercial trends have emphasized production of aerated CT (ACT) by mechanically infusing air into the liquid during the first 18-36 hr of the brewing period (105-108, 207) followed by immediate use on crops or soils. This trend is particularly suited to commercial users interested in the relatively quick production and usage of ACT (within 24 hours). Static or non-aerated CT (NCT) production methods, which involve steeping of compost in water over a range of time periods from several days to several weeks, are still used by some farmers to make CT on the farm. The addition of various supplements to the liquid phase at the start of the brewing period is another recent trend advocated by some practitioners and CT equipment vendors (207). Some of these supplements are nutrients designed to encourage a rapid increase in the concentration and metabolic activity of bacteria and fungi evolving from the compost matrix (106, 207). The number of published (13, 44, 45, 112, 123) and unpublished reports (Brinton, personal communication; E. Ingham, personal communication) on CT and compost/water mixtures are limited and have yielded conflicting results concerning the growth dynamics of *E. coli* and *Salmonella* in CT. Because CT is sprayed onto plant parts that could be consumed

raw, it is important to understand the potential for pathogen growth in CT. When various supplements are added to the CT liquid at the start of the brewing process, the growth of pathogenic microbes that may have survived composting and are inadvertently introduced into the brewing process, could be a concern.

One approach to controlling pathogen growth is to ensure that high quality compost devoid of pathogens is used for CT production. The National Organic Standards Board recommends preparation of compost tea from compost that meets thermophilic, time-temperature standards for a Process to Further Reduce Pathogens (PFRP) as described in the U.S. EPA regulations for composting (57). Raw manure, *partially* composted manure, or commercial food waste are not suitable for CT production within the current USDA certified organic program.

While thermophilic compost is suitable for application to gardens, lawns, and soils, it cannot be considered a sterile product. Therefore, even a few surviving bacterial pathogens in a batch of thermophilic compost, might grow to large numbers when favorable conditions are available, such as those possibly present in some CT.

This research describes the effects of two CT production processes, aerated, and static or non-aerated and amended in several ways, on growth and survival of foodborne pathogens and fecal coliforms. Four compost tea production systems were evaluated for their effects on microbial populations (heterotrophs, gram negatives, coliforms, *E. coli*, *Enterococcus* and fungi) in 24 hour aerated compost tea produced using the same feedstocks. Seven commercially available CT nutrient supplements and their individual components were tested to determine their individual and combined effects on growth of selected pathogens and indicator organisms. Finally,

this research examined the effect that heterotrophic populations, pH, electrical conductivity, and total organic carbon content of ACT collected throughout the brewing cycle, on the ability of *E. coli* O157:H7 to survive and compete with indigenous populations for available nutrients under aerobic (ACT) conditions.

Materials and Methods

Bacteria and culture conditions.

Escherichia coli isolate 427 (serotype O157:H7) donated by X. Jiang, Clemson University, originated from cattle feces, and had ampicillin resistance (100 µg/ml) and plasmid-related green fluorescent protein traits (117). Isolate 435, a spontaneous mutant of *E. coli* ATCC 43895 that resists nalidixic acid (50 µg/ml), (implicated in a foodborne disease outbreak and isolated from ground beef) was donated by J. Meng, Univ. Maryland, College Park. *E. coli* isolate 466 was isolated from dairy manure compost at USDA Beltsville, Maryland. *S. enteritidis* isolate 430 was a spontaneously resistant mutant to ampicillin (100 µg/ml) was donated by X. Jiang. *Salmonella senftenberg* isolate 695, a spontaneously resistant mutant to nalidixic acid (50 µg/ml), was donated by J. Meng, Univ. Maryland College Park. *Enterococcus faecalis* isolate 476 was isolated from poultry manure-based compost at USDA, Beltsville Maryland. All bacteria used in this study were identified and confirmed using standard microbiological methods, including biochemical and serological methods (6). Cultures were stored at -80^{o}C in Trypticase Soy broth (TSB, Difco, Becton Dickinson, Sparks, Md.) containing 30% glycerol. *E. coli* isolates were grown and enumerated on MacConkey's agar (Difco), supplemented with either 100 µg/ml ampicillin (Sigma Chemical Co., St. Louis, Mo.) or 50 µg/ml nalidixic acid (Sigma). *Salmonella* isolates were grown and enumerated on XLT4 agar, supplemented with either 50µg/ml nalidixic acid (Sigma) or 100µg/ml ampicillin. The *Enterococcus* isolate was grown and enumerated on modified Enterococcus Agar (Difco,, supplemented with an additional 1.5% Agar (Difco).

Compost Source and Nutrient Supplements.

Compost used throughout the study was commercially available and marketed specifically for CT production (Rexius, Eugene, Oregon), and was a proprietary blend of several thermophilic composts, produced from animal manure and yard trimmings. Compost was collected from a mature pile at the production facility and stored indoors at ambient temperature until used.

The three nutrient treatments were selected because they represented approaches currently used by many growers producing CT on their farms. Treatment A used basic CT without addition of commercially available supplements. The only nutrients available in this treatment were those naturally present in the 500 g compost placed in the brewing assembly sock. Treatment B was identical to treatment A, except that 0.5% (vol:vol) "Bacterial Nutrient Solution" (Soil Soup, Inc., Seattle, Washington) was added at the start of the brewing cycle to enhance bacterial growth purported to be beneficial in controlling foliar phytopathogens (as stated by the practitioners and the manufacturer). This bacterial nutrient solution is a proprietary blend of molasses, bat guano, sea bird guano, soluble kelp, citric acid, epsom salts, ancient seabed minerals, and calcium carbonate. Treatment C was identical to treatment A, except that the following supplements were added in accord with recent recommendations by CT practitioners and vendors: 0.12% (w:v) powdered soluble kelp (Maxicrop, Elk Grove Village, Illinois), 0.25% (w:v) liquid humic acids (Humax, JH Biotech, Inc., Ventura, California), 0.96% (v:v) glacial rock dust (Gaia Green Products Ltd., Grand Forks, British Columbia, Canada) per 15 liters, to encourage growth of microbes purported to aid in biocontrol of phytopathogens.

Compost Tea Method (ACT, NCT) Comparison

Compost tea was prepared with aeration (ACT) and without aeration (NCT) using a brewing time within the range typical for each process, 24-36 hr ACT and 7-8.5 days NCT. Each ACT was made with a Bio-blender^{TM} (Soil Soup, Inc.), which was immersed and actively infused air into the CT throughout the brewing process. Tap water (15 L/bucket) was aerated for 2 hr to de-chlorinate the water prior to use. Compost with inocula was added to the liquid by completely immersing the sock containing the compost into water which contained the designated nutrient supplements (described above). To assist extraction of soluble materials and microorganisms from the compost, the filter sock was lifted above the water and allowed to drain into the bucket for 15 sec, then re-immersed for 30 sec. This was done 3 times with the filter sock left in the liquid, while being aerated, for the remainder of the production cycle.

The NCT process represented the simplest approach used for on-farm preparation of CT without aeration, where no commercial apparatus was used. Each NCT was made by allowing 15 L tap water (20-22^{o}C) to passively de-chlorinate overnight before the compost (500 g) was mixed directly with the water. Compost socks were typically not used for NCT production to facilitate microbial extraction, but the final product was strained prior to spraying. Nutrient supplements were then added as designated for each of the treatments. Contents were vigorously stirred for 20 sec with a sterile glass rod and then left undisturbed for 8.5 days. Each complete set of teas was prepared, once a month for three months, resulting in three replications over time.

Isolates were grown individually overnight in 250 ml Erlenmeyer flasks with 150 ml TSB at 37^{o}C, centrifuged at 2,683 x *g*, washed three times and then re-suspended in 100 ml sterile phosphate buffered saline, pH 7.4 (PBS). For the CT component bioassay, each isolate (*E. coli* # 435 and *S. sentftenberg* # 695) was serially diluted in sterile PBS and enumerated on MacConkey's agar. All cultures were maintained on wet ice until inoculated into each nutrient source (maximum 18 hr).

For the ACT/NCT study, pathogen inocula (*E. coli* #'s 435, 427, 466, and *S. enteritidis* #430, and *E. faecalis* #476) were conditioned to grow on the compost substrate to avoid abrupt nutritional and consequent metabolic shifts that can accompany growth when changing from synthetic nutrient media to complex natural compost nutrients. By conditioning the inocula on the compost substrate prior to introduction of a small aliquot into the mixed microflora of a larger mass of compost, the inoculum would simulate as closely as possible a natural occurrence of a small contaminated mass that might survive in an inadequately heated portion of a compost pile. The conditioning involved steam-pasteurizing a portion of compost for 1 hr on each of three consecutive days, after which it was dried at 80^{o}C for 24 hr prior to inoculation. No detectable microbes were found in this pasteurized compost prior to inoculation. Steaming avoided the potential production of reduced forms of iron, manganese, and other elements that can develop and impart toxicity to the substrate when composts or soils are autoclaved (280). Cell suspensions of each isolate (100 ml, prepared as above) were inoculated in individual 100 g portions of the oven-dried, steam-pasteurized compost to achieve 50% moisture content (wt/vol). Inoculated

compost was incubated for 72 hr at 22^{o}C to allow isolates to adapt to growth (without competition) within the pasteurized compost matrix, and then bacterial populations were enumerated, as described below. Inoculated compost was stored at 4^{o}C until used.

When used for CT production tests, inoculated compost was diluted 10-fold with stored, uninoculated, unsteamed, compost "as-received" from the supplier to achieve the target starting concentrations (approximately 10^1, 10^2, 10^3 CFU/g) for CT brewing. For example, the inoculated compost (10 g) was added to 90 g of uninoculated compost in sealed plastic bags and homogenized by vigorously shaking and manually massaging for 5 min. After determining that the target concentrations of pathogens were present, a small portion (~5 g) of the pathogen-containing compost was placed in the center of a larger mass (approximately 495 g) of uninoculated, unsteamed, "as-received" compost and the entire 500 g was loaded into autoclaved 100 μm mesh socks marketed for CT production (Soil Soup, Inc.). Socks were placed into sterile plastic bags, sealed, and overnight shipped in insulated secure coolers with frozen gelpacks to Corvallis, Washington, for subsequent brewing.

All buckets containing human pathogens were brewed in an ambient temperature growth chamber to simulate on-farm conditions. Samples (50 ml) were aseptically drawn (36 hr for ACT and 8.5 days for NCT) from the center of each bucket 10 cm below the liquid surface. Samples were stored at 4^{o}C overnight prior to analysis.

Component Bioassays.

For the CT component bioassays, seven different materials currently marketed as possible alternatives to molasses in CT production were evaluated for their ability to support growth of *E. coli* O157:H7 isolate 435 and *Salmonella* senftenberg isolate 695. Components were diluted 0.5% (v:v) in sterile deionized water and inoculated with either *E. coli* at (1.35 $\log_{10}$ CFU/ml) or *Salmonella* at 1.42 ($\log_{10}$ CFU/ml). After 24 hr incubation at 22^{o}C, microbial populations were enumerated as described below, and each bioassay was performed in triplicate.

Analysis of four Compost Tea Brewing Systems.

In collaboration with Matthew Ryan of The Rodale Institute in Kutztown, Pennsylvania, four aerated compost tea brewing systems (A, B, C, D) were evaluated for their ability to propagate total coliforms, *E. coli*, and enterococci that were indigenous in a standardized feedstock used for each brewer. No marked strains or inoculated compost were used in this comparison. All brewing systems involved mechanical agitation of the compost/water as well as an impeller device that infuses air throughout the tea. Brewer "A" was a bio-blender™ (Soil Soup, Inc., Edmonds, Washington) which produced approximately 5 gal per batch of CT (Fig. 3.5). Brewer "B" was an ETB-22 (Sustainable Agricultural Technologies, Inc., Cottage Grove, Oregon) which produced 22 gal per batch of CT (Figure 3.6). Brewer "C" was a Bob's Bitti brewer (Bob's Brewers, Seattle Washington) which produced 10 gal of CT (Figure 3.7). Brewer "D" was a homemade bucker bubbler system which produced 25 gal per batch of CT in a 33 gal Rubbermaid trash can using an air

compressor to "bubble" air throughout the liquid using a braided PVC hose containing 1/16 inch holes (Fig. 3.8). The compost tea recipe used for each brewer was designed in collaboration with Paul Wagner of the Soil Foodweb, Inc., laboratory in New York. The recipe included a mixture of three compost sources (two manure-based and one vermicompost-based), as well as several supplements including Soluble Humic Acid (0.14% w:v); Soluble Kelp (0.10% w:v); and Fish Hydrolysate (0.07% w:v). The recipe was adjusted to achieve the same concentrations (w:v) of compost and supplements for each brewing system. In each system, the compost was contained inside of either mesh containers (Brewer A, B, C) or a burlap sack (Brewer D), and the additives were made directly to the liquid at the start of the brewing process. All 24 hr ACT was prepared at Rodale Institute and transported overnight to Beltsville for analysis. The 200 ml samples were packaged in wet ice to stabilize microbial contents during shipment. Each tea was prepared in duplicate and the experiment was repeated twice.

Competition Study

Two batches of CT (15 L each) were prepared using the Bio-Blender™ as described previously, one batch with and one batch without the 0.8% (v:v) Bacterial Nutrient Solution (Soil Soup, Inc., Edmonds, Washington). In addition, a fish-tank air bubbler system infused air into each batch of tea throughout the 24 hr brewing cycle to maintain dissolved oxygen concentrations above 6 ppm. The compost feedstocks included 167 g each of three types of "Class A" composts to produce a tea containing a diverse array of microorganisms. For each compost source there were no detectable levels of indigenous *E. coli*. The pH (Model 150: IQ Scientific

Instruments, San Diego, California), Electrical Conductivity (Model 933100; Hanna Instruments, Woonsocket, Rhode Island), and Total Organic Carbon content (Phoenix 8000 analyzer, Teledyne Tekmar, Mason, Ohio) were determined for each CT at 30 min (0.5 hr), 8 hr, 20 hr and 24 hr.

Samples (25ml) of each batch of CT was collected at 0.5 hr, 8 hr, 20 hr and 24 hr and placed into sterile 50 ml conical tubes. Each tube was inoculated with washed cells [1.12 Log cfu/ml] of *E. coli* O157:H7 (UMD#435) that were resistant to 50µg/ml Nalidixic Acid to determine the ability of the inoculum to compete with the indigenous microbiota contained in the CT. The initial concentration of cells per CT reaction tube was 1.12 log cfu/ml of *E. coli* O157:H7. All CT tubes were incubated at room temperature (20-22^{o}C) with rotary agitation (100 rpm) for 24 hr and plated onto MacConkey's agar supplemented with 50 ppm Nalidixic Acid. Each CT tube was also infused with atmospheric oxygen via a fish-tank bubbler system

Microbiological Characterization of Compost Teas

Samples of compost and CT were serially diluted in buffered peptone water (Becton Dickinson) and plated in duplicate (50 µl each plate) using a WASP-II spiral plating instrument (Don Whitley Scientific, Ltd., England) to enumerate bacterial populations. The detection limit for this procedure was calculated as 10 CFU/ml. Inoculated isolates of *E. coli* were enumerated on MacConkey's agar, containing antibiotics and incubated for 24 hr at 37^{o}C and distinguished as pink colonies; fecal coliforms were enumerated as pink colonies on MacConkey's agar incubated at 44.5^{o}C for 18-24 hr; inoculated *Salmonella* were enumerated on XLT4 agar

containing antibiotics and incubated at 37°C for 24 to 48 hr. *Salmonella* isolates appeared as black colonies on XLT4. *Enterococcus spp.* were enumerated on modified *Enterococcus* agar (Difco, Becton Dickinson), supplemented with 1.5% agar) incubated at 37°C for 48 hr and appeared as red or purple colonies. Indigenous, baseline levels of *E. coli* and *Salmonella* were determined by enriching CT, prepared using uninoculated and unsteamed compost and streaking the enrichments onto the media.

Physical and Chemical Characterization of Compost

At the final sampling event (36 hr for ACT or 8.5 day for NCT), temperature, pH (Model 150: IQ Scientific Instruments, San Diego, California), electrical conductivity (EC), (Model 933100; Hanna Instruments, Woonsocket, Rhode Island), and dissolved oxygen (DO), (Model 600; Engineered Systems and Design, Newark, Delaware) were recorded for each batch. Moisture content of compost samples was determined by oven-drying approximately 50 g samples at 105 °C overnight; results were used to calculate dry weight for reporting bacterial content in the compost.

Statistical analyses

A two-way analysis of variance (ANOVA) with individual *t* tests were performed to determine significant differences between microbial population means in response to nutrient, supplement, and brewing conditions. The ANOVA model included type of tea (ACT, NCT) and nutrient supplement (No nutrient "A", Nutrient "B" and Nutrient "C") as fixed effects. Comparisons of mean log values for each

strain were made using the least significant difference separation technique in Mixed ANOVA using SAS/STAT software (Version 9.2, SAS Institute Inc., Cary, North Carolina).

Results

ACT/NCT Compost Tea Comparison Study

Comparative analysis of all inoculated pathogen populations across all treatments revealed a very significant fixed effects for treatments B (bacterial nutrient supplement) ($P<0.0001$), and C, the kelp, humic, and rock dust supplement ($P < 0.0001$), whereas Treatment A (no nutrients added) was insignificant ($P>0.1$). When individual isolates were analyzed, results showed that in response to treatment A, *E. coli* O157:H7 (#435 and #427), and *Enterococcus* #476 (both ACT and NCT), and S. enteritidis #430 (NCT only) populations were reduced below inoculum concentrations (Figs. 3.1 and 3.2). In 36 hr ACT treatment A, *Salmonella* and fecal coliforms showed approximately a 5-fold mean increase from the inoculum level (Fig 3.1), however these results were not statistically significant ($P>.05$) from the inoculum. In contrast, the pooled populations of *E. coli* O157:H7 and fecal coliforms (ACT and NCT) in response to treatments B ($P<0.0065$) and C ($P< 0.0001$), were significantly higher in concentration than the inoculum (Fig 3.1 and 3.2). In comparing growth between ACT and NCT, the populations of *E. coli* O157:H7, *S. enteritidis* #430 and *Enterococcus* #476 were always at lower concentrations in the NCT than in the ACT production systems.

For the 36 hr ACT preparations (Fig 3.1), in comparison with inoculated levels, *Salmonella* showed a 100-fold growth increase for treatment B and a 10-fold growth increase with treatment C (Fig 3.1). In response to treatment A (no nutrients), *E. coli O157:H7* isolates were undetectable; *Salmonella* survived but did not produce a statistically significant growth response from the inoculum level ($P>0.05$). *E. coli* O157:H7 and fecal coliforms grew 100- to 1000-fold in response to treatments B and C (Fig 3.1). *Enterococcus* levels decreased approximately 5-fold in response to treatments C, but increased 10-fold with treatment B. Across all ACT treatments, the mean dissolved oxygen concentration was 8.25 ppm, the pH was 6.9 and electrical conductivity (EC) was 3.52 (210).

For the 8.5 day NCT (Fig. 3.2), both *Salmonella* and *Enterococcus* were undetectable in treatments A and B, while none of the strains were detected in treatment A. *Salmonella* showed a 10-fold growth increase (from inoculum levels) in response to treatment C. *E. coli* O157:H7 populations also increased, but to a greater extent (1000-fold) with treatment C than with treatment B. Fecal coliform populations survived and grew more than 1000-fold in response to both treatments B and C, but they were undetectable in treatment A. Across all NCT treatments, the mean dissolved oxygen concentration was 2.6 ppm, the pH was 5.7 and electrical conductivity (EC) was 0.87 (210).

No indigenous strains of fecal coliforms, *E. coli*, or *Salmonella* were recovered on the test media (described in the Materials and Methods section above) streaked with CT produced from unsteamed, uninoculated "as-received" compost. Across all treatments for NCT and ACT, the total heterotrophic, aerobic bacterial

population was significantly greater (P=0.0057) with 36 hr ACT (~7.6 log cfu/ml) in comparison to 8.5 day NCT (~6.7 log cfu/ml) (not shown). In the ACT, treatments B and C produced comparable, but significantly higher concentrations of heterotrophs than treatment A (P<0,05). In the NCT, treatment C produced statistically significant higher concentrations of heterotrophs than treatment B (P<.05) but neither were largely different from treatment A (not shown).

Component Bioassays

When populations in the component bioassays were enumerated individually, results showed that the inoculated *Salmonella* and *E. coli* grew above inoculum levels (Table 3.1), even in treatments supplemented with materials that are not considered to be nutrient sources, such as humic acid.

Brewer Comparison

The four compost tea brewing systems selected in this study represented a wide range of commercially available systems (A,B,C) as well as a low-cost "do-it-yourself" approach (D). There is much debate among various compost-tea websites that discuss and advocate the benefits of one brewing system over another based on rates of mechanical aeration, extraction efficiencies, the ability to propagate pathogens, beneficial bacteria and fungi and ease of use.

This study was designed to analyze the microbiological quality of ACT produced with four brewing systems using the same recipe for each system, which included small quantities of kelp, fish hydrolysate and humic acid. As shown in

Table 3.1, *E. coli* and *Salmonella* had the ability to use all of these CT amendments as nutrient sources for propagation although seaweed and humic acid were the least supportive.

The levels of total coliforms and *Enterococcus* were statistically the same for brewing systems A, B and C and CT, however, brewing system D resulted in lower concentrations of each microbe (Fig. 3.3). There was no statistical difference between the levels of recovered *E. coli* for each brewing system (<10 cfu/ml) (Fig. 3.3). The physico-chemical conditions necessary to sustain the maximum population density of heterotrophs (>10^8 cfu/ml) was achieved in each 24 hr brewing system, suggesting comparable microbiology, nutrient extraction and aeration efficiencies of CT for each system. There were no significant differences (P>0.05) between the total heterotrophic, gram negative or fungal populations in any of the brewing systems (Fig. 3.4) . The four compost tea brewing systems are illustrated in Figures 3.5 – 3.8.

Competition Study

In the ACT supplemented with the nutrient solution (0.8% v:v), *E. coli* O157:H7 cells inoculated into the CT at each time period (0.2 hr, 8 hr, 20 hr, 24 hr) during the brewing cycle were able to grow well above inoculum levels in 24 hr (Table 3.2). Despite high levels of competing organisms (e.g. >10^8 cfu/ml heterotrophs at 24 hr) the conditions in ACT that were also supplemented with nutrients provided conditions conducive to *E. coli* survival and growth when inoculated at even very low levels (13 cfu/ml). In contrast, the conditions in ACT without any nutritional supplementation prevented re-growth of the *E. coli* O157:H7

inoculum after 0.5 hr (Table 3.3). The *E. coli* O157:H7 inoculum that was recovered at the 20 hr ACT sample indicated survival but not growth. The chemical properties of the CT at each time period are shown in Tables 3.2 and 3.3. The pH readings were slightly higher in the non-supplemented tea (mean of 7.50) versus the nutrient supplemented tea (mean of 7.0), but all readings were well within the proper range for good quality compost and compost tea. The mean EC readings for all time periods were lower in the non-supplemented tea (872 µS/cm) than in the supplemented tea (1445 µS/cm) but all were within the parameters for good quality compost and compost tea.

The TOC content of nutritionally supplemented ACT gradually decreased 1.5 fold over the 24 hr brewing cycle (Figure 3.9) from 1267 ppm to 824 ppm and was inversely correlated with the total heterotrophic population (r^2 = -0.966). This was not surprising, as the heterotrophic population is expected to use the available nutrients as food sources, thereby increasing in population size as the TOC levels decrease. The *E. coli* O157:H7 population, however, positively correlated with TOC levels (r^2 = 0.968). In the early collected samples of CT, the 0.5 hr and 8 hr populations of *E. coli* O157:H7 increased dramatically by 3 and 2 logs, respectively in the supplemented CT.

The TOC content of ACT without additional nutrient supplements gradually increased over two-fold (Figure 3.10), from 40.7 ppm (0.5 hr) to 99.8 ppm (24 hr). This increase was attributed to the gradual release of indigenous nutrients contained within the compost feedstocks. In this experiment we can assume that the difference between the TOC levels for each ACT w/nutrients and the TOC levels for ACT

without nutrients was due solely to the addition of the nutritional supplement. The addition of 0.8% (v:v) supplemental nutrients added at least 1200 ppm TOC to the CT. The total heterotrophic population in the non-supplemented tea, while achieving much lower population levels than in supplemented ACT, still produced approximately 10^7 cfu/ml density in 24 hr which was positively correlated to TOC levels ($r^2 = 0.798$, Figure 3.10). The O157:H7 inoculum had a growth response only in the 0.5 hr CT sample, where the heterotrophic population (Log 5.41 cfu/ml) was lowest in concentration. In all further time points, the inoculum was unrecoverable, suggesting that the *E. coli* O157:H7 inoculum could not respond to increasing amounts of TOC due to increasing heterotrophic competition.

There is a dynamic relationship between the survival and growth of *E. coli* cells, the total organic carbon content and the heterotroph population in CT. Figure 3.11 shows negative correlation effects between the heterotrophic populations and *E. coli* O157 cells in both supplemented ($r^2 = -0.989$) and non-supplemented ($r^2 = -0.416$) ACT. A three-dimensional scatter plot containing all these factors (Fig. 3.12) suggests that, even with the maximum heterotrophic population of $>10^8$ cfu/ml in 24 hr nutrient-supplemented ACT (TOC = 824 ppm), *E. coli* O157:H7 populations can not only survive *but will increase in concentration* by almost 1 Log in 24 hr. Comparatively, non-supplemented ACT resulted in eight-fold lower TOC levels in 24 hr, while maintaining a high heterotrophic population of approximately 10^7 cfu/ml, which in turn seemed to be able to reduce the *E. coli* O157:H7 population to below detection limits.

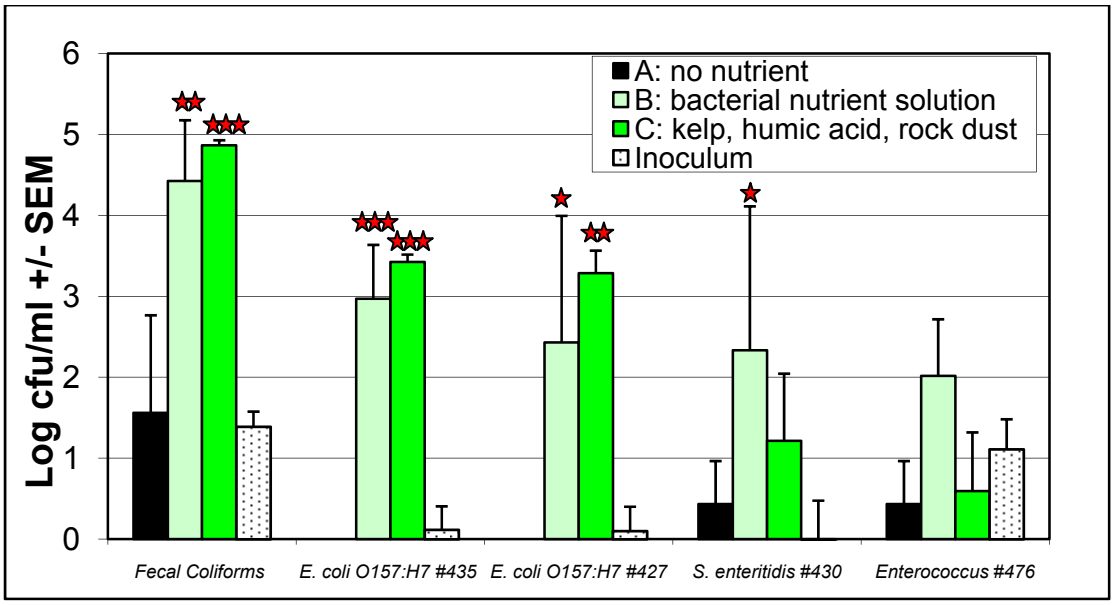

Figure 3.1 : Foodborne pathogen response in 36 hr aerated compost tea (ACT), prepared using three treatments: A) no nutrients were added; B) bacterial nutrient solution (0.5% v:v); C) soluble kelp (0.12% w:v), humic acid (0.25% v:v) and rock dust (0.96% w:v). A symbol above the treatment indicates statistically significant differences from the inoculum level of each species. '★ P < 0.05;'★★ P < 0.01; '★★★P < 0.001

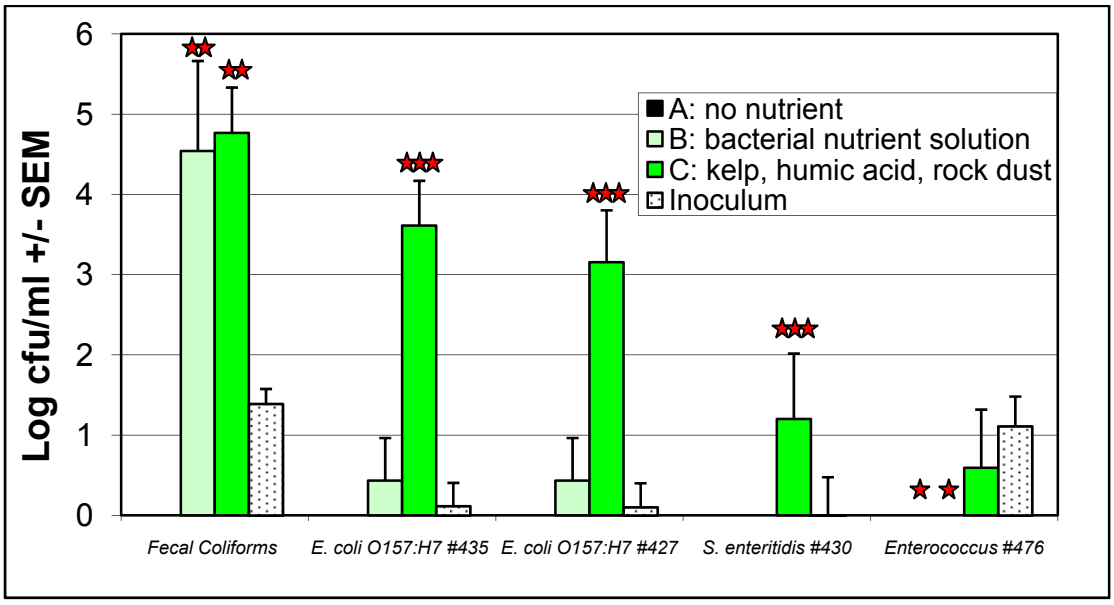

Figure 3.2 : Foodborne pathogen response in 8.5 day non-aerated compost tea (NCT), prepared using three treatments: A) no nutrients were added; B) bacterial nutrient solution (0.5% v:v); C) soluble kelp (0.12% w:v), humic acid (0.25% v:v) and rock dust (0.96% w:v). A symbol above the treatment indicates significant difference from the inoculum level of each species. '★' P < 0.05;'★★ P < 0.01; '★★★P < 0.001

Table 3.1 : Populations of *E. coli* O157:H7 (USDA 435) and *Salmonella senftenberg* #695 after 24 hr incubation at 22°C with 0.5% components (v:v in sterile distilled deionized water)

Component	*E. coli* O157:H7[a] Log_{10} CFU/ml	SEM[b]	*Salmonella* senftenberg[c] Log_{10} CFU/ml	SEM
Fish Hydrolysate #1	5.83	0.30	5.54	0.04
Soil Soup	5.69	0.12	5.90	0.42
Seaweed	3.79	0.11	4.42	0.03
Fish + Seaweed Hydrolysate	6.84	0.09	6.33	0.02
Humic Acid	2.29	0.12	2.46	0.17
Kelp	4.49	0.02	3.78	0.01
Fish Hydrolysate #2	6.54	0.12	5.62	0.13

[a] Initial population of *E. coli* O157:H7 (USDA#435) was 1.35 (Log_{10}) +/- 0.03 SEM (N=4)
[b] SEM – standard error of the mean
[c] Initial population of *Salmonella senftenberg* (USDA #695) was 1.42 (Log_{10}) +/- 0.03 (N=4)

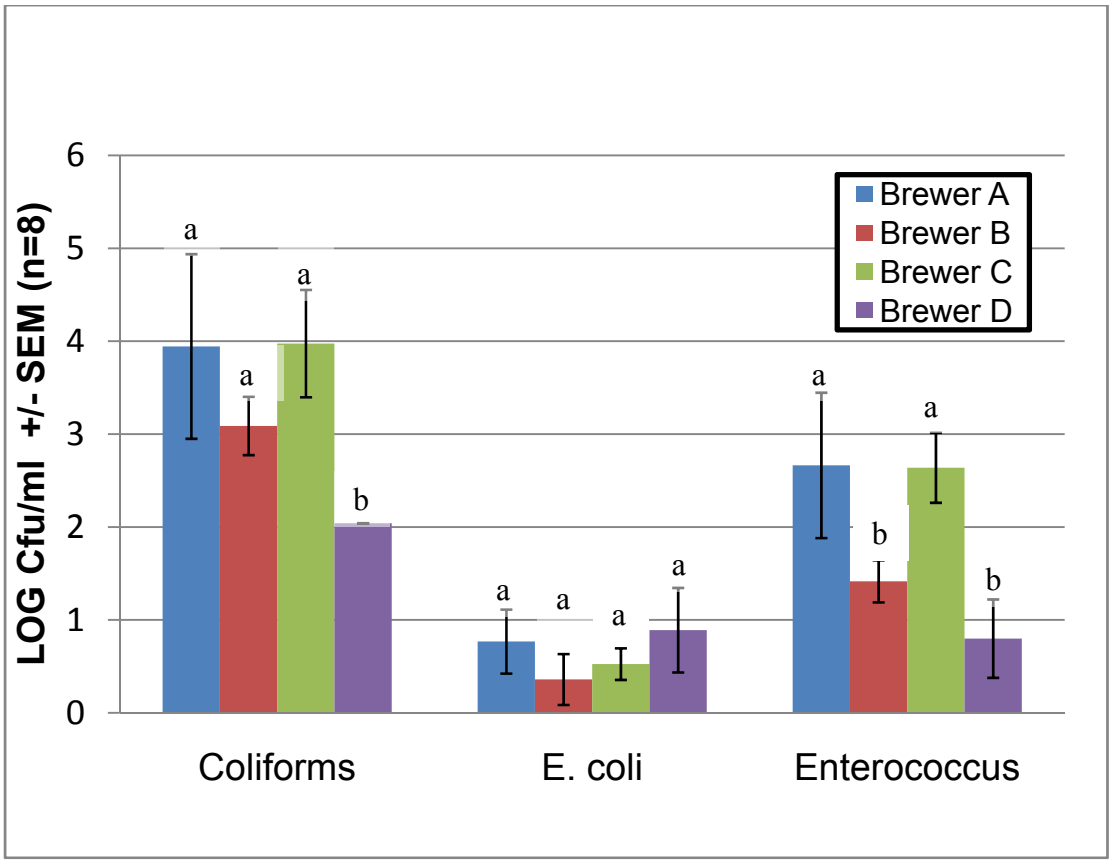

Figure 3.3 : Examination of coliform, *E. coli* and *Enterococcus* content from aerobic compost tea (ACT) produced in four brewing systems. Within each microbial population, bars with different letters indicate statistically significant differences using LSD mean separation technique at P=0.05.

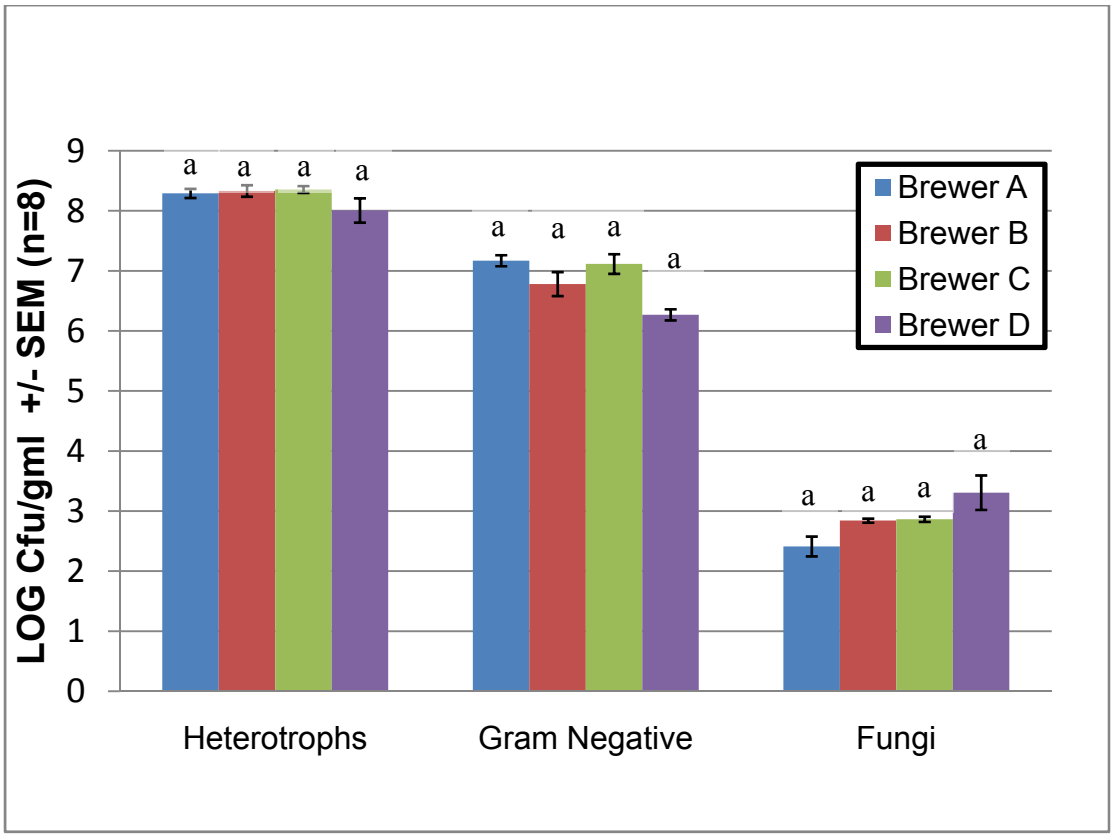

Figure 3.4 : Examination of heterotrophic, gram negative and total fungi content from aerated compost tea (ACT) produced in four brewing systems. Within each microbial population, bars with the same letters indicate no significant differences using LSD mean separation technique at P=0.05

Figure 3.5 : <u>Brewer A</u>. Bio-BlenderTM ACT brewing system (Soil Soup, Edmonds, WA)

Figure 3.6 : Brewer B. ETB-22 ACT brewing system (Sustainable Agricultural Technologies, Inc., Cottage Grove, OR)

Figure 3.7 : Brewer C. Bob's Bitti brewer ACT brewing system (Bob's Brewers, Seattle WA)

Figure 3.8 : <u>Brewer D</u>. Do-it-Yourself ACT brewing system; Air compressor and attached PVC aeration tubing not shown

Table 3.2 : Effects of various microbial populations and chemical properties of 24 hr ACT supplemented with 0.7% nutrient solution on the growth potential of inoculated *E. coli* O157:H7

	Mean Log cfu/ml[a]			
	0.5 hr	**8 hr**	**20 hr**	**24 hr**
Heterotrophs[b]	5.23	5.99	8.57	8.98
Gram Negative[b]	1.30	2.94	5.48	5.61
Fecal Coliform[b]	0.00	0.00	2.56	3.34
Enterococcus[b]	0.00	0.00	2.53	2.24
Fungi[b]	3.17	2.92	1.30	1.30
O157:H7 Inoculum[c]	1.12	1.12	1.12	1.12
O157:H7 Final[d]	4.50	3.74	2.60	1.99
	Chemical properties			
TOC (ppm)[e]	1267	1248	988	824
pH	6.90	6.07	6.99	7.08
EC (µS/cm)[f]	1542	1335	1448	1455

a = Means of log cfu/ml reported for two samples of compost tea collected over a period of 24 hr.

b = Heterotrophs, gram negatives, fecal coliform, *Enterococcus* and fungal counts reflect the specific microbial populations that were in direct competition for nutrients with inoculated cells of *E. coli* O157:H7.

c = Log 1.12 cfu/ml cells of *E. coli* O157:H7 were inoculated into each ACT sample

d = Concentration of *E. coli* O157:H7 population after 24 hr incubation in ACT at room temperature (20-22^{O}C)

e = Total organic carbon (TOC)

f = Electrical conductivity (EC)

Table 3.3 : Effects of various microbial populations and chemical properties of 24 hr ACT (not supplemented with nutrients) on the growth potential of inoculated *E. coli* O157:H7

	Mean Log cfu/ml[a]			
	0.5 hr	**8 hr**	**20 hr**	**24 hr**
Heterotrophs[b]	5.41	5.54	7.05	6.93
Gram Negative[b]	1.99	2.22	3.84	4.02
Fecal Coliform[b]	0.00	0.00	0.00	0.00
Enterococcus[b]	0.00	0.00	0.00	1.84
Fungi[b]	3.35	3.30	2.52	2.52
O157:H7 Inoculum[c]	1.12	1.12	1.12	1.12
O157:H7 Final[d]	1.73	0.00	1.18	0.00
	Chemical properties			
TOC (ppm)[e]	40.7	83.3	96.3	99.8
pH	7.68	7.26	7.45	7.63
EC (µS/cm)[f]	901	727	917	942

a = Means of log cfu/ml reported for two samples of compost tea collected over a period of 24 hr.

b = Heterotrophs, gram negatives, fecal coliform, *Enterococcus* and fungal counts reflect the specific microbial populations that were in direct competition for nutrients with inoculated cells of *E. coli* O157:H7.

c = Log 1.12 cfu/ml cells of *E. coli* O157:H7 were inoculated into each ACT collected (0.5 hr, 8 hr, 20 hr and 24 hr).

d = Concentration of *E. coli* O157:H7 population after 24 hr incubation in ACT at room temperature (20-22^{O}C)

e = Total organic carbon (TOC)

f = Electrical conductivity (EC)

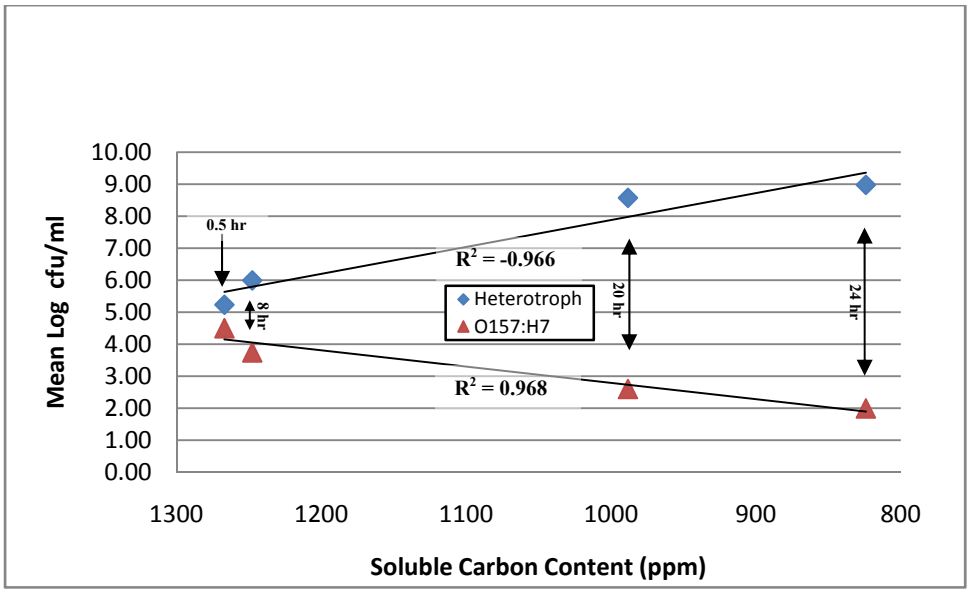

Figure 3.9 : Effect of Total Organic Carbon (TOC) content on the bacterial heterotrophic population and *E. coli* O157:H7 populations in ACT supplemented with 0.8% (v:v) nutrient solution (Soil Soup, Inc.). Statistically significant (P<0.05) Pearson correlation coefficients were produced using the Proc Corr procedure in SAS version 9.2 (Sas Institute, Cary NC)

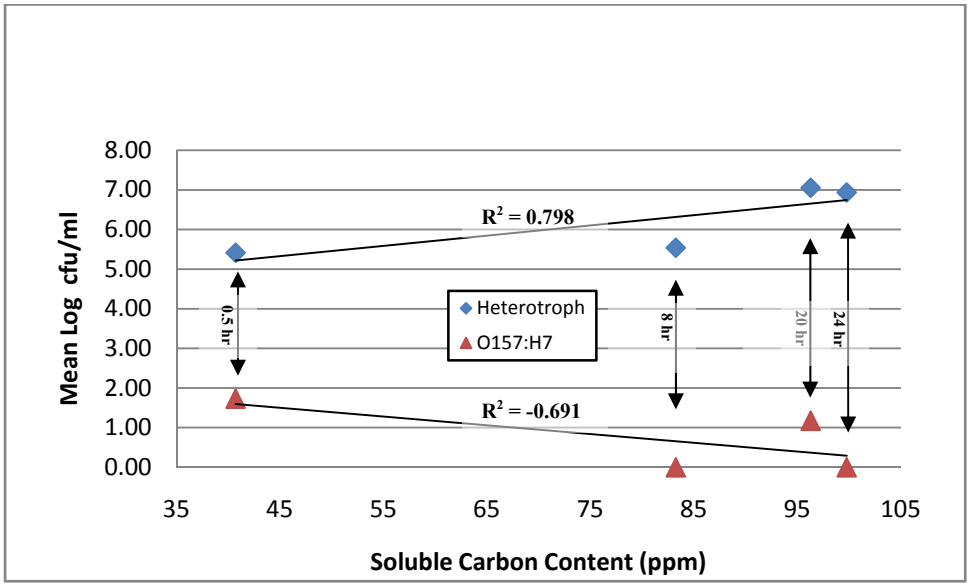

Figure 3.10 : Effect of Total Organic Carbon (TOC) content on the bacterial heterotrophic and *E. coli* O157:H7 populations in ACT without supplemental nutrients. Statistically significant (P<0.05) Pearson correlation coefficients were produced using the Proc Corr procedure in SAS version 9.2 (Sas Institute, Cary NC)

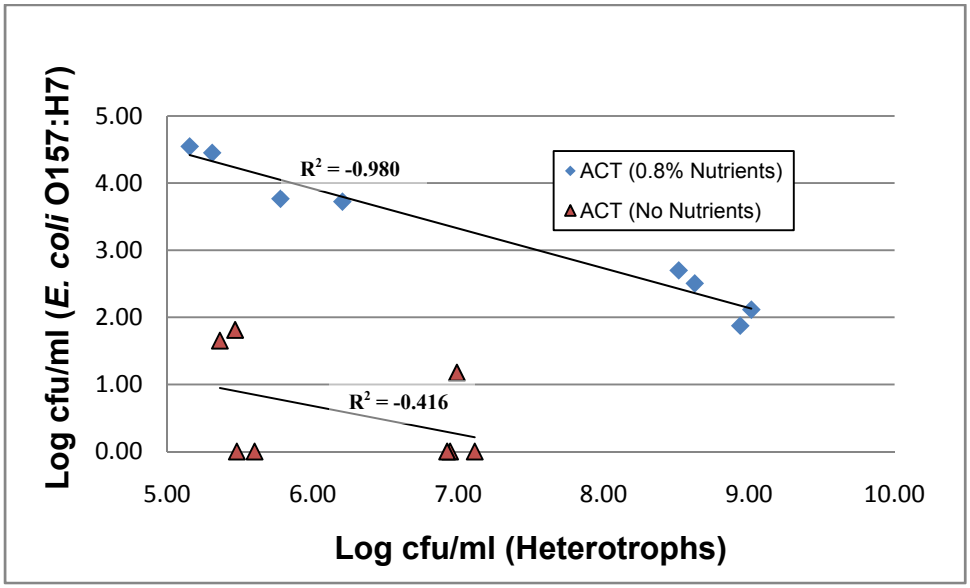

Figure 3.11 : Effect of the bacterial heterotrophic population on the survival and re-growth potential of inoculated *E. coli* O157:H7 in nutrient-amended and non-amended ACT (Soil Soup, Inc.) Statistically significant (P<0.05) Pearson correlation coefficients were calculated using the Proc Corr procedure in SAS version 9.2

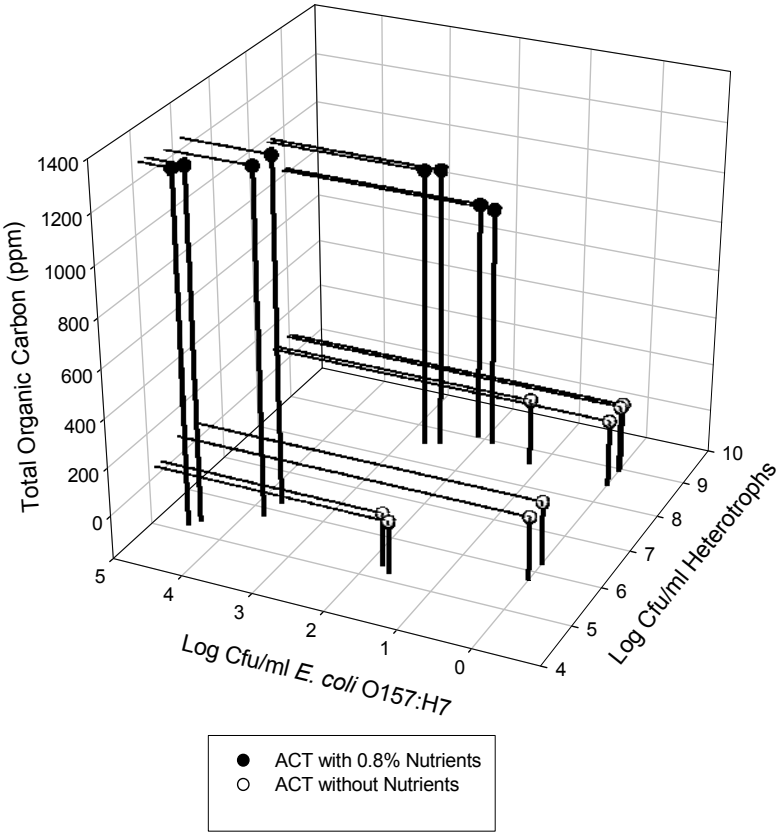

Figure 3.12 : Interactions among total organic carbon and heterotrophic populations in aerated compost tea on the re-growth potential of *E. coli* O157:H7

Discussion

The results of this study demonstrate that adding certain supplements during the ACT and NCT brewing cycles (treatment B and C, Fig. 3.1 and 3.2) contributed to the propagation of *E. coli* O157:H7, *S. enteritidis*, *Enterococcus* and fecal coliforms in the final CT product. Data support the conclusion that avoiding the use of supplements in CT production is likely to reduce the possibility of foodborne pathogen propagation. *E. coli* O157:H7 concentrations were reduced to undetectable levels in all ACT and NCT where nutrient supplements were not added (treatment A, Figs 3.1 and 3.2). In general, where nutrient supplements were used (treatments B and C, Figs 3.1 and 3.1), the concentrations of fecal coliforms, *E. coli* O157:H7, *Enterococcus* and *S. enteritidis* were always greater for the ACT than for NCT. In contrast, Kannangara et al. (123) reported increased concentrations of *E. coli* K-12 in their NCT as compared to their ACT systems with molasses and kelp supplements. Interestingly, their results with *E. coli* K-12 in ACT agree with our results with *E. coli* O157:H7 that show no growth in the absence of nutrient-supplementation. Furthermore, the *E. coli* K-12 inoculum levels that Kannangara et. al. (123) used, 10^6 -10^7 cfu/ml, were unlikely to occur in thermophilic, composted manure, and the compost tea systems used were not reflective of current technology by industrial or farm practitioners. As determined in this study, compost alone may contain sufficient inherent nutrients (treatment A) to support survival and/or growth of *Salmonella* along with fecal coliforms, *and Enterococcus spp.* up to 36 hr ACT. The NCT, on the other hand, resulted in no detectable fecal coliforms, *Enterococcus spp*., *E. coli* O157:H7 or *S. enteriditis* in response to the no-nutrient supplement (treatment A).

The efficacy of CT relative to phytopathogen control and plant-growth promotion remains unclear and the mechanisms by which various diseases are reduced likely vary (208). One possible explanation is that the phylloplane microbiota is overwhelmed by the increased number of microbes from the CT spray that competitively excluded and/or inhibited phytopathogens. Another theory is that physical barriers are created from the microbial biofilm that formed when CT is sprayed onto the foliage. However, as is the case with any water source used for crop irrigation and sprays, the presence of human pathogens in the water supply poses a potential hazard for the consumption of fresh produce. Foodborne pathogens can use biofilms as a protective barrier against environmental stressors and microbial competitors (174).

Comparison of CT produced from four different aerobic compost tea production systems using identical feedstocks resulted in comparable populations of heterotrophs, gram negative bacteria, coliforms, *E. coli* and total fungi. All of the brewing systems were able to favorably enhance growth of indigenous *E. coli* contained in the compost feedstock. Although concentrations were low (<10cfu/ml), *E. coli* in the resulting tea transferred to the foliar surfaces of crops could ostensibly cause foodborne disease if the guidelines for harvesting food crops sprayed with CT are not met.

The feedstocks used for the brewer-comparison studies, when tested for indigenous *E. coli*, were found to be negative using enrichment methods (detection limit <0.04 cfu/g). The discovery of *E. coli* in all final CT products suggests that at least one of the three composts used in the CT recipe contained indigenous *E. coli*.

The low levels of *E. coli* recovered in the tea suggest that the initial population was very low and/or that the available nutrient sources during the CT manufacture were insufficient for the *E. coli* population to further propagate beyond the recovered levels (<10 cfu/ml). Adding a molasses-based nutrient source may result in levels of *E. coli* several log cfu/ml higher than observed here. However, the fact that less than 10 cfu/ml *E. coli* survived after 24 hr, despite the enormous microbial competition for nutrients in the CT, suggests that *E. coli* is particularly suited for survival in this matrix. This study provided further evidence that all compost feedstocks used to brew CT, and the final CT products where nutrients are added, should be tested for pathogens. Regular pathogen testing should be included in any good agricultural practice for the production of compost tea, regardless of the brewing system used to manufacture the tea.

The quality of CT produced by each brewer and the effects on phytopathogen control are beyond the scope of this study. Interestingly, Ryan et. al. 2005 (200), observing the effects of foliar and soil-drench applications of CT produced with the same recipe and brewing systems used (Brewer B) on yield and disease incidence on vineyard, pumpkin and potato crops, found evidence that in some cases the CT treatments were able to decrease the severity (but not eliminate early powdery mildew infestation) (200).

Based on the similarities to the microbial content data from all brewing systems in this study, it is reasoned that the CT produced by each brewing system would be similar in their ability to help control phytopathogen-related disease.

A final experiment was conducted to explore anecdotal evidence that suggests that E. *coli* may not be able to survive with high counts (i.e., > 10^6 cfu/ml) of indigenous bacterial heterotrophic populations under aerobic ACT conditions (123, 141). Examination of the ability to propagate *E. coli* O157:H7 cells in ACT collected at various time periods over a 24 hr brewing cycle resulted in increasing *E. coli* populations in all samples where nutrients were added to the CT feedstock. The inoculated O157:H7 population did not increase in un-supplemented tea where the heterotroph population was above log 5.54 cfu/ml and the total organic content in the tea was below 100 ppm. The total organic carbon (TOC) content is an indirect measure of organic molecules present in water measured as carbon and is one indication of available nutrients for the microbial populations. TOC is a popular indicator of water quality, and is measured in this study to assess correlation of available nutrients in ACT with the re-growth potential for *E. coli* O157:H7. Several reports (13, 44, 123, 267) that discuss the potential effects of nutrient amendments on the *E. coli* and *Salmonella* populations refer only to amounts of amendments on a g/L basis.

This is the first report to quantify available nutrients in ACT over time in terms of total organic carbon and study the effects on inoculated *E.coli* O157:H7 cells (13 cfu/ml). Results of this investigation show that *E. coli* O157:H7 is well suited for growth when the TOC levels are above 1200 ppm and can effectively compete for available nutrients even with high levels of competing heterotrophic populations (>10^5 cfu/ml). Further study is warranted to determine if a lower inoculum concentration of *E. coli* would behave in similar fashion. Similarly, it would also be

interesting and potentially useful to understand the *E. coli* population dynamics in response to nutrient supplemented and non-supplemented NCT.

If human pathogens are present even in small numbers in compost used to prepare CT, and conditions of CT production allow them to grow, then the likelihood of contaminant microbes being retained on foliage increases substantially when the CT is used as a foliage-applied spray. There is substantial evidence to suggest that using contaminated CT as a spray, soil drench, or a hydroponic nutrient source, may lead to the internalization of pathogens into the root systems and edible portions of plants (11, 101, 115, 232, 234, 266). Therefore, control of foodborne pathogen growth during production of CT is essential to reducing the potential hazard from on-farm introduction of these pathogens through CT application. Quality control and assurances for composting, compost product, as well as the CT production process would help to significantly address the problem of undesirable bacterial contaminants in sprays and crop/soil drenches used for fresh produce crops.

Effects of nutrient supplementation on the phytopathogen biocontrol efficacy of CT remain a topic for further research. Presently, no published peer-reviewed reports have substantiated *reliable* benefits of nutrient-supplemented ACT on biocontrol efficacy . However, several studies (20, 23, 33, 44, 108, 202, 207, 208, 210) document biocontrol benefits from watery extracts of compost in the absence of molasses-based nutrient supplements.

Nutrient supplementation has been shown to increase the general heterotrophic bacterial populations in CT (105-108). No unequivocal evidence currently exists to support the conclusions that supplements can provide significantly

better and reliable plant-protection, biocontrol, or plant-growth benefits (128, 129, 210). While the practice of amending CT with nutrients or supplements has expanded and engaged many fresh produce growers in the organic production sector, the results of this study give support to the presently limited evidence that there are potential hazards in this practice (13, 22, 44, 112, 123).

This research is believed to be the first "realistic" investigation into the potential hazards in current CT production methods. It concludes that amending CT with nutrient supplements increases the likelihood that populations of pathogenic bacteria will colonize on fruit and vegetables, thus leading to the possibility of greater exposure to those organisms and perhaps eventually to a higher prevalence of foodborne illness in humans. This increased pathogen potential is important even when very low numbers (< 2.0 cfu/g) of *E. coli* O157:H7 and *Salmonella* are present in the starting compost used to produce CT. Several commercially available CT supplements (e.g., humic acid and kelp), with documentation by Duffy and Kannangara, et. al. (44, 123), give credence to the conclusion that growth of *E. coli* O157:H7 and *Salmonella* during CT production is a continuing concern despite the fact that such supplements were purportedly added more for their mineral and physical attributes rather than their carbonaceous nutrients. This research suggests that commercially available nutrient supplements have non-target pathogen growth supporting capabilities.

Another adverse effect observed during this study when supplements were incorporated (treatments B and C) into the initial liquid phase was the development of a substantial, easily visible biofilm on all the brewing equipment (including the

bucket, sock and aeration device). Microbial biofilms are known to protect microbes against the deleterious effects of certain disinfectants, mechanical washing, and antibiotics (174). While the equipment used in this study was thoroughly sterilized prior to CT production, it is essential that on-farm producers be informed and educated about the necessity of sanitizing their CT equipment with biocides to prevent microbial cross-contamination between batch preparations, particularly where supplements are being used. Biofilms generated in the CT vessel or on the aeration equipment have the ability to cross-contaminate the next batch of CT, if the equipment is used without proper sanitization. If such biofilms contain pathogenic microbes, such a scenario would enhance the likelihood of introducing foodborne illness pathogens in a crop production system.

When CT is produced with compost or supplements that are known to support the growth of *E. coli* O157:H7 or *Salmonella*, the resulting CT should be tested for the presence of human pathogens before being used for spraying or soil drenching of fresh produce. Further field research is necessary to determine the ability of foodborne pathogens to survive on foliar surfaces of crops in field conditions when contaminated compost tea is applied.

CHAPTER 4

EFFECT OF COMPOST AND COMPOST TEA ON THE MICROBIOLOGICAL SAFETY AND HARVEST QUALITY OF STRAWBERRY FRUITS

Abstract

Compost tea (CT) is considered by some organic and conventional growers to be a cost-effective, biologically-based control for several foliar and root diseases when used as a spray or soil drench. CT has been shown to enhance strawberry fruit yields as well as reduce disease severity of *Botrytis cinerea* (grey mould), a fungal fruit rot. However, CT may be a source of foodborne pathogens if the ingredients or the brewing processes are not properly controlled. This study determined in a controlled fashion the effects of two compost tea treatments on (1) *E. coli* contamination, (2) yield, and (3) plant disease of strawberry fruits grown in black root-rot infested soil and compost socks. Two nutrient-supplemented aerated compost tea treatments (CT and CT amended with three yeast isolates known as biocontrols for fungal rot of fruit). Along with a water spray control, these two CT treatments were applied in a split-split plot treatment design to four strawberry cultivars (Sparkle, Chandler, Northeastern, Allstar) grown in Maryland in either sandy loam soil with a history of black root rot or in poultry litter compost-filled socks. The CT used in this study was naturally contaminated with 2.73 cfu/ml commensal *E. coli* and was applied at a rate of 250 ml per linear meter of plant bed (approximately 40 ml per plant). Strawberry yields, percentage of diseased fruits and microbiological quality (total heterotrophs, gram negative bacteria, total yeast and fungi, enterococci, coliforms and *E. coli*) were

determined for seven fruit harvest events in June 2005. Compost tea treatments did not have significant effects on either harvest yield or percentage of diseased fruits when compared to water spray controls. Although each plant was thoroughly sprayed with CT, resulting in deposition of ~100 cfu *E. coli* per plant, *E. coli* was not detected on any fruits that matured four days post CT application. Three cultivars (Allstar, Chandler and Northeastern) produced greater yields when grown in compost socks (272.9, 146, 124.6 g/lin-m, respectively) than in soil (148.7, 88.1, 93.7 g/lin-m, respectively). Furthermore, cultivars grown in compost tended to have fewer (2-10%) diseased fruits than those grown in unamended soil beds, although there was no significant statistical difference between the two findings. Composts can be possible sources of pathogens when parts of the composted mass are inadequately exposed to lethal thermophilic temperatures or are incompletely stabilized whereby sufficient nutrients are present to support pathogen regrowth. In this study, when strawberries were grown organically on stabilized, composted poultry-litter, not only were berry yields enhanced but fruit disease incidence and *E. coli* die-off on CT sprayed berries remained equivalent to that of berries harvested from unamended soils. These results showed that growth of naturally present *E. coli* from low concentrations in CT sprayed directly on intact strawberry fruits and leaves was not enhanced by the presence of composted manure in the planting beds or by the trace nutrients in CT. Other factors, such as solar radiation exposure, desiccation, microbial competition and predation, present in the plant/fruit microenvironment likely contributed substantially to die-off of *E. coli* on the berry fruits.

Introduction

Organic production systems do not have the benefit of using chemical pesticides to control the multitude of foliar and soil-borne diseases, weeds and insects that can dramatically reduce crop yields. Research has provided a variety of organic alternatives, but no one approach has evolved as a "state of the art" practice. Each organic farming system should develop an approach that is unique to its environmental, soil, and plant conditions to achieve goals related to fruit quality, quantity and management styles (82).

Compost is widely used as an organic amendment to improve the nutrient and physical properties of soil (67), as well as to reduce the negative effects of soil-borne fungal pathogens by increasing the microbial diversity (7, 197). Current research suggests that while the nutrient (including antioxidant content) and organoleptic qualities of fruits were not significantly affected by organic vs conventional systems, strawberry yields were improved when compost was used as a soil-amendment (86, 160, 224, 265). The application rates needed (3 to 10 tons/acre) can be cost prohibitive for many small farming operations. At the same time, however, large compost application rates may not necessarily be key to increasing yields. Arancon, et. al, (2004) showed increases (35%) in marketable field-strawberry yields and a reduction of disease prevalence using vermicompost application rates as low as 2 and 4 ton/acre, and Singh, et. al., (224) found a rate of diminishing return when vermicompost was applied above 7.5 ton hectare^{-1}, i.e., there were no increased benefits. Controlling for macro-nutrient availability across all treatments, Arancon, et. al.(5) surmised that the growth promotion benefits of vermicompost were due to

microbiological or chemical growth-promoting characteristics contained within the vermicompost, rather than existing nutrient availability. These findings were further substantiated by Malandraki, et. al, 2008 (150) when verticillium wilt was suppressed using a manure-based compost as compared to a sterilized compost control that failed to prevent disease progression.

To reduce the cost of using compost as an amendment or alternative to black plastic mulch and soil-fumigation, compost application is typically restricted only to the rows intended for planting. While compost can be conservatively applied and incorporated in this fashion, the compost becomes less effective as it is diluted with the surrounding soil. Depending on the initial application rate, yearly inputs of compost are usually required to maintain effectiveness as a plant growth promoter and weed and disease control amendment. Furthermore, analysis of fungal and bacterial communities in the rhizosphere of compost-amended soils suggest that both communities converge back to their original states one year after application (282).

This study introduced a novel and cost-effective system for planting strawberries in 100% compost growth medium, referred to as GroExx™ compost socks. Filtrexx International LLC (Grafton, Ohio) has commercialized the use of compost filter sock systems, termed FilterSoxx™, SiltSoxx™ and InletSoxx™ as silt-fence alternatives for controlling erosion, revegetating slopes and streambank restoration. These methods involve filling mesh-tubes containing mixtures of compost to provide three-dimensional filtration media to capture nutrient and organic pollutants contained in storm water, sediment and agricultural runoff while providing a natural growth medium for re-vegetation. The GroExx™ system used in this study

involved filling 8 inch polyethylene mesh tubes with 100% compost produced at the USDA-ARS Beltsville compost research facility. These GroSoxx™ compost socks were placed directly on top of non-fumigated soil with a history of Black Root Rot and strawberry plants were planted directly into the compost. Preliminary research suggested that GroSoxx™ may be a low-cost and simple method to introducing an organic approach into a conventional farming system while benefiting from the intensive, targeted supply of nutrients, instant disease and weed control and higher first-year strawberry yields (160).

Compost tea is a relatively inexpensive and simple approach designed to introduce the diverse microbiological microbiota and soluble micronutrient components of compost into an aqueous extract, which is then applied to the phyllosphere of crops in an attempt to control foliar diseases (132, 171, 207, 208). Scientific evidence is slowly accumulating to verify the efficacy and safety of compost tea in controlling fungal phytopathogens in a variety of farming systems (23, 129, 207, 271).

The optimal method of compost tea preparation *for disease suppression* is still in question, however. In a two-year study, Hargreaves, 2008 (86) determined that while non-aerated compost teas did not significantly increase strawberry yields, the tea foliage treatments had effectively provided enough macro and micro-nutrients to produce similar yields compared to both municipal solid waste compost and inorganic fertilizers (87). Prokkola and Kivijarvi, 2007 (187) also failed to show significant effects on grey mould or fruit yields when using compost extracts during strawberry plant cultivation. Another two-year study comparing non-aerated and aerated

compost teas showed that while both teas were effective in suppressing grey mould (*Botrytis cinerea*), only aerobic tea was able to increase fruit yields (268). The proper dilutions and rates of compost tea application also remain in question. Welke, 2004 (268) determined that compost teas applied at two dilutions (4:1 and 8:1; v:v with potable water) had, in both cases, similar and greater positive effects on reducing grey mould as compared with water spray controls. Cronin et. al (1996) and Elad and Shtienberg (1994) determined, on the other hand, that only highly concentrated compost tea extracts were effective in reducing disease (33, 50).

The use of compost teas in farming practices today, as they bear on food safety issues, are among the most controversial and least understood. Several reports indicate that very low levels (<100 cfu) of *E. coli*, O157:H7 in particular, have the ability to propagate during both aerated and non-aerated produced CT when certain additives (e.g. molasses) are used to brew the tea (44, 112, 123). These studies were performed by inoculating teas or composts with known concentrations of bacteria (e.g. *E. coli*) to study the various effects of compost tea preparation methods on these populations. To provide effective (and convincing) evidence to develop guidelines for the safe preparation and use of compost tea, controlled studies are needed where compost teas are prepared with compost known to be naturally contaminated with foodborne pathogens. Many of the studies performed thus far, including this one, have used *E. coli* as a surrogate for foodborne pathogens such as *Salmonella*. Studies involving the fate of these pathogens through application of naturally contaminated compost teas onto produce surfaces are also needed to provide effective guidelines for CT usage. Welke, in 2004 (268), conducted the first study to detect fecal coliforms on

the surfaces of strawberry fruits treated with compost teas, but was unable to detect any culturable fecal coliforms from the fruits. It is likely, however, that the teas did not contain *E. coli* based on their low total coliform content (2.3-93 cfu/ml) in the tea. Welke's study, in accordance with the NOSB and Good Agricultural Practice guidelines, terminated foliage CT applications as soon as the plants began to produce flowers which may have reduced the potential for the fecal coliform populations to survive the environmental conditions in the field study (268).

This research is the first to assess the fate of initial low levels of indigenous *E. coli* propagated and applied in ACT to the surfaces of strawberry fruits. The CT used throughout this study was naturally contaminated with *E. coli* using compost feedstocks that were determined to be effectively negative originally for *E. coli* using standard quantitative testing. Trace *E. coli* populations were able to resuscitate and proliferate during the CT brewing process, however. The levels of *E. coli* and *Enterococcus* in the final CT product in this study were 2.73 cfu/ml and 128 cfu/ml, respectively (applied at a rate of 42 ml per plant during each CT application event). These levels are well above the acceptable limits with the current NOSB recommendations (171) which follows the current EPA standards for recreational water (56), set at 126 cfu/100ml for *E. coli* and 33 cfu/100 ml for enterococci. Additionally, one experiment attempted to produce an "enhanced" CT product by introducing three bio-control yeast isolates into the final CT product with the intent of increasing fruit yields by reducing the incidence of foliar disease.

Materials and Methods

Strawberry plot design and Statistical Analysis

This experiment has a split-split-plot treatment structure and was conducted in a randomized block design. The compost (whole-plot growth media) was applied in all 3 blocks and the soil (whole-plot growth media) applied in 2 of the 3 blocks (Table 4.1). Sub-plot foliage treatments (Compost Tea, Compost Tea+Yeast, Water) were randomized within each row. Sub-Sub-plot cultivars (Sparkle, Chandler, Northeastern and Allstar) were randomized within each sub-plot foliage treatment. ANOVA was performed using PROC MIXED in SAS version 9.2(SAS Institute, Cary N.C. The model for strawberry weight and disease ratios included the block, growth medium (Soil, Compost), foliage treatment (CT, CT+Yeast, Water), and cultivar (Sparkle, Chandler, Northeastern, Allstar) as fixed effects, in addition to all interactions. Random variance components were included in the model for the day of harvest (Day 1, 4, 10, 18, 22, 25, 32), block*media, and all block*media*foliage treatment interactions. The ANOVA model for microbial data analysis was analyzed similarly, but as a split-plot design because the cultivars were pooled for analysis of each foliage treatment (Trt1: Water Spray, Trt2: Compost tea, Trt3: Compost tea+yeast). All of the data were analyzed for homogeneity of variances and normality prior to ANOVA calculations.

Table 4.1 : Randomized split-split treatment design for compost tea strawberry field trials

Block 1		Block 2	Block 3	
Row 1 Compost sock	**Row 2 Soil**	**Row 3 Compost Sock**	**Row 4 Compost Sock**	**Row 5 Soil**
Sparkle Chandler Northeastern Allstar	Allstar Chandler Northeastern Sparkle	Sparkle Allstar Chandler Northeastern	Chandler Allstar Sparkle Northeastern	Northeastern Chandler Allstar Sparkle
Chandler Allstar Northeastern Sparkle	Sparkle Allstar Chandler Northeastern	Chandler Allstar Sparkle Northeastern	Northeastern Sparkle Allstar Chandler	Chandler Sparkle Northeastern Allstar
Allstar Northeastern Sparkle Chandler	Chandler Sparkle Allstar Northeastern	Allstar Chandler Northeastern Sparkle	Sparkle Allstar Chandler Northeastern	Chandler Northeastern Sparkle Chandler

Field Rows (Row 1- Row 5) were randomized in a field in the USDA Beltsville North Farm. Strawberries were planted directly into Compost growsoxx (Rows 1,3,4) or Soil (Rows 2,5). Three Sub-plot foliage treatments include water control (Trt 1: Yellow), Compost Tea (Trt 2: Pink), and Compost Tea with Yeast (Trt 3: Orange). Four cultivars (Sparkle, Chandler, Northeastern, Allstar) were randomized within each sub-plot.

Strawberry field preparation

The field plot in this experiment was located on the north farm of USDA-ARS Beltsville campus and had sandy loam soil with a history of a variety of phytopathogens that affect strawberry plants. During the pre-planting season, the field was treated with herbicide to kill existing vegetation and tilled to prepare the soil for planting. Drip-line irrigation system, with emitters spaced 30.5 cm apart and an emitter flow rate of 0.055 liter/min–linear-meter (4.5 gal/min-1000 linear ft) was installed in the middle of each row and secured with metal landscape pins. "Fertigation" (a technique describing the incorporation of fertilizer into the irrigation

system) was used equally, and on an "as needed" basis, equally, to all the rows using 1 Tbs/Gallon Peter's fertilizer (20-11-15). In the soil-based rows (Row 2 and 5), strawberries were planted directly into raised-soil to create matted-row beds. For compost-based rows (Rows 1, 3 and 4), a new raised-bed growing method using 100 percent compost was implemented. This system (GrowExx) involved filling 20 cm polyethelene-mesh socks (GrowExx/Filtrexx inc., Grafton Ohio) with mature, screened poultry litter compost produced at the USDA Composting Facility, Beltsville Maryland. The compost was screened to <0.32 cm prior to filling the socks. The GrowExx socks were placed directly on top of un-fumigated soil, with irrigation tape secured along the top of each sock with metal landscape pins.

Four June-bearing cultivars of strawberry plants were obtained from Indiana Berry, Inc., (Huntington, Indiana). All plants were received as traditional soil-propagated bare-root transplants and maintained in a moist, $4^{o}C$ environment until planted. Cultivars were selected for their varied resistances to red stele, verticillium wilt, gray mold (*Botrytis cinerea*) and powdery mildew (*Sphaerotheca macularis)*. All crowns were planted directly into the soil or GrowExx compost socks along each drip-line fertigation tapes with 12" spacing between crowns. Six crowns were planted for each cultivar (per sub-sub plot) for a total of 18 crowns of each cultivar/ row for a total of 360 plants. Planting occurred in the spring of 2004 for harvesting the following June (2005).

Preparation and Application of Compost Tea (Foliage Treatments)

Three foliage treatments were used in this experiment. Treatment 1 of Aerated Compost Tea was prepared using Bio-blender™ (Soil Soup, Inc.) to actively infuse air into the CT throughout the brewing process. Additionally, fish-tank bubblers were used to infuse air into each batch of tea using sterile polypropylene tubing during each 24 hr brewing cycle. Prior to use, tap water (15 liters/bucket) was aerated for 2 hr to de-chlorinate it. A sock containing the compost was completely immersed into the water with the designated nutrient supplements. To enhance extraction of soluble materials and microorganisms from the compost, the filter sock was lifted above the water and allowed to drain into the bucket for 15 sec, then re-immersed for 30 sec. This was done a total of 3 times with the filter sock left in the liquid, while aerating for the remainder of the production cycle. The compost tea recipe used included a mixture of three mature, "Class A" compost sources (two manure-based and one vermicompost-based), along with supplements including Fish Hydrolysate (0.08%v:v), Humic Acid (0.14% w:v), Kelp (0.10% w:v), and a molasses-based nutrient (Soil Soup Nutrient, 120ml/5gal). Peanut oil (1.0 ml) was added at the start of each batch to reduce the amount of "frothing" that normally occurs during the brewing cycle. All 24 hr ACT was sprayed within three hours after preparation. All compost sources used in this study were determined to be below detectable levels (< 0.04 cfu/g) for *E. coli* using enrichment methods (i.e. 1 cell in 25grams = 0.04 cfu/gram).

Treatment 2 (CT+Yeast) was prepared as described for treatment 1, but with the addition of a concentrated yeast population that was prepared separately and

homogenized into the CT after brewing and immediately prior to foliage application. *Aureobasidium pullulans* (10, 147, 164), *Metschnikowia pulcherrima* (80, 83, 143, 183)and *Sporobolomyces roseus* (65) were chosen for their bio-control properties of certain phytopathic diseases of fruits and vegetables. Yeasts were a kind gift from Janisiewicz, W.J. (U.S. Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station, Kearneysville, West Virginia). All yeast inocula were prepared in individual 1000 ml flasks containing 750 ml Yeast Malt Extract broth (Difco, Becton Dickinson) and incubated on a rotary shaker 25-26^{o}C for 24 hr. Yeast cells were collected by centrifugation at 1500 x g for 10 min, washed twice and resuspended in 100 ml sterile water before being immediately added to the compost tea to prepare treatment 2 (CT+Yeast). The tea was homogenized by mixing with a sterile glass rod.

The Compost Tea foliage treatments were applied as un-diluted solutions via dedicated Round-Up™ style pressurized pump sprayers and applied at a rate of 1.5 L/sub-plot (6 linear meter) or 250 ml / linear meter. A dedicated sprayer was used for each treatment. Each sprayer was manually shaken during application to achieve equal distribution of the microbial content. This rate of application was more than sufficient to completely cover the entire surface of each plant (stem, leaves, fruit and flowers) as well as drench the soil or compost surrounding the stem. Each plant was sprayed until saturation was achieved. All sprayers were rinsed thoroughly after application with distilled water and inverted to air-dry.

Foliage treatments began immediately after planting and continued weekly throughout the summer months in 2004 (June, July, August) of the planting season,

and applied twice in 2005, on June 1 and June 9,, after all cultivars had good flower coverage. All foliage treatments were applied after the first harvest on June 1st and before the harvest on June 9th (vide infra in analysis for further details). All foliage applications were performed with special attention to full coverage of the flowers and fruit. On June 1 (pre harvest) and on June 9 (Day Zero), the foliage treatments were applied to the entire plant including all stem/soil interfaces. Special attention was applied to ensuring all red, ripened, as well as immature green fruits and flowers were completely covered with CT.

Microbiological and Chemical analysis of Compost Tea

Immediately prior to application, 30-40 ml of each foliage treatment (1, 2, 3) was directly collected during the spraying operation into sterile 50 ml conical tubes and maintained on wet ice in an insulated cooler to preserve the microbial content until analysis the following day. Samples of CT were serially diluted, if necessary, in buffered peptone water (Becton Dickinson) and plated in duplicate (100 µl each plate) using a WASP-II spiral plating instrument (Don Whitley Scientific, Ltd., England) to enumerate specific bacterial populations (total aerobic heterotrophs, gram negative bacteria, coliforms, fecal coliforms, *E. coli*, enterococci, and yeast). The MPN system was also used to detect low counts of viable fecal coliforms, *E. coli* and enterococci. The detection limits for these procedures were calculated as 5 CFU/ml for spread plating and <3 MPN/ml for the MPN. Fecal coliforms and *E. coli* were enumerated on MacConkey's agar containing MUG (37°C or 44.5°C, respectively for 24 hr), as well as in the MPN system using LT, EC and EC+MUG medium (the methodology is described in Chapter 2). All presumptively positive *E. coli* and

Enterococcus colonies were confirmed using protocols also described in Chapter 2. *Enterococcus spp.* were enumerated on modified *Enterococcus* agar (Difco, Becton Dickinson), adjusted to 1.5% Agar concentration and incubated at 37^{o}C for 48 hr. Total yeast counts were obtained using Special Yeast and Mold Agar (SYMM, Difco, Becton Dickinson), supplemented with 50 ppm Streptomycin, Chlortetracycline and Chloramphenicol (Sigma) to eliminate bacterial contaminants. All SYMM plates were incubated at 28^{o}C for 48 hr. Each CT batch was monitored for temperature, pH, EC, dissolved oxygen, and total organic carbon content at the end of each 24 hr brewing cycle.

Marketable and Non-Marketable Strawberry Harvest

The strawberry plants produced the first fruits on May 23rd and continued until the last week of June. Fruit harvests occurred on May 23, 26, June 1, 9, 13, 16 and 20. For each day, all red, ripe and rotten fruits from each plot (6 plants for each of 60 plots) were harvested individually and consolidated into buckets. The berries were weighed using a calibrated field balance (Adventurer model, Ohaus Corp., Pine Brook, New Jersey) for the total harvest (g) for each plot. The harvest for each plot was then separated into numbers of marketable and non-marketable fruits. Marketable fruits included those that were excellent quality and fit for point-of-sale operations. Non-marketable fruits included those that were not fit for sale due to phytopathogen disease, insect and/or wildlife damage. The weights of total harvest were used to determine effects of compost and soil growth conditions as well as foliage treatments. The percentage of the number of non-marketable to the number of

the total harvest was used to determine the effects of the above parameters on fruit quality.

Harvesting procedure for the microbiological analysis of strawberry fruits

Four "excellent quality" and marketable strawberries were aseptically harvested from each plot (4 berries per cultivar). The strawberries were excised from the plants using a sterile knife to cut through each stem approximately 5 mm from the calyx. Strawberries were collected and placed directly into sterile tared whirl-pak bags (Nasco, Fort Atkinson, Wisconsin). Weights (for each plot) were recorded and all samples were placed in coolers containing ice packs for transport to the laboratory for analysis. Strawberries were harvested for microbial analysis five times during the growing season. The first analysis was performed on the fruits prior to the foliage treatment on June 1 (T-1). The microbiological content of the berries on this day represented the native epiphytic microbiota contained on the fruit surfaces that developed without any additional foliage treatments, since the prior treatments in August of the previous year (2004). The second microbiological analysis was performed immediately *after* the second application of foliage treatments on June 9 (Time Zero). The third, fourth and fifth microbial analyses were performed on June 13 (Time 4), June 16 (Time 7) and June 20 (Time 11). Times 4, 7 and 11 refer to the number of days after the foliage treatments were applied (Time Zero). It should be noted that, on each day after the select strawberries were harvested for microbial analyses, all red- ripe and rotten strawberries were removed from the plants and recorded to collect data for the total harvest. After each harvest, the plants contained

several small green immature fruits as well as flowers from which fruits would develop the following week. Several small, green strawberries that were treated on Day Zero were analyzed for microbial content the following harvest day (Day 3) after ripening.

Microbiological Analysis of Strawberry Fruits

Strawberries from each foliage treatment/row consisted of four whirl-pak bags (each bag containing 4 fruits). For each foliage treatment, the four whirl-pak bags were infused with 1:1 (w:v) Buffered Peptone Water (Difco) containing 0.1% Sodium Pyruvate (Sigma). Each bag was placed into a sonicating water bath for two min, and then removed every 30 sec to manually massage the surfaces of the fruits.. The sonication facilitated removal of surface debris and microbes into the liquid. The surface washes from each of the four bags were consolidated into a sterile 500 ml screwcap bottle and homogenized via manually shaking for one min and then placed on ice until analyzed. This procedure was performed on each set of berries (n=16) to produce 15 samples that represented the microbiological microbiota from the surfaces of one serving size (approximately 166 grams, 1 cup, or 16 strawberries) of pooled strawberries exposed to each foliage treatment.

Each sample was homogenized and 50 ml was used for the Colilert™ system (IDEXX Laboratories, Inc., Westbrook, Maine) to detect coliforms and *E. coli*. 50 ml was used for the Enterolert™ system (IDEXX) to detect enterococci. The remaining sample was spiral plated onto Trypticase Soy Agar, MacConkey's Agar, MacConkey's Agar w/MUG, Modified Enterococcus Agar and Special Yeast and

Mold Medium to quantify the total heterotrophic bacterial population, gram negative bacterial population, coliforms, fecal coliforms, enterococci, and yeast populations, respectively. The methodologies for plating and MPN techniques are described in Appendix A. Methods not previously described are the Colilert™ and Enterolert™ Quantitray™ MPN systems that process the sample in self-contained heat-sealable units to enrich and detect the target populations in 24 hr using patented defined substrate technology. All Quantitray™ results were confirmed using biochemical confirmation of isolated bacteria from each positive reaction vessel.

Results

Effect of Compost GroExx™ and Foliage Treatments on Strawberry Yields (Table 4.2)

There was a marginally significant effect for Cultivar*growth medium (F=33.36, P=0.0886), which was due to one cultivar (Allstar) that produced statistically significant (P=0.0385) more fruit when grown in the compost socks (272.9 g/lin-m) than in the soil (148.7 g/lin-m respectively, Table 4.2). Chandler and Northeastern cultivars also produced greater yields when grown in compost (146, 124.6 g/lin-m, respectively) than in soil (88.1, 93.7 g/lin-m, respectively), although these differences were not significant (Table 4.2). There were no statistically significant main effects for the foliage treatments (Water Control, Compost Tea, or Compost Tea+Yeast, F=0.89, P=0.411), or the growth medium (Soil/Growsoxx; F=0.78, P=0.3770) on the pooled marketable yield of all strawberry cultivars (Allstar, Chandler, Northeastern, Sparkle) harvested in 2005 compared to conventional growth methods.

Table 4. 2 : Effect of foliage treatments and growth medium on marketable yield of four cultivars of strawberry yields grown in field conditions

Treatment[a]	Growth Medium[b]	Marketable Yield[c] (g/linear-m row)				Pooled Means[e]
		Allstar[d]	Chandler[d]	Northeastern[d]	Sparkle[d]	
Control	Compost	283.5 a	150.5 a	143.5 a	435.0 a	**253.1 a**
	Soil	185.2 ab	118.6 a	84.6 a	458.4 a	**211.7 ab**
CT	Compost	261.7 a	150.1 a	103.2 a	361.1 a	**219.0 ab**
	Soil	200.5 ab	102.9 a	155.1 a	507.3 a	**241.5 ab**
CT+Yeast	Compost	273.4 a	137.4 a	127.1 a	420.6 a	**239.6 ab**
	Soil	60.4 b	42.8 a	41.5 a	447.1 a	**147.9 b**
Pooled Means[f]	**Compost**	**272.9 a**	**146.0 a**	**124.6 a**	**405.6 a**	237.3 a
	Soil	**148.7 b**	**88.1 a**	**93.7 a**	**470.9 a**	200.4 a

[a] Three foliage treatments applied weekly to strawberry plants after planting in May 2004 until first harvest May 2005: Control (water spray), CT (aerated compost tea), CT+Yeast (compost tea amended with *Aureobasidium pullulans*, *Metschnikowia pulcherrima*, and *Sporobolomyces roseus)*.

[b] Strawberry plants were planted into soil or compost socks (GrowExx™).

[c] Marketable yield included all fruits that were red, ripe, not injured and contained no visible evidence of insect or animal damage. Yields shown are the means of three replicates each over seven harvests (May 23,26, June 1, 9, 13, 16 and 20).

[d] For each cultivar, yield means for all treatments followed by the same letter are not statistically different at $P \leq 0.05$ using the Least Significant Difference mean separation technique.

[e] Yield means (g/linear m row) were pooled across all cultivars to display growth medium*foliage treatment effects. Means with the same letter are not significantly different at $P \leq 0.05$.

[f] Yield means (g/linear m row) were pooled across all foliage treatments to display growth medium (compost/soil) effects for each cultivar. Means with the same letter are not significantly different at $P \leq 0.05$.

Effect of Compost Socks and Foliage Treatments on Disease (Table 4.3)

Each cultivar (Allstar, Chandler, Northeastern and Sparkle) produced a lower ratio (lower disease index) for all plants grown using the compost socks (23%, 21%, 23% and 26%, respectively) rather than in the bare soil beds (29.33%, 25.59%, 24.92% and 36.74%, respectively). This corresponds with a lower incidence of black root rot in Chandler and Allstar cultivars using compost socks as compared to non-fumigated soil plots in a multiple-farm study (160). However, there were no significant main effects for the foliage treatments ($F=0.55$, $P=0.5777$) or growth medium ($F=2.89$, $P=0.3369$) on the incidence of disease of the pooled cultivars during the 2005 harvest season. The incidence of disease refers, in this research, to the ratio of non-marketable berries to the total harvest. This ratio does not quantify specific foliar or root-borne diseases, but uses an observational index by providing a look at the cumulative effects these diseases have on the quality and quantity of the total fruit yield.

Table 4.3 : Effect of foliage treatments and growth medium on percentage of diseased fruits of four cultivars of strawberries grown in field conditions

Treatment[a]	Growth Medium[b]	Percentage of Disease[c] (#non-marketable berries / #Total Harvest)*100				Pooled Disease[e]
		Allstar[d]	Chandler[d]	Northeastern[d]	Sparkle[d]	
Control	Compost	25.20 a	21.14 a	21.74 a	29.70 a	**24.18 a**
	Soil	27.69 a	25.38 a	19.64 a	34.43 a	**25.91 a**
CT	Compost	25.15 a	22.30 a	23.19 a	21.92 a	**23.17 a**
	Soil	29.17 a	35.23 a	30.81 a	38.49 a	**33.04 a**
CT+Yeast	Compost	18.42 a	20.61 a	24.09 a	27.64 a	**22.67 a**
	Soil	30.98 a	16.88 a	24.62 a	37.30 a	**26.61 a**
Pooled Disease[f]	**Compost**	**23.11 a**	**21.33 a**	**23.00 a**	**26.49 a**	23.34 a
	Soil	**29.33 a**	**25.59 a**	**24.92 a**	**36.74 a**	28.58 a

[a] Three foliage treatments applied weekly to strawberry plants after planting in May 2004 until first harvest May 2005: Control (water spray), CT (aerated compost tea), CT+Yeast (compost tea amended with *Aureobasidium pullulans*, *Metschnikowia pulcherrima*, and *Sporobolomyces roseus)*.

[b] Strawberry plants were planted into soil or compost socks (GrowExx™).

[c] Percentage of Disease was determined, for each plot, by counting the number of non-marketable strawberries and dividing by the total number harvested. Non-marketable berries included fruits that were affected by phytopathogens or visibly injured due to insect or animal infiltration. Percentages shown are the means of three replicates each over seven harvests (May 23, 26, June 1, 9, 13, 16 and 20).

[d] For each cultivar, yield means for all treatments followed by the same letter are not statistically different at $P \leq 0.05$ using the Least Significant Difference mean separation technique.

[e] Pooled Disease Percentages were pooled across all cultivars to display growth medium*foliage treatment effects. Percentages with the same letter are not significantly different at $P \leq 0.05$.

[f] Pooled Disease Percentages were pooled across all foliage treatments to display growth medium (compost/soil) effects for each cultivar. Means with the same letter are not significantly different at $P \leq 0.05$.

Microbiological content of foliage treatments (CT and CT+Yeast)

The microbiological analysis of each compost tea batch was performed on samples taken immediately prior to application to the strawberry plants. Each treatment (Treatment 2: Compost Tea; Treatment 3: Compost Tea + Yeast) was identical except for the yeast added to treatment 3 (Figure 4.1). The heterotrophic, gram negative, coliform and enterococci content of the CT reflected the populations of bacteria generally available in 24 hr compost tea that was brewed with added nutrients. The microbial content of both treatments was identical except for the concentration and type of yeast. The yeast content in treatment 3 (log 5.98) reflected a 3.51 log increase over that of treatment 2 (log 2.47) and was due to the added yeast isolates after the brewing process was complete. The higher yeast content in Treatment 3 was an attempt to produce a value-added compost tea containing known antagonists to agents that cause root and foliar diseases. The yeast content in treatment 2 (log 2.47) reflected the population of yeast and fungi that were extracted and/or further propagated from the original compost during the CT brewing process.

Good quality vermicompost and thermophilically-processed manure-based compost that meet time and temperature requirements for proper composting should not contain viable *E. coli*, fecal coliform or *Salmonella* populations. Compost prepared with feedstocks containing biosolids are required to show that the final product contains less than 3000 MPN/g fecal coliforms and < 3 MPN/4g of *Salmonella*, in order to meet EPA "class A" regulations (57). All of the compost feedstocks used in this study to produce compost teas were non-detectable for *E. coli*

and *Salmonella* via enrichment methods (detection limit < 0.04 cfu/g), thus were in compliance with the EPA "class A" requirements for fecal coliforms content.

E. coli population

The compost teas used throughout this study contained a mean of 2.73 cfu/ml *E. coli*, however, suggesting that one of the compost feedstocks contained trace amounts of *E. coli* (below detection limits.) The compost teas in this study were produced using molasses-based nutrients which have been shown to enrich and propagate even trace levels of *E. coli* in the compost feedstocks to potentially hazardous concentrations (112). The mean level of *E. coli* in the compost tea (2.73 cfu/ml) was over the maximum concentration set by the current best practices for irrigation water (based on the current EPA regulations for *E. coli* content in recreational water of 126 cfu /100 ml or *1.26 cfu/ml in a single sample*) (56). This seemingly small concentration of *E. coli* contained in the foliage treatments had the potential to cause disease in humans if the population included pathogenic serotypes (such as *E. coli* O157:H7) which has an extremely low infectious dose (<30 cfu for *E. coli* O157:H7 (14).

Enterococcus population

The *Enterococcus* populations in the compost tea treatments were approximately 128 cfu/ml, which deposited approximately 3 Log cfu *Enterococcus* onto each plant surface, including the stem, leaf, flowers and fruits on T0 (day of application). Currently, there are no standards for the allowable amounts of

Enterococcus in irrigation water; however, the amount contained in the foliage treatments used in this study was well above the 0.33/ml allowable concentrations set by the EPA for recreational water usage (56).

If potentially pathogenic microbes in the compost tea were applied to the surfaces of fruits, and these microbes survived and/or propagated in protective niches on those surfaces, after which the product was consumed raw, there exists the possibility that the foliage treatments could contribute to human foodborne illness.

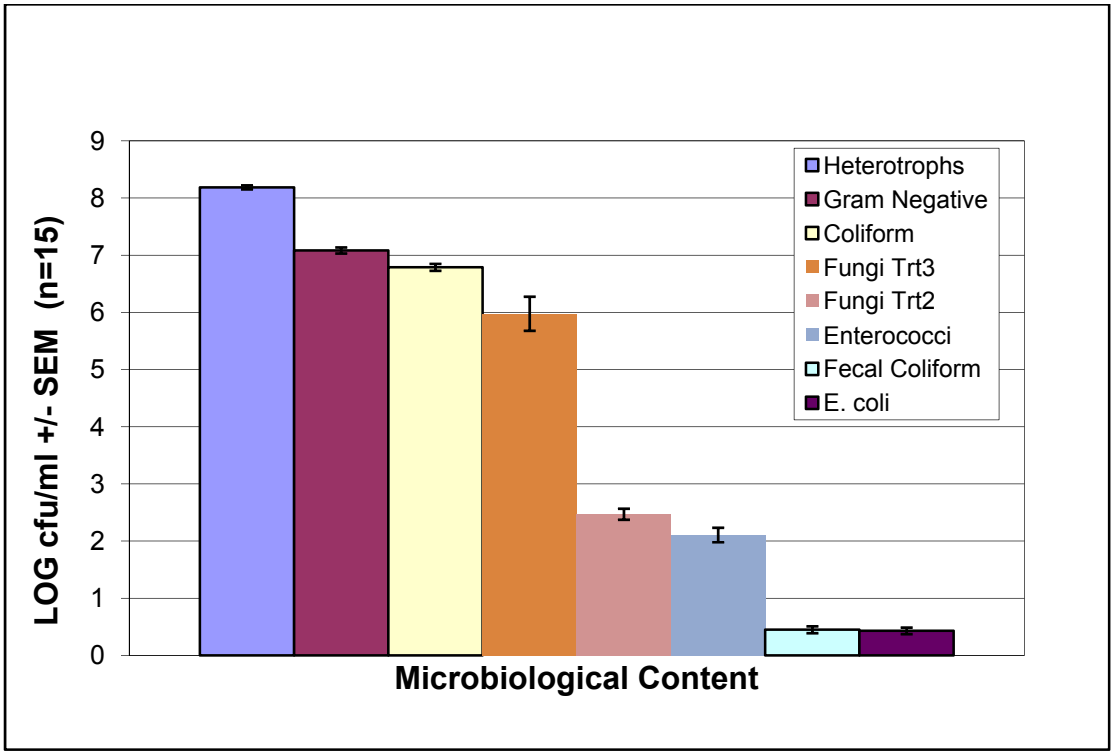

Figure 4.1 : The average microbiological content of 15 batches of compost tea (CT). CT was applied weekly to strawberry plants after planting and up to the first day of harvest

Microbiological Quality of Strawberry Fruits

For each foliage treatment (Control, Compost Tea, Compost Tea+Yeast), sixteen marketable strawberries (four from each cultivar) were harvested and

processed for determination of the microbial content of the surface flora. Sixteen berries consisted of approximately 166 grams and considered to be one serving size of whole berries (http://www.urbanext.uiuc.edu/strawberries/nutrition.html). The surface concentrations of the following microbial populations were determined for a single serving size: Aerobic bacterial heterotroph population (Figure 4.2), gram negative population (Fig 4.3), coliform population (Figure 4.4), Enterococcus (Figure 4.5), and yeast (Figure 4.6).

The differences in microbiological content between strawberry fruit grown in either compost or soil were not significant ($F=0.75$, $P=0.5561$); therefore, all microbiological (log cfu) data were pooled for both compost and soil grown strawberries for the graphical depiction of the microbial data (Figs 4.2-4.6). Surprisingly, the foliage treatments (CT, CT+Yeast, Water Control) had no overall effect on the tested microbiological populations ($F=1.26$, $P=0.2639$) of the strawberry surfaces. This finding suggested that the compost tea treatments in this study contributed minimally to the establishment microbial populations that could form sustainable communities on the strawberry fruits over the course of 11 days after inoculation. This study, however, provides only concentration data of specific groups of microbial populations and does not include information regarding specific genera, class or species of bacteria or fungi that might indeed be influenced by the different foliage treatments.

Heterotrophic Populations

The heterotrophic populations (Fig 4.2), for example, show very similar concentrations throughout the sampling times (T-1 through T11). However, these numbers do not reflect the specific types of bacteria present on the strawberry fruit surfaces. Neither does this study include microbiological data on the internalized microbiota (endophytes) of the strawberry fruits, or on the strawberry plant including the roots, stem, leaf and flower tissues where the compost teas were also applied. That is, only the fruits analyzed on Day Zero provide direct quantification of the microbial populations after the foliage treatments were applied directly to already ripened strawberry fruits. Time 4 (4 days after foliage treatment) also included the analysis of fruits that were directly applied with the compost tea treatments. However, these fruits were small, green and not yet ripened during the time of compost tea application. Eleven days after compost tea treatments, most of the fruits that were harvested for microbial analysis had developed from flowers that were sprayed with compost tea on Day 0.

Observing the recovered microbial populations over time (T-1, T0, T4, T7, T11), there were significant differences ($P<0.0001$) in all population counts except for yeast (Fig. 4.6) which remained stable from T-1 to eleven days after compost tea application ($P=0.4427$). The heterotrophic populations (Fig 4.2) of the fruits were not significantly affected by any foliage treatments except for immediately after the day of application (T0). Both CT and CT+Yeast treatments significantly increased the heterotrophic content on the fruits ($P<0.05$) as compared to the water spray on T0. Interestingly, the levels of heterotrophs for all other time points were statistically

similar (P>0.05), which suggested that a significant portion of the microbial biomass contained in the CT treatments did not survive on the fruit surfaces after four days (T4).

Total Gram Negative Populations

The total gram negative populations of the fruits followed a similar pattern (Fig 4.3). As expected, both compost tea treatments on T0 introduced significantly different populations on the fruit surfaces as compared with the water spray control. At the end of the harvest, 11 days after CT applications, the fruits contained the same gram negative content as the T-1 samples. In fact, for all other time points besides T0 (day of application), the gram negative counts were similar for all treatments. The compost tea treatments had little effect on the quantity of total yeast, aerobic heterotrophs, or gram negative populations of the harvested strawberry fruits harvested four (T4), seven (T7) or eleven (T11) days following compost tea application.

Enterococcus Populations

Enterococcus populations on the fruit surfaces were at their highest concentrations for all treatments on the fruits harvested prior to application of the foliage treatments (T-1). All foliage treatments on T0 reduced the surface concentrations of *Enterococcus* by over 0.5 log, suggesting that the foliage treatments (including the water spray control) were able to reduce the indigenous populations either by diluting, competing or dislodging the indigenous flora. Although the

concentrations of enterococci populations on the fruits were always greater (except for T11) for the CT treatments than water sprays, these differences were not significant. The compost tea treatments increased, but did not significantly affect the populations of *Enterococcus* on the fruit surfaces (8.8 cfu/serving, CT and 32.9 cfu/serving, CT+Yeast) when compared to the water spray controls (3.85 cfu/serving). This is not the first study to have found enterococci on strawberry fruits, but it provides the first quantitative evidence that enterococci may be introduced into the food chain through the consumption of fresh strawberries. These isolates were not identified to species; however, McGowan, in 2006, recovered mostly *E. casseliflavus* and *E. mundtii* isolates from 17% of the strawberries sampled in his extensive qualitative prevalence study (156). While these isolates are not considered pathogenic, they have been determined to have virulence profiles that could cause opportunistic disease (155). Even with the low concentrations found in our study (<10 cfu/ serving), the possibility exists for these opportunistic microbes to cause disease if consumed raw by infants or immuno-compromised patients. Furthermore, Johnston, in 2004 (119), determined a prevalence of *E. faecium, E. faecalis*, and other *Enterococcus* species, 52%, 21%, and 27%, respectively, on fruit surfaces. He found that 91% of the recovered *E. faecium* isolates were resistant to at least one antibiotic, excluding intrinsic resistance.

Total Coliform Populations

The total coliform content (Fig 4.5) of strawberry fruits was highly variable across all treatments and time points. Although higher coliform counts were expected on all CT treatments in comparison to water controls, the populations were not statistically different ($P<0.05$). The compost teas distributed approximately 8 Log cfu total coliforms and 100 cfu of *E. coli* onto each plant surface, soil, fruits and flowers at T0; however, there was no evidence that this influx of coliform populations was able to sustain any level of viability on the fruit surfaces, or to thrive on fruits that developed from inoculated flowers.

E. coli, however, was recovered from fruits harvested at T0, immediately after CT application. The recovered *E. coli* populations (Fig 4.5) on fruits sampled at T0 were 6.2 MPN/serving and 1.24 MPN/serving (CT and CT+Yeast, respectively). All other sampling times were negative for *E. coli*. As an aside and interestingly, all strawberry samples that contained *E. coli* were grown in the compost socks and not in soil. Since these samples were harvested immediately after CT application, it is difficult to theorize why the growing medium would have any influence on these results.

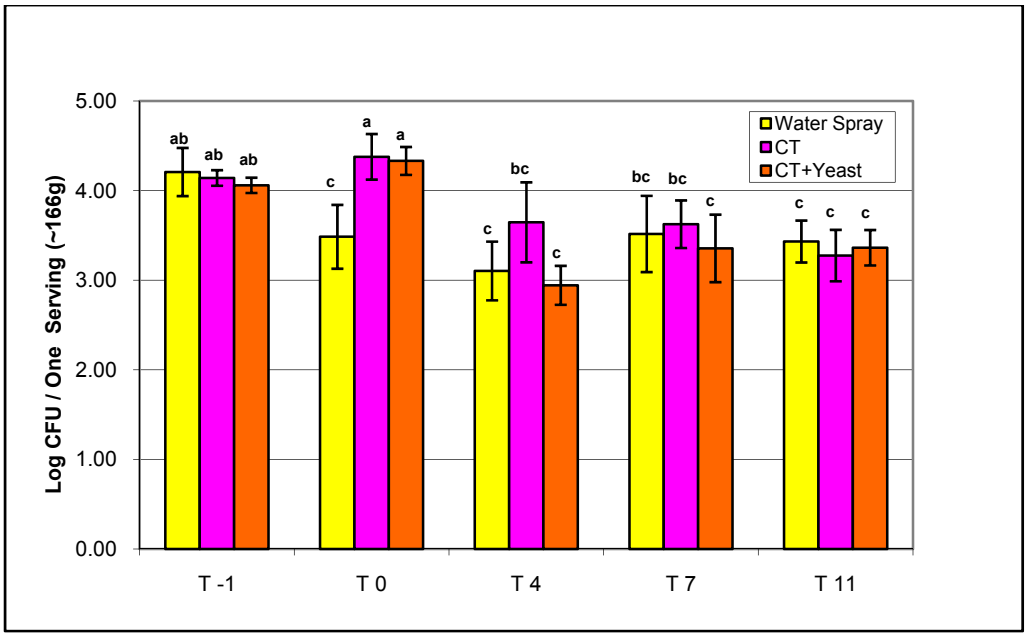

Figure 4.2 : Total aerobic bacterial heterotrophic population from the surfaces of one serving of marketable strawberry fruits (n=16, ~166g) sprayed at time Zero (T0) with compost tea, compost tea amended with yeast, or a water spray control. Strawberries were sampled five times, from pre-foliage application until 11 days after foliage application (T-1, T0, T4, T7 and T11).Different letters indicate significant differences at the P<0.05 level (using the least significant difference means separation technique, SAS version 9.2, Cary, NC)

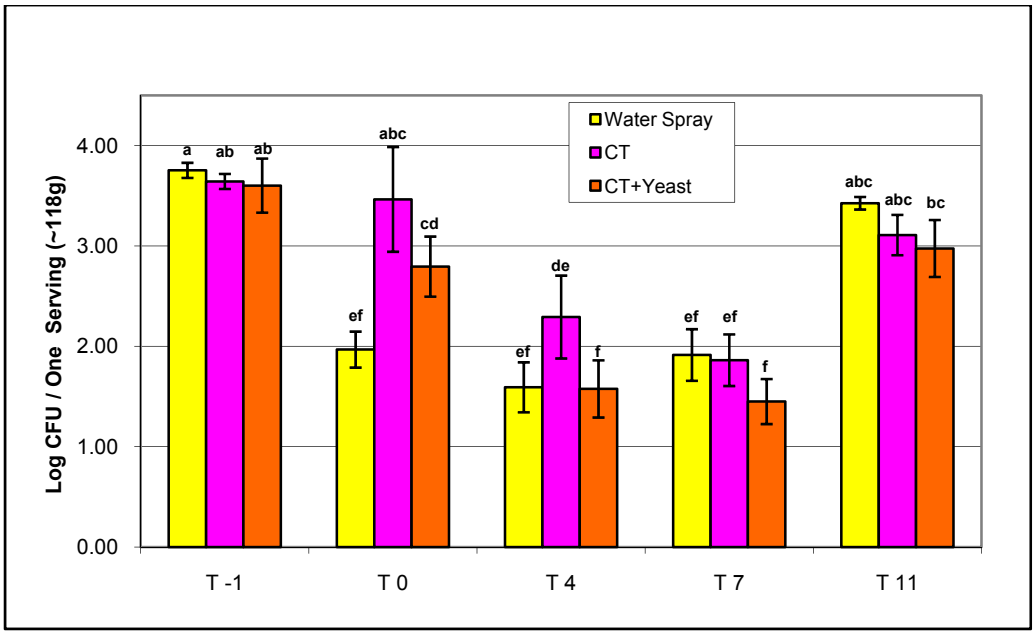

Figure 4.3 : Gram negative bacterial population from the surfaces of one serving of marketable strawberry fruits (n=16, ~166g) sprayed at time Zero (T0) with compost tea, compost tea amended with yeast, or a water spray control. Strawberries were sampled five times, from pre-foliage application until 11 days after foliage application (T-1, T0, T4, T7 and T11). Different letters indicate significant differences at the P<0.05 level (using the least significant difference means separation technique, SAS version 9.2, Cary, NC)

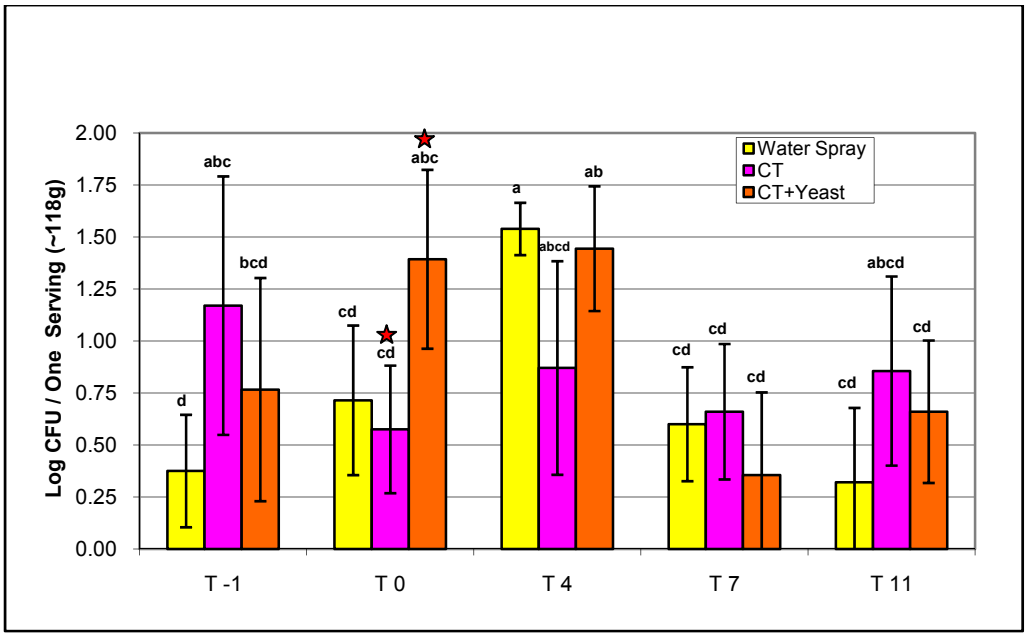

Figure 4.4 : Total coliform and *E. coli* population from the surfaces of marketable strawberry fruits (n=16, ~166g) sprayed at time Zero (T0) with compost tea, compost tea amended with yeast, or a water spray control. Strawberries were sampled five times, from pre-foliage application until 11 days after foliage application (T-1, T0, T4, T7 and T11). ☆' : indicates that *E. coli* was confirmed at T0 samples in both CT: 6.2 MPN/serving and CT+Yeast: 1.24 MPN/serving. All other samples were negative for the detection of *E. coli*. Different letters indicate significant differences at the P<0.05 level (using the least significant difference means separation technique, SAS version 9.2, Cary, NC).

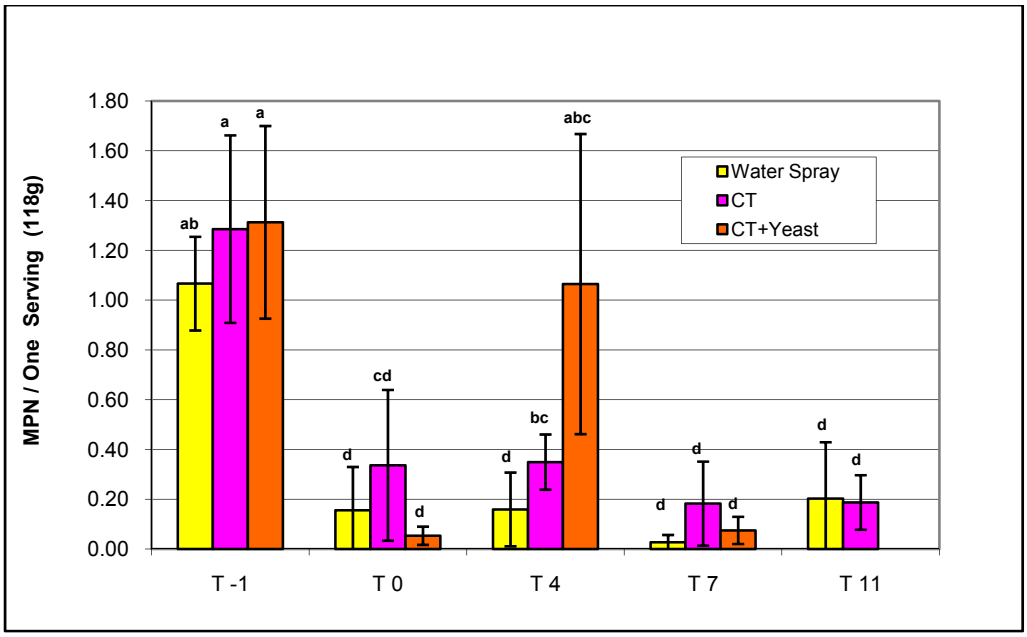

Figure 4.5 : *Enterococcus* population from the surfaces of marketable strawberry fruits (n=16, ~166g) sprayed at time Zero (T0) with compost tea, compost tea amended with yeast, or a water spray control. Strawberries were sampled five times, from pre-foliage application (T-1, T0, T4, T7 and T11). Different letters indicate significant differences at the P<0.05 level (using the least significant difference means separation technique, SAS version 9.2, Cary, NC)

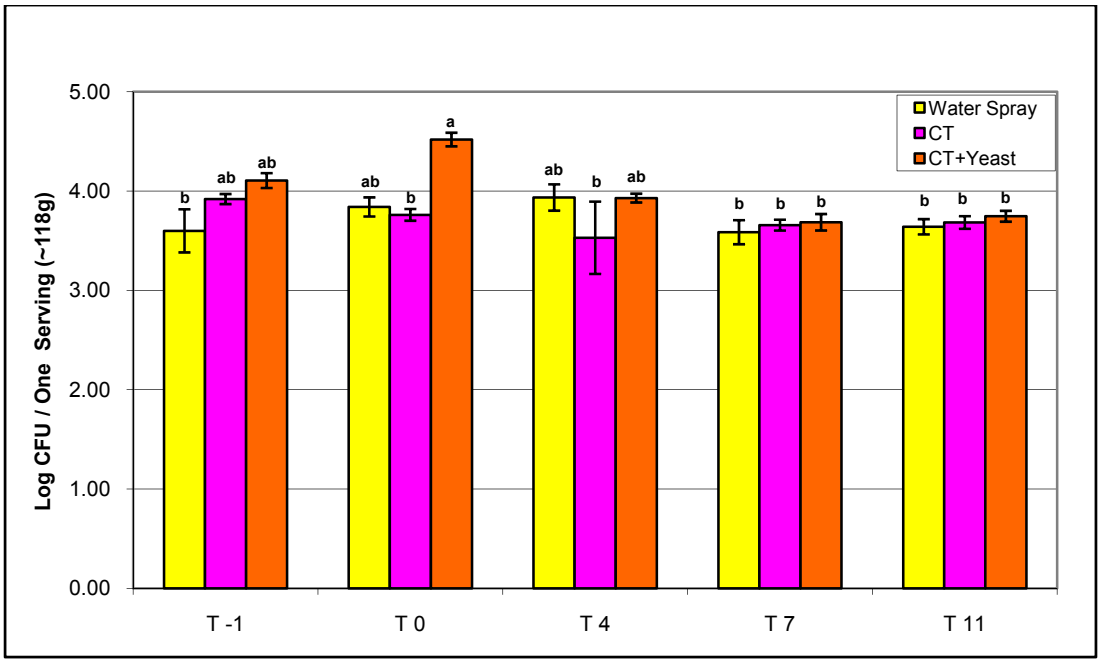

Figure 4.6 : Total yeast population from the surfaces of marketable strawberry fruits (n=16, ~166g) sprayed at time Zero (T0) with compost tea, compost tea amended with yeast, or a water spray control. Strawberries were sampled five times, from pre-foliage application (T-1, T0, T4, T7 and T11). Different letters indicate significant differences at the P<0.05 level (using the least significant difference means separation technique, SAS version 9.2, Cary, NC)

Discussion

ACT has been reported to promote as much as 20% greater strawberry fruit yields, while both ACT and NCT reduced the incidence of foliar diseases when compared to water spray controls (268). In our field trial, however, neither of the ACT treatments (CT and CT supplemented with three biocontrol yeast strains) had a statistically significant effect on strawberry yield, nor on disease incidence, when compared to the water spray controls. The biocontrol yeast isolates in this study were chosen for their effects on various phytopathogens and foliar diseases: *Aureobasidium pullulans*: fire blight (147), Penicillium expansum (164), blue mold (10); *Metschnikowia pulcherrima*: fruit rot (80); *Sporobolomyces roseus*: *Botrytis cinerea* (65). Leverentz, 2006 (143) also showed that *Metschnikowia pulcherrima* provided post-harvest reduction of both *Listeria monocytogenes* and *Salmonella* on fresh cut apples during storage.

The field experiments were conducted in un-fumigated soil within an area having a seven-year history of black root rot (BRR) and red stele, a foliar disease caused by *Phytophthora fragariae* Hickman. Black root rot (BRR) is caused by an interaction of one or several plant pathogens and favorable soil conditions that result in poor plant vigor and decreased yields. While the CT foliar treatments did not have a significant effect on disease rates, three out of four cultivars used in this study produced greater yields, when grown using the compost socks as opposed to growing in bare soil. One cultivar, Allstar, produced yield differences that were statistically significant (Table 4.2). This difference is attributed to greater plant vigor, plot coverage, longer runners and greater numbers of flower buds (data not shown).

Many regional growers that host strawberry "U-Pick" operations rely on conventional methods of covering soil with black plastic mulch and solarizing and/or fumigating with methyl bromide (MeBr) to eliminate any residual soilborne pathogens, insect pests and control weeds. Most of these raised-bed plasticulture systems are annual operations that require removal of plants, plastic and irrigation systems at the end of each season. These methods require large annual start-up costs for the materials, machinery and time needed to set up the field, as well as contributing to large amounts of landfill-destined waste. Although MeBr is purported to be an excellent fumigant that controls a variety of plant diseases, the effect is only temporary for BRR which can re-establish within several months. MeBr is currently being banned from use in agriculture because it has been concluded that MeBr contributes substantially to the destruction of the ozone layer. Compost has been an attractive and sustainable alternative method to both conventional and organic farmers for the control of plant pathogens. Further, it reduces the need for chemical fertilizer by enhancing the physical and chemical properties of soil. Costs, however, for producing and/or purchasing and broadcasting compost under organically approved guidelines onto the entire field can be prohibitive (82). Research suggests that while conventional systems may produce more yield per acreage than organic alternatives, organic operations can still be more profitable (74).

The compost sock method used in this study provided a simple and effective method for introducing an organic-based farming approach which yielded greater numbers of marketable strawberry fruits during the first growing season. This system eliminated the need to prepare soil via fumigation, and tilling and mounding the soil

into planting rows. The compost socks also eliminate the requirement for black plastic mulch which is commonly used to reduce the need to actively control weeds. Good quality vermicompost and theromophilically processed manure compost should not contain viable weed seeds, thereby reducing the necessity for manual or chemical weed control. The compost socks provided a raised, 3-dimensional planting medium with excellent drainage and showed no symptoms of BRR in the roots, which remained inside the socks (160). The plant "runners" were easily established along the top and sides of the socks.

The compost tea treatments used in this study were prepared using a molasses-based nutrient and thermophilically produced poultry compost which was determined to be negative for *E. coli.* They were applied as an un-diluted foliar spray to the entire plant and soil, or compost, around each root ball. Both CT treatments did not significantly affect the concentrations of total aerobic heterotrophs, gram negative bacteria, total coliforms, or yeast populations on the strawberry fruit surfaces four days after CT application when compared with water spray controls. Low concentrations of *E. coli* were recovered from the fruits in both CT treatments, but only when harvested immediately following CT application (T0).

Although properly prepared, thermophilic composts should not contain viable foodborne pathogens (142), Gong in 2005(79) determined that a few *E. coli* cells were able to survive the composting process at 54-67C in low moisture conditions (40%). Droffner 1995 (43) concurred that the destruction of pathogens in compost may not rely on the temperature profiles alone. Even a few remaining cells of *E.coli* in the CT feedstock may stimulate the production of a dangerous batch of tea when

molasses-based nutrients are used (112). This information, closely reviewed by the National Organics Standards Board, influenced the eventual recommendations for compost tea manufacture and usage (171). Where nutrients are used to prepare compost teas, the tea should be tested and determined to meet the EPA standards for recreational water (<135 cfu/100ml *E. coli* and <33 cfu/100ml Enterococci). CT that does not meet these standards is still acceptable for foliage application, provided the application event is at least 120 days prior to the first harvest. In effect, CT that fails to meet this standard is required to follow the same rules that apply to the use of raw manure.

The compost teas used in this study contained very low levels of *E. coli* and *Enterococcus* contamination. These levels were above the EPA limits, providing the first study to determine the fitness of low levels of *E. coli* populations in CT foliage application events on strawberry fruits. *E. coli* was not determined to be particularly well suited for survival under field conditions on strawberry fruits, and was not detected on any fruits in as little as four days after CT application. This suggests that the current NOSB recommendations for CT application, regarding *E. coli* levels and/or days-to-harvest application timing, might perhaps be slightly too stringent. Four days of solar radiation, desiccation and competition with indigenous epiphytic microbiota may have contributed to the elimination of the additional microbial populations applied by the CT treatments (122). It should be noted, however, that this study did not preclude the possible presence of endophytic *E. coli* that may have been introduced and further propagated inside the fruits via contamination of the flowers through CT application. Enteric pathogen internalization into tomato fruits

by direct inoculation of the flowers prior to fruit development was demonstrated by Shi et. al, in 2007 (219).

In a related project, we harvested strawberries from eight local farms in Maryland to determine *E. coli, Salmonella* and *Listeria spp*. content. We found two farms that contained strawberry samples with *E. coli* contamination and one farm with *Listeria spp*. (unpublished data). The concentrations of *E. coli*, however, were below 0.02 cfu per serving (16 berries) and *Listeria spp.* was not quantified. Neither farm incorporated CT into their farming practices. A large survey of fresh produce in Minnesota included 11 strawberry samples that showed a mean coliform content of 2.7 Log for organic and 4.2 Log for conventionally grown fruits, although no *E. coli* were recovered from the strawberry fruits harvested in this study (165). Enterococci concentrations were not determined, however, and more information is needed to determine the levels of enterococci that are introduced into the human population through the consumption of fresh strawberries. Furthermore, are these isolates contributing to the increasing concerns about antibiotic resistance of foodborne pathogens? Future research should contribute to determining how foodborne pathogens infiltrate strawberry fruits because sugar contents may provide a means for their survival and, indeed, growth.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

"Absence of evidence is not evidence of absence"

- Dr. Carl Sagan

The frequency of foodborne outbreaks associated with fresh produce in the United States. is increasing. One understandable and unfortunate consequence is a dramatic decline in consumer confidence in the safety of farm produce. In response, government regulatory agencies and commercial growers have imposed regulations and guidelines across the farm-to-fork continuum to reduce the introduction of enteric pathogens into the food supply and, eventually, to restore consumer confidence in produce safety. The most practical way to reduce the incidence of illnesses caused by these pathogens is to increase our understanding of point-source contamination and dissemination of enteric pathogens that can occur in both pre-harvest (i.e. on-farm production) and post-harvest (i.e. sanitization, packaging and transport) practices.

Prior to the research for this dissertation, the results of a comprehensive examination of two commonly used organic inputs in both organic and conventional farm operations (i.e. compost and compost teas) has not been published. This research provides the first intensive food-safety related investigation into these two organic inputs, which may inadvertently be contributing to the on-farm survival and dissemination of foodborne pathogens.

When practiced in accordance with current standards, composting has become generally accepted as an effective means of eliminating foodborne pathogens that

may be present in foodwaste, biosolids, manures and greenwastes. Despite recent evidence to suggest otherwise, there is generally an assumed "no risk" label on widely distributed compost products for use in home gardens, organic and conventional farming operations. Few studies have looked at the microbiological quality of such finished "Class A" composts in terms of human pathogen content, and the reported studies are either regional and/or feedstock-specific. This dissertation investigated "Class A" composts produced from a variety of feedstocks (both biosolids and non-biosolids-based) available to consumers in all regions of the United States. By correlating pathogen content with specific factors used for measuring compost maturity (e.g., moisture, EC, pH, C:N, Soluble Carbon), this research design assessed pathogen content, and potential for pathogen content, in compost. Furthermore, through repeated sampling in the same compost-producing facilities over several months, seasonality trends (Spring, Summer and Winter) were considered as possible determinants of the incidence of potential human pathogens in finished compost products.

This research provided compelling evidence that even "Class A" composts cannot be considered pathogen free. *Salmonella*, *E.coli* (including the recovery of a single Shiga-toxigenic isolate) and high levels of fecal coliforms were detected with alarming frequency especially in the Summer and Winter months of compost production. Analysis of physico-chemical parameters suggested that soluble carbon and electrical conductivity content of the compost samples may be a predictor for potential pathogen content because each was positively correlated with *E. coli* populations, and enterococci populations, respectively. In addition, principal

component analysis of *E. coli* FAME profiles provided a means for differentiating isolates based on feedstock origin. This provides a potentially powerful tool for source-tracking the origin of *E. coli* populations from human, domestic animal or wildlife sources.

Routine pathogen testing of all consumer-destined composts should be required, including composts constructed from green waste and other non-manure sources. As currently stated, time/temperature requirements are of questionable value because pathogens have been shown to survive the current EPA Part 503 standards. Future research should focus on determining appropriate temperature monitoring methods and sampling protocols for composting operations to ensure that all particles in the compost are exposed to the required time/temperatures disinfection continuum. Consumer awareness concerning the potential for pathogen-content in commercially available compost should be addressed, especially farming operations that use compost in the production of fresh produce, but also for composts destined for the production of compost teas.

This study also investigated the potential for the propagation and dissemination of *E. coli* using current compost tea production methods in a field study with strawberries. This was designed to test a widely held theory concerning aerated tea production methods: namely, that even when low levels of *E. coli* and *Salmonella* are present in compost, the introduction of these microbes into the extremely diverse and microbiologically competitive ACT environment will quickly die-off because they are not able to survive in highly aerated and heterogeneously diverse microbiota environments. Current practitioners maintain that, provided proper attention is given

to the maintenance of oxygen levels above 6ppm (even when nutrient additives are used), the few remaining *E. coli* or *Salmonella* present in compost will be quickly eliminated during the production of ACT due to competition, inhibition and/or predation of the heterotrophic microbiota extracted from compost.

Using a novel inoculation protocol designed to simulate naturally contaminated compost and using typical CT production methods, the research in this dissertation contradicts this theory. When nutrient additives were used, both ACT (>6ppm O^2) and NCT (<6ppm O^2) provided conditions conducive for the survival and even propagation of *E. coli* and *Salmonella*. Furthermore, it was shown that, even without nutrient additives, the populations of inoculated *Salmonella*, *Enterococcu*s and fecal coliforms were able to survive after 36 hours of ACT production.

Industry has responded to a booming "buyers market" by developing several competing ACT brewing systems. Each brewing method purports to have the ability to produce superior ACT in terms of quality and quantity of extracted bacteria and fungi leading, in turn, to exaggerated and unsubstantiated statements concerning product suppression of phytopathogens. The research in this dissertation determined that four of the tested devices produced essentially identical results in terms of recovered microbiota. Furthermore, using compost naturally contaminated with extremely low levels of *E. coli* (<0.04 cfu/g), each of the four systems resulted in recoverable *E. coli* in the final 24hr ACT products , even when nutrient additives were not used.

Although recovered concentrations of *E. coli* were low (< 10 cfu/ml), all ACT produced by the four brewing systems would be prohibited from application to

produce using the current standards of the Good Agricultural Practices (GAPs) set by the NOSB concerning compost tea that are consistent with the EPA recreational water guidelines (which require less than 1.26 cfu/ml for *E. coli* and less than 0.33 cfu/ml for enterococci).

Another critical contribution of this dissertation points to the need for investigations determining the extent to which ACT, when used in farming operations, may be introducing foodborne pathogens into the fresh produce supply. An *E. coli* population from a naturally contaminated compost was tracked through the production of ACT by being applied weekly to: 1) several cultivars of organically and conventionally grown strawberries, and 2) to the surfaces of harvested "ready to eat" strawberries. *E. coli* was recovered only from fruits harvested immediately following ACT application. Berries that were harvested four days after ACT application did not contain any culturable *E. coli* from the fruit surfaces. While more research is warranted to determine the extent to which contaminated CT may introduce pathogens into a variety of produce farming operations, it is clear from this study that the extracted microbiota, organic matter, and other constituents extracted from compost did *not* enhance the ability of *E. coli* to survive on the surfaces of strawberries in field conditions.

Given our current understanding of the transient fitness of foodborne pathogens to the epiphytic growth and survival on fresh produce and the increasing frequency with which produce is implicated in foodborne outbreaks, it is paramount to prevent produce contamination *at the source*. This emphasis on safety means that regulators should focus on farming practices where and when produce is cultivated.

Epidemiological evidence linking compost or compost teas to outbreaks involving produce is sorely lacking, but this research suggests that the introduction of compost and compost teas may indeed be a significant source of on-farm introduction of foodborne pathogens.

Although practical suggestions are made as part of this research to reduce the incidence of pathogens in compost and compost teas, reliance on manufacturing process standards for the production of these two organic inputs cannot realistically guarantee pathogen-free products. Routine microbiological testing of compost and compost tea products should become standard practice to provide stronger assurance that only high quality, and truly pathogen-free organic products are introduced into all plant production systems.

APPENDIX A

Testing Methods for the Examination of Composting and Compost (TMECC)

The methods included in this appendix were authored by David T. Ingram for inclusion in the TMECC manual for the U.S. Composting Council and are under review for publication by the Government Printing Office (GPO) : Section 1a (Coliforms), 1b(Fecal Coliforms), 1c(*Escherichia coli*), Section 2 (*Salmonella*), Section 3 (enterococci).

The TMECC manual is available for purchase in CD-ROM format:
Thompson, W., P Leege, P Millner, M Watson. 2002. Test Methods for the Examination of Composts and Composting, 500 pp.: CD-ROM by U.S. Compost Council.
(http://www.compostingcouncil.org)

Referenced Documents

TMECC:
TMECC Method 02.09-A Total Solids and Moisture at 70±5°C

Other Sources:
Eaton, A.D., L.S. Clesceri, and A.E. Greengerg, Standard Methods for the Examination of Water and Wastewater. 19th ed., ed. A.E. Greenberg. 1995, Washington, D.C.: American Public Health Association. 539.

Murray, P.R., Medical Microbiology. 3rd ed. 1998, St. Louis, MO. Mosby. x, 719.

Joklik, W.K., *et al*., Zinsser Microbiology. 20th ed. 1992, Norwalk: Appleton & Lange. 1294

1A; 1B; 1C: Coliform Bacteria

This section covers detection and quantification techniques for coliform bacteria.

Method 1A Total Coliforms.
Method 1B Fecal Coliforms.
Method 1C Escherichia coli.

The methodologies described in this section do not purport to address all safety concerns, if any, associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use. Aseptic techniques and sterile materials and apparatus should be used throughout all methods in this section.

Terminology

coliform, n—a lactose-fermenting member of the family Enterobacteriaceae. commonly associated with the intestinal tract of animals, including humans, fish, birds and insects. However, many are also known and reported to be free-living in the environment and associated with plants and soil. While most coliforms are not medically significant, all are opportunistic pathogens and able to cause disease in the very young and old, and immunologically compromised individuals.

fecal coliforms, n—indicator organisms for fecal pathogens; a subset of coliforms (lactose-fermenting enterics) that are considered to be associated almost exclusively with the intestinal tracts of animals and insects (few strains are associated with plant material).

Escherichia coli, n—the classical example of a fecal coliform; found in feces from all animals, hence its presence in compost is evidence of fecal contamination. Most strains of *E. coli* are opportunistic pathogens and are unable to cause disease in healthy humans. However, some strains are pathogenic. Good compost manufacturing procedures are able to reduce the numbers of *E. coli* in the final product to a level that will protect public health and the environment. Specific time-temperature conditions for the various methods of composting must be met in order to achieve pathogen reduction and satisfy any standard limits that jurisdictions may impose.

Indicator organism, n—microbes that are generally not pathogenic, but co-exist in habitats with pathogens. Detection and quantification of an indicator organism in a sample is presumptive evidence that pathogens may also be present in the habitat from which the sample was obtained. Detection and quantification of indicator organisms is often much simpler and less costly than detecting/quantifying specific pathogens.

Summary of Test Methods

Method 1A Total Coliforms—The method described herein will determine the presence and quantity of total coliforms in a compost sample. Using a combination of traditional culture methods, spread plating and a Most Probable Number method (MPN), this system has the ability to quantify coliforms over a very broad range of numbers (<3.6 MPN g^{-1} to 10^6 cfu g^{-1}) while surpassing several inherent limitations associated with each method. A brief discussion may be found in the Interference and Limitations section of this manuscript (Section 6.1)

Method 1B Fecal Coliforms—The method described herein will determine the presence and quantity of fecal coliforms in compost. Using a combination of traditional culture methods, spread plating and a Most Probable Number method (MPN), this system has the ability to quantify fecal coliforms over a very broad range of numbers (<3.6 MPN g^{-1} to 10^6 cfu g^{-1}) while surpassing several inherent limitations associated with each method. A brief discussion may be found in the Interference and Limitations section of this manuscript (Section 6.2)

Method 1C <u>*Escherichia coli*</u>—This method described herein will determine the presence and quantity of *E. coli* in compost. Using a combination of traditional culture methods, spread plating and a Most Probable Number method (MPN), this system has the ability to quantify fecal coliforms over a very broad range of numbers (<3.6 MPN g^{-1} to 10^6 cfu g^{-1}) while surpassing several inherent limitations associated with each method. A brief discussion may be found in the Interference and Limitations section of this manuscript (Section 6.3)

Significance and Use

Method 1A Total Coliforms—The detection of coliforms indicates the possible presence of enteric pathogens. A finding of total coliforms indicates that the compost does not contain growth inhibitors for enteric bacteria. Many coliforms are not exclusively associated with fecal material; their presence should not be used or interpreted as an indication of the presence of pathogens. Lauryl Tryptone Broth (LT) is used as a non-selective, resuscitative medium for quantification of total coliforms in the coliform MPN (detects <3.6 – 1,100 MPN g-1). MacConkey's agar is used as a selective and differential medium for the quantification of total coliforms when incubated at 35C for 18-24hr. Spread plate counts may provide quantification information between 10^3 – 10^6 cfu g^{-1}.

Method 1B Fecal Coliforms—Fecal coliforms are indicators of fecal contamination. A finding of fecal coliforms, which are 'indicator organisms', implies that pathogens may be present in the sample. Fecal coliforms have the distinction of growing and surviving at higher temperatures and in the presence of bile salts than other coliforms. The EC-MUG medium is the selective medium used in the fecal coliform MPN (1B), which contains bile salts and is incubated at $44.5^{+/-}$0.2C to enhance the selectivity. Duplicate MacConkey's agar may also be used to estimate fecal coliform cfu when incubated at $44.5^{+/-}$0.2C. Fecal coliforms are significantly reduced during the thermophilic phase of the composting process. Fecal coliforms are quantified in the finished compost to show that the recommended composting temperatures have been achieved, and that potential enteric pathogens have been killed. The mere presence of fecal coliforms does not indicate that the compost sample is dangerous or unfit for use. For example, amounts of fecal coliforms up to 2,000,000 MPN per gram total solids in biosolids compost are considered by the US EPA (40CFR Part 503) to be acceptable for land application at remote agricultural sites where public access is restricted and specific management practices are observed. For compost to be distributed to the general public, fecal coliforms should not exceed 1,000 MPN per gram total solids in the final product.

Method 1C <u>*Escherichia coli*</u>—*E. coli* is the predominant fecal coliform found in human and animal fecal matter. Its presence indicates the potential presence of enteric pathogens. A finding of *E. coli* in compost must be interpreted in the context of the concentration. The presence of trace amounts of *E. coli* can sometimes be found in 'finished' compost that has been properly processed. However, trace amounts in a few samples simply may indicate that contamination (from birds or

other wildlife) may have occurred after the compost completed proper temperature cycling. The *E. coli* MPN (1C) system is performed using same medium provided by the fecal coliform MPN (1B). Simply observing the positive fecal coliform MPN tubes under a long-wave ultraviolet (465nm)) light source will determine if *E. coli* is present. *E. coli* produces a fluorogenic compound by cleaving the substrate 4-methyllumbelliferyl-beta-D-glucuronide (MUG).

Limitations

Method 1A Total Coliforms—Most Probable Number (MPN) methods have several limitations including the requirement of time, effort and equipment required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, i.e., MacConkey's agar, has been reported to be inefficient in growing organisms that have been injured or are described as being viable but not culturable. The simultaneous strategy proposed in these methods of using both a limited MPN and spread plates was designed to avoid the massive equipment required for the MPN while eliminating the low sensitivity and cultivability problems with spread plates.

Method 1B Fecal Coliforms—Most Probable Number (MPN) methods have several limitations including the requirement of time, effort and equipment required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, i.e., MacConkey's agar, has been reported to be inefficient in growing organisms that have been injured or are viable but not culturable, especially when attempting to grow them at 44.5^{O}C. The simultaneous strategy proposed in these methods of using both a limited MPN and spread plates was designed to avoid the massive equipment required for the MPN while eliminating the low sensitivity and cultivability problems with spread plates. The fecal coliform MPN uses Lauryl-Tryptone broth (LT, from method 1A) as a non-selective enrichment prior to inoculating the sample in EC-MUG, a selective and differential medium for fecal coliforms and *E. coli.* These techniques allow the resuscitation of any injured organisms as well as eliminate the possibility of including auto-fluorescent materials into the EC-MUG medium, which would interfere with the interpretations of the test.

Method 1C <u>Escherichia coli</u>— This test, which is a supplemental method to the fecal coliform method, is based on the ability of *E. coli* to produce the enzyme β-glucuronidase (GUD). 94% of *E. coli* have been reported to produce GUD. GUD cleaves the substrate 4-methylumbelliferone-β-D-Glucuronide (MUG), producing 4-methylumbelliferone (MU) which is fluorescent under long wave ultraviolet light (465nm). A limitation of this method includes the possibility 'counting' false positive organisms based solely on the ability to fluorescence. However, most fluorescent organisms (other than *E. coli*) will not grow under the growth conditions and growth medium described in the methods (i.e. EC-MUG medium at $44.5^{+/-}$0.2C). It must be noted that this method will not detect *E. coli* O157:H7, a serotype responsible for significant foodborne illnesses. *E. coli* O157:H7 has been reported to

be sensitive to elevated temperatures (e.g. 44.5C, and does not produce the GUD enzyme required to make the medium fluoresce.

Sample Handling

Samples at as-received moisture content are used for these tests. Moisture analysis of a parallel sample aliquot must be conducted so that data can be calculated and reported on a dry weight basis (refer to Method 03.09 Total Solids and Moisture). If delays in analysis are anticipated, store compost samples in sealed containers at approximately 4°C. Large compost samples must be homogenized and mixed thoroughly before the subsamples for microbial analysis are collected. Thorough mixing helps overcome heterogeneous distribution of microbes.

1A Total Coliforms

Reagents and Materials:

Most Probable Number Technique:
culture tubes—16-mm × 150-mm, screw-top tubes (e.g., Fisherbrand).
dilution tubes—16-mm × 150-mm, screw-top tubes filled with 9ml Buffered Peptone Water (BPW)
incubator—set at 36 ± 1°C.
inverted gas tubes—6-mm × 50-mm, (e.g., Fisherbrand).
strainer bag—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).
buffered peptone water—BPW, (e.g., Difco, Becton Dickinson).
Lauryl-Tryptose broth—LT, (e.g., Difco, Becton Dickinson).

Spiral Plating:

spiral-plating machine—optional alternative to conventional spread plating method, (e.g., Spiral Biotech).
agar plates—MacConkey's Agar, (e.g. Difco)

Procedure for Most Probable Number Technique:

Prepare 10^{-1} Homogenate—Place 25 g of compost into sterile stomacher bag. Bring weight up to 225 g with the addition of buffered peptone water (BPW) for a 1:10 dilution (10^{-1}).
Homogenize for 60 sec at 260 rpm on a Stomacher machine or manually massaging for two minutes.
Prepare three additional dilutions by performing three 1:10 serial dilutions in sterile BPW. This can be done by adding 1 mL sample homogenate (10^{-1}) to 9 mL BPW, vortexing for 5-10 sec, and continuing this dilution scheme two more times.
Prepare nine screw-top culture tubes, each containing 9 mL sterile Lauryl-Tryptose broth (LT) each containing an inverted gas tube.
Aseptically transfer 1 mL of the 1:10 (10^{-1}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.
Aseptically transfer 1 mL of the 1:100(10^{-2}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.
Aseptically transfer 1 mL of the 1:1000 (10^{-3}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile LT.
Incubate tubes for 24 h to 48 h in a 36° ± 1 °C incubator.
Observe the inverted gas tubes for the presence of small air bubbles. Gas formation indicates a 'positive' result for lactose fermentation, and is therefore a positive result for a coliform. Record the number of tubes in each dilution set that is positive for gas formation. This number will be used to calculate the MPN g^{-1} (Most Probable Number per gram sample) for total coliforms

Procedure for Spiral Plating:

Prepare two MacConkey's (MAC) agar plates. Air-dry the surface of the plates by maintaining them covered at room temperature for one day, or place into a laminar flow hood for 10 minutes with the lids ajar.
Plate 100µl from the 10^{-2} dilution used during the MPN protocol above onto each MAC plate. Incubate for 18 to 24 h at 36 +/- 1^{O}C.

Observe the agar surface for colonies that are bright pink or red. These colonies are considered to be coliforms (gram negative, lactose fermenting members of the family Enterobacteriaceae).

Calculations

Most Probable Number technique— Record the number of positive tubes in each dilution set, and compute the MPN Score using standard MPN tables or MPN software (The freeware MPN calculator (VB6 version; Michael Curiale) (www.i2workout.com/mcuriale/mpn/index.html). Adjust the MPN g^{-1} score to reflect the sample on a dry weight (dw) basis. For example, if there were two tubes positive in the 10-1 dilution set, one tube positive in the 10-2 dilution set, and zero tubes positive in the 10-3 dilution set, the MPN score would be '2-1-0', reflecting an MPN score of 7 MPN g^{-1}.

Quantify the total coliforms as colony forming units (cfu g^{-1}) using the protocols included for the spiral-plater.

Depending on the concentration of coliforms in the sample, results will be reported in either cfu g^{-1} (via spread plating technique) or MPN g^{-1} (via Most Probable Number technique); or both.. Results should be reported on a dry weight (dw) basis for all quantification tests.

1B Fecal Coliforms

Reagents and Materials

Most Probable Number Technique:
culture tubes—16- x 150-mm, screw-top tubes (e.g., Fisherbrand).
dilution tubes.
incubator—set at 44.5ºC.
inverted gas tubes—6- x 50-mm, (e.g., Fisherbrand).
strainer bag—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).
buffered peptone water—BPW, (e.g., Difco, Becton Dickinson).
EC-MUG—E. Coli Medium plus 4-methylumbelliferone-β-D-Glucuronide (e.g., Difco, Becton Dickinson).

Spiral Plating:
spiral-plating machine—optional, (e.g., Spiral Biotech).
agar plate—MacConkey's, (e.g. Difco, Becton Dickinson).

Procedure

Most Probable Number Technique:

Prepare nine screw-top culture tubes, each containing 9 mL sterile *E. Coli* Medium plus MUG (EC-MUG).
For each positive LT tube (*from method 1A*), aseptically transfer 20µl - 40µl into a culture tube containing 9ml EC-MUG.
Incubate all EC-MUG tubes at 44.5 +/- 0.2^{O}C for 18 to 24 h.
Observe the EC-MUG gas tubes for presence of air bubbles. Gas formation indicates a 'positive' result from lactose fermentation. All EC-MUG tubes that contain gas are considered POSITIVE for growth of fecal coliforms. Record the number of positive tubes in each dilution, as this number will be used to calculate the MPN g^{-1} for fecal coliforms.

Spiral Plating Technique:

Prepare two MacConkey's (MAC) agar plates Air dry the surface of the plates by maintaining them at room temperature for one day, or place into a laminar flow hood for 10 minutes with the lids removed.
Plate 100µl from the 10^{-2} sample dilution. Incubate for 18 to 24 h at 44.5 +/- 0.2^{o}C.
Observe the agar surface for colonies that are bright pink or red and that have a hazy appearance. These colonies are considered to be fecal coliforms (gram negative, lactose fermenting members of the family Enterobacteriaceae that grow at 44.5C and tolerate bile salts. Depending on the concentration of fecal coliforms in the sample, results will be reported in either cfu g^{-1} (via spread plating technique) or MPN g^{-1} (via Most Probable Number technique); or both.. Results should be reported on a dry weight (dw) basis for all quantification tests.

1C *Escherichia coli*

Reagents and Materials:

culture tubes—16- x 150-mm, screw-top tubes (e.g., Fisherbrand).
dilution tubes
incubator—set at 35 to 37ºC.
inverted gas tubes—6- x 50-mm, (e.g., Fisherbrand).
strainer bag—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).
EC-MUG—E. Coli Medium plus MUG, (e.g., Difco, Becton Dickinson).
MacConkey's Agar (MAC-MUG).
Eosin-Methylene Blue Agar (EMB, Difco, Becton Dickinson) plates.
Indole reagent (DIFCO, Becton Dickinson).
Triple Sugar Iron Agar (TSI, Difco) slant.
Motility Indole Lysine Agar (MIL) deep.

Procedure

Most Probable Number Technique:
Observe the EC-MUG tubes (from Method 1B, fecal coliform MPN) under long wave ultraviolet light (~465nm). Any tube that fluoresces AND that contains gas in the inverted gas tube is considered POSITIVE for *Escherichia coli.* Note the number of tubes that fluoresce AND contain gas in each dilution. This number will be used to calculate the MPN g^{-1} for presumptive *Escherichia coli*.
Each presumptive positive tube for *Escherichia coli* must be biochemically confirmed.

Biochemical Confirmation of *Escherichia coli*

Prepare three MacConkey's Agar containing MUG (MAC-MUG) and three Eosin-Methylene Blue Agar (EMB) plates. Use one MAC and one EMB plate per each dilution set. Divide each plate into three sections, labeled A, B and C so that each tube within each dilution set has a corresponding section on both EMB and MAC plates. Using a sterile loop, remove one loopful of culture from each positive EC-MUG tube and, using the same loop, streak for isolation on both identically labeled MAC-MUG and EMB sections.
Incubate EMB plates at 36+/-1^{o}Cfor 18 to 24 h, and MAC-MUG plates at 44.5C for 18-24hr.
.
Observe growth—Escherichia coli produces a deep pink coloration on MAC plates, and the medium surrounding this culture should have a 'fuzzy' pink appearance due to the precipitation of bile salts and low pH (due to lactose fermentation). Fluorescence of the growth on MAC-MUG plates under long wave ultraviolet radiation indicates presence of *E. coli*. Growth on EMB should be metallic green within 18-24hr, but can also appear dark purple.

Prepare one Triple Sugar Iron Agar (TSI) slant one Motility Indole Lysine Agar (MIL) deep for each isolate to be tested. Pick three colonies from the MAC plate that are pink, have precipitated bile salts AND that have a corresponding sector of EMB that has a metallic green sheen (or appears dark purple).. Using the same needle for each medium, streak the surface and then stab the bottom of the TSI tube, then stab the MIL tube twice. Incubate the TSI and MIL tubes for 18-24 hours at 36 +/- 1^{o}C.5-37C. Place two drops of Indole reagent (DIFCO, Becton Dickinson) onto the surface of each MIL tubes (DO NOT SHAKE THE TUBE).

Escherichia coli should exhibit the following biochemical characteristics:

TSI—Acid Slant (A/Yellow), Acid Butt (A/Yellow), Gas production (bubbles) throughout the medium.

MIL—Basic Slant (K/Purple), Basic Butt (K/Purple), Motility (medium is cloudy), and Indole production (Red band at the top of the tube, after the addition of Kovac's Reagent).

E. coli will be reported via the MPN score (MPN g^{-1}). Results should be reported on a dry weight (dw) basis for all quantification tests. Report only those tests that have been confirmed biochemically for the presence of *E. coli.*

2 *SALMONELLA*

This test covers the detection and quantification techniques for *Salmonella* spp. in compost samples

Method 2A and 2B Enrichment and quantification of *Salmonella* spp. in compost samples, respectively.

Method 2C Confirmation Protocols for presumptive *Salmonella* isolates.

The methodologies described in this section do not purport to address all safety concerns, if any, associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use. Aseptic techniques and sterile materials and apparatus should be used throughout all methods in this section.

Terminology

Salmonella, n—Any of various rod-shaped bacteria of the genus *Salmonella*, all of which are pathogenic to humans and animals. Salmonellosis includes a wide variety of pathologies, including: foodborne infection, typhoid, and paratyphoid fever.

Enrichment procedure—Because of the time, effort and expense of the quantification method, a preliminary detection screening analysis is first conducted to rule out 'negative' samples prior to processing the samples through the quantification system. The first step is to perform an enrichment procedure to detect a single cell of viable *Salmonella* in a 25-g sample (Detection limit: 0.04 cfu g^{-1}). If any viable cells are recovered and confirmed as salmonellae, then an additional 25 g is used to quantify how many cells per gram of salmonellae are in the sample.

Quantification procedure—This strategy uses a three-tube Most Probable Number (MPN) for quantifying low numbers (3.6 – 1,100 cfu g^{-1}), as well as a spiral plating technique that can quantify larger numbers (2×10^3 cfu g^{-1} to 2×10^8 cfu g^{-1}) of *Salmonella* spp. in compost. The MPN is a robust system for resuscitating, selectively enriching, and simultaneously quantifying *Salmonella* spp. in compost. It is a five-day procedure. Spread plating requires only two days of labor (one day to perform and a second day to confirm isolates). The spread plating technique is not as robust as the MPN method because organisms that have been injured often are inhibited on plating medium. The MPN system can be adapted to capture larger numbers of organisms by adding more dilutions and more tubes. This would be a labor and time-intensive solution for processing many samples, so the MPN system is limited to a 3-tube system to capture lower numbers of organisms while the spread plating system will capture larger numbers of organisms.

Significance and Use

All species of *Salmonella* are considered true pathogens. That is, any viable cells of *Salmonella* spp. found in the finished compost are *potentially* pathogenic to animals or humans. Because *Salmonella* spp. is among the leading causes of foodborne illness in the United States, any potential sources of food-supply contamination should be examined and eliminated. This is the overall rationale behind the strict standards (< 3 salmonellae per 4 g of total solids (dry weight) that the US EPA has established for public distribution of composted biosolids.

Salmonella spp. are very susceptible to heat and other environmental stressors such as low moisture and low water activity (A_w). For these reasons, we can conclude that the presence of viable salmonellae in finished compost indicates that the compost has not been properly heat pasteurized, or that the 'finished' compost has been re-inoculated from some outside source, (e.g. rodent or other pest droppings or transferred from contaminated equipment).

It is important to note that finding salmonellae in compost does not imply that the compost is hazardous. The amounts present and the manner in which compost will be distributed and used are important determinants in the outcome. The presence of this pathogen in soil or composts must be considered carefully when deciding to use the product in ways that may lead to its contact with water or foods since these are two pathways that can lead to disease

In the case of post-consumer cafeteria residues, the potential exists for pathogens to have entered the residuals stream; these must be destroyed or reduced to virtually undetectable levels via the composting process in order for the compost to be distributed and used by the general public (this would include use in bulk landscaping situations where the public may come into contact with the product). All these conditions are practices put in place to protect public health and the environment from contact with pathogens. Several conditions must be met in order for disease to occur: 1) The pathogen must reach a susceptible host; 2) The pathogen must be ingested in sufficient quantity to cause disease; and 3) the person must be susceptible to the amount ingested. For salmonellae, thousands of cells are needed in order for the organism to cause disease in humans.

Compost samples that are 'positive' for salmonellae contamination should be re-composted, re-tested, and determined to meet the US EPA standard before release or sale to the general public or before use in production of fresh market vegetables and fruits that might touch the compost during growth.

Sample Handling

Samples at as-received moisture content are used for this test. If delays in isolation are anticipated, store compost samples in sealed containers at approximately 4°C. Because of the high ratio of coliform bacteria to pathogens, large compost samples (1 L or 1,000 cm^3) are required.

Incubator—convection, capable of maintaining 37±0.5°C.
Glassware—autoclavable.
Pipettes—disposable, sterile, 1 and 10 mL.
Stomacher bag, (e.g., Model 400C, Seward Medical).
Conical tube—50-mL, (e.g., Bluemax, Corning).
Vortex mixer
Spiral-Plating Machine—Optional, (e.g. Spiral Biotech).
Reagents, Materials and Media for Method B
Buffered Peptone Water, (e.g., Difco, Becton Dickinson).
Tetrathionate Broth, (e.g., TT-Hajna, Difco).
Xylose-Lysine Tergitol 4 agar, (e.g., XLT4, Difco).
Culture Tubes—screw top, 16- × 150-mm, (e.g., Fisherbrand).

Enrichment Procedure for Method A

Weigh a 25 g sample (as-received basis) directly into a sterile stomacher bag.
Add 225 mL of Buffered Peptone Water and blend at 260 rpm for 60 sec.
Place bag into a convection 35ºC incubator for 18 to 24 h.
Manually homogenize the bag by gently shaking and massaging.
Aseptically transfer 5 mL homogenate into a sterile 50-mL conical tube.
Add 45 mL Tetrathionate Broth (Hajna formulation) and vortex for 10 sec.
Place tube into a 35ºC incubator for 18 to 24 h.
Vortex the tube for 5 to 10 sec, and aseptically transfer two loopfuls onto XLT4 agar. Streak for isolation.
Incubate XLT4 plates for 24 h at 35ºC. If no black colonies are seen, the plates are then incubated for an additional 24 h (total of 48 h).

Presumptive Positive—All red colonies, red colonies with black centers, and black colonies are considered presumptive positive *Salmonella* spp.
Pick three [3] presumptive positive colonies from the XLT4 plates and perform the biochemical and serological confirmation procedure (2C).

Quantification Procedure for Method B

Most Probable Number System
Place 25 g compost into a sterile stomacher strainer bag. Bring the weight up to 225 g with approximately Buffered Peptone Water (BPW). This prepares a 1:10 dilution of the sample.
Homogenize using a Stomacher machine at 260 rpm for one minute or manually massaging for two minutes.
Prepare 1:100 and a 1:1000 dilution of the original sample homogenate (1:10) each in 9 mL BPW dilution blanks.
Aseptically transfer 1 mL of the 1:10 sample homogenate into each of three screw top culture tubes containing 9 ml sterile BPW.

Aseptically transfer 1 mL of the 1:100 dilution into each of three screw top culture tubes containing 9 mL sterile BPW.
Aseptically transfer 1 mL of the 1:1000 dilution into each of three screw top culture tubes containing 9 mL sterile BPW.
Incubate all 9 mL tubes in a 35-37ºC incubator for 18-24 hours.
Vortex each 9 mL culture tube and aseptically transfer 1 mL from each into a 9 mL culture tube containing Tetrathionate (TT-Hajna) broth.
Vortex and incubate the TT tubes overnight for 18 to 24 h at 35 to 37°C.
Vortex each tube. Transfer a loopful of enrichment from each tube onto a sterile, surface-dried XLT4 agar plate.
Incubate XLT4 overnight for 24-48 h.

Follow the Serological and Biochemical Confirmation protocol (Method 2C) on three presumptive positive isolates from each plate. Calculate the MPN g^{-1} score using only data from tubes containing confirmed isolates.

Calculations for Method B:

Most Probable Number Technique: Record the number of positive tubes (biochemically confirmed from Method C) in each dilution set, and compute the Most Probable Number (MPN) using standard MPN tables or MPN software (The freeware MPN calculator (VB6 version; Michael Curiale) (www.i2workout.com/mcuriale/mpn/index.html). Adjust the MPN g^{-1} score to reflect the sample on a dry weight (dw) basis.

Spiral plating technique: Quantify the total presumptive positive salmonellae cfu g^{-1} using the protocols included for the model spiral-plater used.

Incubator—convection, capable of maintaining 37±0.5°C.
Inoculation Loops
Reagents, Materials and Media for Method C
Biochemical/Serological Procedure
Prepare two-biochemical media: Triple Sugar Iron Agar (e.g., TSI, DIFCO) and Motility Indole Lysine Agar (e.g., MIL, DIFCO). Both can be prepared according to the manufacturers instructions, and in screw-top culture tubes. TSI should be made into slants with a generous slant on the top of the tube. MIL should be made into agar deeps (enhancing oxygen deprivation). Prepare each medium according to the manufacturers instructions, (e.g., Difco, Becton Dickinson).
Kovac's reagent, (e.g., Difco, Becton Dickinson).
poly-O *Salmonella* antiserum, (e.g., Difco, Becton Dickinson).

Biochemical Serological Confirmation Protocol for *Salmonella* spp.
Pick a colony of presumptive salmonellae from the XLT4 plate using a sterile inoculation needle and inoculate the medium in the following manner: Inoculate sugar iron agar (TSI) by first streaking the slant, then stabbing into the solid non-slant end of the agar deeper in the tube (the area known as the butt of the agar slant). With the same needle, stab the butt of the motility indole lysine agar (MIL) deep twice. Incubate both TSI and MIL tubes for 18hr at 35°C.
Observe coloration of the medium. Presumptive salmonellae on TSI will have an acid butt (yellow) and basic slant (red). Many will also produce Hydrogen Sulfide, which will be present as a black coloration in the butt. This color will often mask the yellow coloration in the butt. Presumptive salmonellae on MIL will exhibit a purple coloration on both top and bottom of the tube, and salmonellae will also have swarmed throughout the medium (the stab-line should not be visible). Add two drops of Kovac's reagent. Salmonellae do not produce Indole, as exhibited by a red-band formation on the surface of the medium after the addition Kovac's reagent.

For each presumptive positive isolate, perform serology using a slide-agglutination technique by using the cell material from the TSI slant.. This is performed using poly-O *Salmonella* antiserum as well as specific antiserum groups A-E, and is performed according to the manufacturer's instructions.

NOTE—Salmonellae remain presumptive positive until the antiserum is used to confirm the isolates.

3. Enterococci

This test covers the determinations for fecal streptococci and enterococci in compost . *Method 07.03-A Enterococcus.*

The methodologies described in this section do not purport to address all safety concerns, if any, associated with their use. It is the responsibility of the user of these methods to establish appropriate safety and health practices, and to determine the applicability of regulatory limitations prior to their use. Aseptic techniques and sterile materials and apparatus should be used throughout the method. Aseptic techniques and sterile materials and apparatus should be used throughout all methods in this section.

Enterococci, n pl—Gram-positive bacteria that were formerly classified as Group 'D' streptococci. In 1984, several members of the Group 'D' streptococci were reclassified as a new genus, *Enterococcus*, for clinical reasons. Enterococci now represent a small portion of organisms that constitute the fecal streptococci. Enterococci may be used as 'indicator organisms' for fecal contamination in the same way that Group 'D' streptococci were. Enterococci, or 'enteric cocci', are commonly found in fecal material of humans and a variety of animals. These organisms can survive harsh conditions for longer periods of time in the environment than either total or fecal coliforms, *E. coli* or salmonellae. For example, enterococcus can grow in the presence of 6.5% sodium chloride and at 45°C, and also survive at temperatures as high as 60°C.

indicator organisms, n—Microbes that are generally not pathogenic, but co-exist in habitats with pathogens. Detection and quantification of an indicator organism in a sample is presumptive evidence that pathogens may also be present in the habitat from which the sample was obtained. Detection and quantification of indicator organisms is often much simpler and less costly than detecting/quantifying specific pathogens.

Summary of Test Method

Methods described in the Standard Methods for the Examination of Water and Wastewater were adapted for the quantification of *Enterococcus* in compost. The fecal streptococci/enterococci quantification method combines traditional spread plating techniques with an MPN system for more rapid and sensitive quantification. These methods may be easily performed simultaneously with the *Salmonella* and coliform bacteria protocols. Modified *Enterococcus* agar (mEnt) is a presumptive positive medium for *Enterococci*, and the plates may be counted after 48 h of incubation at 35ºC.

Significance and Use

Enterococci are indicator organisms of fecal contamination because they colonize the colon of humans and animals, generally without infection. These organisms and are shed in feces. Enterococcus has become well recognized in the 1990's for its ability to cause life-threatening infections, especially in patients with urinary or intravascular catheters, with intra-abdominal abscesses and in patients that have received broad-spectrum antibiotics; it is the fourth leading cause of hospital-acquired infections. The most significant current concern with *Enterococcus* is its resistance to conventional antibiotics, and the transmission of this resistance to other (more virulent) organisms.

Interference and Limitations

Most Probable Number (MPN) methods have several limitations including the requirement of time, effort and equipment required to handle large quantities of materials per sample. Direct plating onto very selective and differential media, i.e., Modified Enterococcus agar, has been reported to be inefficient in growing organisms that have been injured or are described as being viable but not culturable. The simultaneous strategy proposed in these methods of using both a limited MPN and spread plates was designed to avoid the massive equipment required for the MPN while eliminating the low sensitivity and cultivability problems with spread plates.

Sample Handling

Samples at as-received moisture content are used for this test. Moisture analysis of a parallel sample aliquot must be conducted so that data can be calculated and reported on a dry weight basis. If delays in analysis are anticipated, store compost samples in sealed containers at approximately 4°C. Large compost samples must be homogenized and mixed thoroughly before the subsamples for microbial analysis are collected. Thorough mixing helps overcome heterogeneous distribution of microbes.

Most Probable Number Technique:
culture tubes—16-mm × 150-mm, screw-top tubes, (e.g., Fisherbrand).
dilution tubes
incubator—set at 35 to 37ºC.
inverted gas tubes—6-mm × 50-mm, (e.g., Fisherbrand).
strainer bag—sterile stomacher bag, (e.g., Stomacher Model 400C, Seward Medical).

Spread Plating Technique:
spiral-plating machine—optional, (e.g., Spiral Biotech).
Reagents and Materials for Method A
Most Probable Number Technique:
Buffered Peptone Water—BPW, (e.g., Difco).
Modified Enterococcus Agar—mEnt, (e.g., Difco).

Brain Heart Infusion—BHI, modified broth containing additional sodium chloride (total 6.5% NaCl).
Azide Dextrose Broth—AD Broth, (e.g., Difco).
Spread Plating:
ethanol—70% for sterilization.
Modified Enterococcus Agar –mEnt, (e.g.,Difco).
Procedure for Method A
NOTE 1A—This method can be performed simultaneously with the coliform (TMECC 07.01) and *Salmonella* (TMECC 07.02-B) protocols.
Prepare 10^{-1} Homogenate—Place 25 g of compost into sterile stomacher bag. Bring weight up to 225 g with the addition of buffered peptone water (BPW) for a 1:10 dilution (w:w).
Homogenize by stomaching on a Seward 400C Stomacher machine for 60 sec at 260 rpm. (Note: A sterile laboratory blender may also be used, on 'high' setting for one minute)
Most Probable Number (MPN) Technique: Prepare two additional dilutions by performing three 1:10 serial dilutions in sterile BPW containing, respectively, 10^{-2} and 10^{-3} dilutions of the original compost sample. This can be done by adding 1 mL of the original sample homogenate (10^{-1}) to 9 mL BPW, vortexing for 5-10 sec, and continuing this dilution scheme once more. These will be used to inoculate the MPN tubes described below.

Prepare nine screw-top culture tubes, each containing 9 mL sterile Azide Dextrose broth (AD).

Aseptically transfer 1 mL of the 1:10 (10^{-1}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.
Aseptically transfer 1 mL of the 1:100 (10^{-2}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.
Aseptically transfer 1 mL of the 1:1000 (10^{-3}) sample homogenate into each of three screw-top culture tubes containing 9 mL sterile AD.

Incubate tubes for 24 h in a 35ºC – 37ºC incubator.

Observe the AD tubes for presence of growth (turbidity).Vortex each tube and streak (one loopful) onto the surface of modified Enterococcus Agar plate (mEnt). Simultaneously pipet 20-40µL from each tube into 9mL Brain Heart Infusion Broth containing 6.5% Sodium Chloride (NaCl).

Incubate mEnt plates for 24 – 48 h in a 35ºC – 37ºC Incubator. Record the number of plates in each dilution set that are positive for growth. This number will be used to calculate the MPN g^{-1} (Most Probable Number per gram sample) for fecal streptococci, which includes enterococci.
Incubate the BHI + 6.5% NaCl tubes in a 35ºC – 37ºC for 24 h. Record the number of tubes in each dilution set that are positive for growth. This number will be used to calculate the MPN g^{-1} (Most Probable Number per gram sample) for Enterococci.

Spiral Plating technique:

Prepare two modified Enterococcus Agar (mEnt) plates. Air dry the surface of the plates by maintaining them at room temperature for one day, or place into a laminar flow hood for 10 min with the lids removed.

Using an automated spiral-plating machine, plate 100 µL from the 10^{-2} dilution prepared during the MPN protocol onto two mEnt plates. Incubate for 48 h at 35 to 37ºC.

Observe the agar surface for growth of all colonies, which may appear red, purple or absent of color. All surface growth is considered to be enterococcus.

Calculations

Most Probable Number technique—Record the number of positive tubes in each dilution set, and compute the Most Probable Number (MPN) using the standard tables or MPN calculation software. Adjust the MPN g^{-1} score to reflect the sample on a dry weight (dw) basis.
Spiral-plater protocols—Quantify the enterococci using protocols specific for the model spiral-plater. Adjust the cfu g^{-1} score to reflect the sample on a dry weight (dw) basis

Depending on the concentration of enterococci in the sample, results will be reported in either cfu g^{-1} (via spread plating technique) or MPN g^{-1} (via Most Probable Number technique); or both.. Results should be reported on a dry weight (dw) basis for all quantification tests.

REFERENCES

1. **Abbasi, S. A., and E. V. Ramasamy.** 1999. Biotechnological methods of pollution control, vol. Orient Longman (Universities Press India, Ltd.), Hyderabad, India.

2. **Al-Dahmani, J. H., S. A. Miller, and H. A. J. Hoitink.** 2003. Suppression of bacterial spot of tomato with foliar sprays of compost extracts under greenhouse and field conditions Plant Dis **87:**913-919.

3. **Alam, M. J., and L. Zurek.** 2006. Seasonal prevalence of *Escherichia coli* O157:H7 in beef cattle feces. J Food Prot **69:**3018-20.

4. **Andrews, J. H., and S. S. Hirano.** 1991. Microbial ecology of leaves, vol. 1. Springer-Verlag, New York.

5. **Arancon, N. Q., C. A. Edwards, P. PBierman, C. Welch, and J. D. Metzger.** 2004. Influences of vermicomposts on field strawberries: 1. Effects on growth and yields. Bioresource Technology **93:**145-153.

6. **Bailey, K. L., and G. Lazorovits.** 2003. Suppressing soil-borne diseases with residues management and organic amendments. Soil and Tillage Res **72:**169-180.

7. **Barakan, F. N., S. H. Salem, A. M. Heggo, and M. A. Bin-Shiha.** 1995. Activities of rhizosphere microorganisms as affected by application of organic amendments in a calcareous loamy soil. 2. Nitrogen transformation. Arid Soil Res and Rehab **9:**467-480.

8. **Beck-Friis, B., S. Smars, H. Johnsson, and H. Kirchmann.** 2001. Emissions of carbon dioxide, ammonia and nitrous oxide from organic household waste in a compost reactor under different temperature regimes. Journal of Agric Engin Res **78:**423-430.

9. **Ben-Yephet, Y., and E. B. Nelson.** 1999. Differential suppression of damping-off caused by *Pythium aphanidermatum*, *P. irregulare*, and *P. myriotylum* in composts at different temperatures. Plant Dis **93:**356-360.

10. **Bencheqroun, S. K., M. Bajji, S. Massart, F. Bentata, M. Labhilili, H. Achbani, S. El Jaafari, and M. H. Jijakli.** 2006. Biocontrol of blue mold on apple fruits by Aureobasidium pullulans (strain Ach 1-1): in vitro and in situ evidence for the possible involvement of competition for nutrients. Commun Agric Appl Biol Sci **71:**1151-7.

11. **Bernstein, N., S. Sela, R. Pinto, and M. Ioffe.** 2007. Evidence for internalization of *Escherichia coli* into the aerial parts of maize via the root system. J Food Prot **70:**471-5.

12. **Bertoldi, M. d., and P. A. Vallini.** 1983. The biology of composting: A review. Waste Manage Res **1:**157-176.

13. **Bess, V., M. Manes, B. Richter, and J. Snodgrass.** 2002. *E. coli* survival in compost tea using different nutrient substrates. Proceedings 2002 Internat Symp Compost Sci and Util.

14. **Besser, T. E., B. L. Richards, D. H. Rice, and D. D. Hancock.** 2001. *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission. Epidemiol Infect **127:**555-60.

15. **Bharadwaj, K. K. R.** 1995. Improvements in microbial compost technology: A special reference to microbiology of composting, p. 115-135. *In* S. Khanna and K. Mohan (ed.), Wealth from Waste. Tata Energy Research Institute, New Delhi.

16. **Bishop, P. L., and C. Godfrey.** 1983. Nitrogen transformation during sewage composting. Biocycle **24:**34-39.

17. **Bonanomi, G., V. Antignani, C. Pane, and F. Scala.** 2007. Suppression of soilborne fungal diseases with organic amendments. J of Plant Path **89:**311-324.

18. **Brannen, J. P., D. M. Garst, and S. Langley.** 1975. Inactivation of *Ascaris lumbricoides* eggs by heat, radiation, and thermoradiation. SAND 75-0163. Sandia Labs. Alburquerque, New Mexico.

19. **Brent Tarasoff, P. A., and N. Wiens.** 2005. Energy and natural fertilizer production using latent heat created through composting. Animal Agriculture and Processing: Managing Environmental Impacts. August 31-September 2., St. Louis, MO.

20. **Brinton, W., and D. M.** 1995. The control of plant pathogenic fungi by use of compost teas. Biodynamics **197:**12-15.

21. **Brinton, W. F., P. Storms, and T. C. Blewett.** 2009. Occurrence and levels of fecal indicators and pathogenic bacteria in market-ready recycled organic matter composts. J Food Prot **72:**332-339.

22. **Brinton, W. F., P. Storms, E. Evans, and J. Hill.** 2004. Compost Teas: microbial hygiene and quality in relation to method of preparation. Biodynamics **249:**36-45.

23. **Brinton, W. F., A. Trankner, and M. Droffner.** 1996. Making and using compost teas: Investigations into liquid compost extracts. BioCycle **37:**68-70.

24. **Burge, W. D., and P. D. Millner.** 1980. Health aspects of composting: primary and secondary pathogens, p. 245-264. *In* B. L. Bitton, G. Damron, T. Edds, and J. M. Davidson (ed.), Sludge- Health Effects of Land Application. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

25. **Buyer, J. S.** 2003. Improved fast gas chromatography for FAME analysis of bacteria. J Microbiol Methods **54:**117-20.

26. **Buyer, J. S.** 2006. Rapid and sensitive FAME analysis of bacteria by cold trap injection gas chromatography. J Microbiol Methods **67:**187-90.

27. **Caris-Veyrat, C., M. J. Amiot, V. Tyssandier, D. Grasselly, M. Buret, M. Mikolajczak, J. C. Guilland, C. Bouteloup-Demange, and P. Borel.** 2004. Influence of organic versus conventional agricultural practice on the antioxidant microconstituent content of tomatoes and derived purees; consequences on antioxidant plasma status in humans. J Agric Food Chem **52:**6503-9.

28. **CDC.** 2001. *Salmonella* Surveillance: Annual Summary, 2000. United States Department of Health and Human Services, Centers for Disease Control and Prevention, Atlanta GA.

29. **Chang, J. C.** 1991. Effect of glucose concentration, agar, and amount of inoculum on the 6.5% sodium chloride tolerance test for presumptive identification of enterococci. Zhonghua Min Guo Wei Sheng Wu Ji Mian Yi Xue Za Zhi **24:**36-43.

30. **Chapman, P. A., C. A. Siddons, A. T. Gerdan Malo, and M. A. Harkin.** 1997. A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry. Epidemiol Infect **119:**245-50.

31. **Christensen, K. K., M. Carlsbaek, and E. Kron.** 2002. Strategies for evaluating the sanitary quality of composting. J Appl Microbiol **92:**1143-58.

32. **Craft, C. M., and E. B. Nelson.** 1996. Microbial properties of composts that suppressdamping-off and root rot of creeping bentgrass caused by *Pythium graminicola*. Appl Environ Microbiol **62:**1550-1557.

33. **Cronin, M. J., D. S. Yohalem, R. F. Harris, and J. H. Andrews.** 1996. Putative mechanism and dynamics of inhibition of the apple scab pathogen *Venturia inaequalis* by compost extracts. Soil Biol and Biochem **28:**1241-1249.

34. **Croteau, G. A., and L. M. Zibilske.** 1998. Influence of papermill processing residuals on saprophytic growth and disease caused by *Rhizoctonia solani*. Applied Soil Ecology **10:**103-115.

35. **Das, K., and H. M. Keener.** 1997. Moisture effect on compaction and permeability in composts. J Environ Engineer **123:**275-281.

36. **De Bertoldi, M., and Commission of the European Communities. Directorate-General for Science Research and Development.** 1987. Compost : production, quality, and use, vol. 1. Elsevier Applied Science; Sole distributor in the USA and Canada, Elsevier Science Pub. Co., London ; New York, NY.

37. **De Bertoldi, M., G. Vallini, and A. Pera.** 1983. The biology of composting: a review. Waste Manag and Res **1:**157-176.

38. **De Bertoldi, M., F. Zucconi, and M. Civilini.** 1988. Stabilization research - temperature, pathogen control and product quality. Biocycle **29:**43-47.

39. **Demontet, S., H. Dinel, and S. B. Baloda.** 1999. Pathogen reduction in sewage sludge by composting and other biological treatments: A review. Biol. Agric. Hortic. **16:**409-430.

40. **Diab, H., S. Hu, and D. M. Benson.** 2003. Suppression of *Rhizoctonia solani* on impatiens by enhanced microbial activity in composted swine waste amended potting mixes. Phytopath **93:**1115-1123.

41. **Dick, W. A., and E. I. McCoy.** 1993. Enhancing soil fertility by addition of compost, p. 623-644. *In* H. A. J. Hoitink and H. M. Keener (ed.), Science and engineering of composting: Design, environmental, microbiological and utilization aspects. Renaissance Publications, Worthington, OH.

42. **Diver, S.** 2001. Notes on compost teas: a 2001 supplement to the ATTRA publication compost teas for plant disease control. ATTRA publication, Fayetteville, AK.

43. **Droffner, M. L., and W. F. Brinton.** 1995. Survival of *E. coli* and *Salmonella* populations in aerobic thermophilic composts as measured with DNA gene probes. Zentralbl Hyg Umweltmed **197:**387-97.

44. **Duffy, B., C. Sarreal, S. Ravva, and L. Stanker.** 2004. Effect of molasses on regrowth of *E. coli* O157:H7 and *Salmonella* in compost teas. Compost Sci and Util **v. 12, no. 1:**93-96.

45. **Duffy, B., C. Sarreal, R. Stevenson, S. Ravva, and L. Stanker.** 2002. Regrowth of Pathogenic Bacteria in Compost Teas and Risk of Transmission to Strawberry Plants. Proceedings 2002 International Symposium Compost Science and Utilization.

46. **Ederer, G. M., M. E. Lund, D. J. Blazevic, L. B. Reller, and S. Mirrett.** 1975. Motility-indole-lysine-sulfide medium. J Clin Microbiol **2:**266-7.

47. **Edrington, T. S., T. R. Callaway, S. E. Ives, M. J. Engler, M. L. Looper, R. C. Anderson, and D. J. Nisbet.** 2006. Seasonal shedding of *Escherichia coli* O157:H7 in ruminants: a new hypothesis. Foodborne Pathog Dis **3:**413-21.

48. **Edrington, T. S., W. E. Fox, T. R. Callaway, R. C. Anderson, D. W. Hoffman, and D. J. Nisbet.** 2009. Pathogen prevalence and influence of composted dairy manure application on antimicrobial resistance profiles of commensal soil bacteria. Foodborne Pathog Dis **6:**217-24.

49. **El-Sebaie, O. D., A. H. Hussein, M. M. Abdel-Aty, M. H. Ramadan, and H. A. Helaly.** 2002. Quality monitoring of composting processes of wastewater sludge in Alexandria. J Egypt Public Health Assoc **77:**517-36.

50. **Elad, Y., and D. Shtienberg.** 1994. Effect of compost water extracts on grey mould (*Botrytis cinerea*). Crop Protect **13:**109-114.

51. **EPA.** 1993. 40 CFR Parts 257, 403 and 503 (FRO-4203-3): Standards for the use and disposal of sewage, Final Rule, Fed. Register, 58, 9248. US Government Printing Office, Washington DC.

52. **EPA.** 1999. Control of pathogens and vector attraction in sewage sludge. *In* EPA/625/R-92/013 (ed.). Center for Environmental Research Information, Cincinnati, Ohio.

53. **EPA.** 2003. Control of pathogens and vector attraction in sewage sludge (Including domestic septage) under 40 CFR Part 503. EPA/625/R-92/013. Revised July 2003, United States Environmental Protection Agency, Office of Research and Development, National Risk Management Laboratory, Center for Environmental Research Information. Cincinnati, OH.

54. **EPA.** 1979. Criteria for classification of solid waste disposal facilities and practices. *In* -. Federal Register 44(179) (ed.). Washington D.C.

55. **EPA.** 1994. A Plain English Guide to the EPA part 503 biosolids rule. EPA/832/R-93/003. Office of Wastewater management, Washington, D.C.

56. **EPA.** 1989. Safe Drinking Water. Federal Register. June 29, 1989. Code of Federal Regulations, Title 40, Parts 141 and 142.

57. **EPA.** 1993. Standards for the use and disposal of sewage, final rule; 40 CFR Parts 257, 403 and 503.

58. **EPA.** 1992. Technical support document for Part 503 pathogen and vector attraction reduction requirements in sewage sludge. NTIS No: PB93-110609. National Technical Information Service, Springrield, VA.

59. **EPA.** 1986. Test Methods for Evaluating Solid Waste, Physical/Chemical Methods. 3rd ed.

60. **Epstein, E.** 1997. The science of composting, vol. Technomic Publishing, Lancaster, PA.

61. **Erhart, E., K. Burian, W. Hartsl, and K. Stich.** 1999. Suppression of *Pythium ultimum* by biowaste composts in relation to compost microbial biomass, activity and content of phenolic compounds. J of Phytopath **147:**299-305.

62. **FAO.** 1980. A manual for rural composting. FAO/UNDP Regional Project RAS/75/004 Field Document 15.

63. **Farrell, J. B.** 1993. Fecal pathogen control during composting, p. 282-300. *In* H. A. J. Hoitink and H. M. Keener (ed.), Science and engineering of composting: design, environmental, microbiological, and utilization aspects. Renaissance Publications Worthington, OH.

64. **Feachem, R. G., D. J. Bradley, H. Garelick, and D. D. Mara.** 1983. Sanitation and disease: health aspects of excreta and waste water management, vol. 1. John Wiley & Sons, Washington.

65. **Filonow, A. B., H. S. Vishniac, J. A. Anderson, and W. J. Janisewicz.** 1996. Biological control of *Botrytis cinerea* in apple by yeasts from various habitats and their putative mechanisms of antagonism. Biolog Control **7:**212-220.

66. **Fitzpatrick, G. E., E. C. Worden, and W. A. Vendrame.** 2005. Historical development of composting technology during the 20th century. Hort Technol **15:**48-51.

67. **Follet, R., R. Donahue, and L. Murphy.** 1981. Soil and Soil Amendments, vol. 1. Prentice-Hall, Inc., New Jersey.

68. **Gajalakshmi, S., and S. A. Abbasi.** 2008. Solid waste management by composting: State of the art. Crit Rev in Environ Sci Technol **38:**311-400.

69. **Garcia, C., T. Hernandez, J. Albaladejo, V. Castillo, and A. Roldan.** 1998. Revegetation in semiarid zones: influence of terracing and organic refuse on microbial activity. Soil Sci. Soc. Am. J. **62:**670-676.

70. **Garcia, C., T. Hernandez, and F. Costa.** 1991. Changes in carbon fractions during composting and maturation of organic wastes Environ Manage **15:**433-439.

71. **Garcia, C., T. Hernandez, A. Roldan, J. Albaladejo, and V. Castillo.** 2000. Organic amendment and mycorrhizal inoculation as a practice in afforestation of soils with *Pinus halepensis miller*: effect on their microbial activity. Soil Biol. Biochem **16:**1173-1181.

72. **Garland, G. A., T. A. Grist, and R. E. Green.** 1995. The compost story: from soil enrichment to pollution remediation. Biocycle **36:**53-56.

73. **Gerba, C. P., M. S. Huber, J. Naranjo, J. B. Rose, and S. Bradford.** 1995. Occurrence of enteric pathogens in composted domestic solid waste containing disposable diapers. Waste Manage Res **13:**315-324.

74. **Gliessmann, S. R., M. R. Werner, S. L. Swezey, E. Caswell, J. Cochran, and F. Rosado-May.** 1996. Conversion to organic strawberry management changes ecological processes. Calif Agric **50:**24-31.

75. **Goldstein, N.** 1998. Status of composting in the United States, p. 35-43. *In* S. Brown, S. J. Angle, and L. Jacobs (ed.), Beneficial Co-Utilization of Agricultural, Municipal and Industrial By-Products. Kluwer Academic Publishers, Boston.

76. **Goleuke, C. G.** 1992. Bacteriology of composting. Biocycle **33:**55-57.

77. **Golueke, C.** 1998. Compost Quality Review. Biocycle **39:**28-33.

78. **Gong, C. M.** 2007. Microbial safety control of compost material with cow dung by heat treatment. J Environ Sci (China) **19:**1014-9.

79. **Gong, C. M., I. Koichi, I. Shunji, and S. Takashi.** 2005. Survival of pathogenic bacteria in compost with special reference to *Escherichia coli*. J Environ Sci (China) **17:**770-4.

80. **Grebenisan, I., P. Cornea, R. Mateescu, C. Cimpeanu, V. Olteanu, G. Campenu, L. A. Stefan, F. Oancea, and C. Lupu.** 2008. *Metschnikowia pulcherrima*, a new yeast with potential for biocontrol of postharvest fruit rots. *In* R. K. Prange and S. D. Bishop (ed.), International Symposium on Sustainability through Integrated and Organic Horticulture, vol. 1. ISHS Acta Horticulturae, Seoul.

81. **Greig, J. D., and A. Ravel.** 2009. Analysis of foodborne outbreak data reported internationally for source attribution. Int J Food Microbiol **130:**77-87.

82. **Guerena, M., and H. Born.** 2007. Strawberries: organic production. ATTRA - National Sustainable Agriculture Information Service**:**1-28.

83. **Guinebretiere, M. H., C. Nguyen-The, N. Morrison, M. Reich, and P. Nicot.** 2000. Isolation and characterization of antagonists for the biocontrol of the postharvest wound pathogen *Botrytis cinerea* on strawberry fruits. J Food Prot **63:**386-94.

84. **Haggag, W. M., and M. S. M. Saber.** 2007. Suppression of early blight on tomato and purple blight on onion by foliar sprays of aerated and non-aerated compost teas. J of Food, Agric Environ **5:**302-309.

85. **Hargreaves, J., M. S. Adl, P. R. Warman, and H. P. V. Rupasinghe.** 2008. The effects of organic amendments on mineral element uptake and fruit quality of raspberries. Plant and Soil **308:**213-226.

86. **Hargreaves, J., S. M. Adl, and P. R. Warman.** 2008. The effects of organic and conventional nutrient amendments on strawberry cultivation: Fruit yield and quality. J Sci Food Agric **88:**2669-2675.

87. **Hargreaves, J. C., S. M. Adl, and P. R. Warman.** 2009. Are compost teas an effective nutrient amendment in the cultivation of strawberries? Soil and plant tissue effects. J Sci Food Agric **89:**390-397.

88. **Haug, R. T.** 1993. The practical handbook of compost engineering, vol. 1. Lewis Publishers, London.

89. **Hay, J. C.** 1996. Pathogen destruction and biosolids composting. Biocycle **37:**67-76.

90. **Hayes, J. R., L. E. Carr, E. T. Mallinson, L. W. Douglass, and S. W. Joseph.** 2000. Characterization of the contribution of water activity and moisture content to the population distribution of *Salmonella* spp. in commercial poultry houses. Poult Sci **79:**1557-61.

91. **Hays, B. D.** 1977. Potential for parasitic disease transmission with land application of sewage plant effluents and sludges. Water Research **11:**583-595.

92. **He, X. T., T. J. Logan, and S. J. Traina.** 1995. Physical and chemical characteristics of selected U.S. municipal solid waste composts. J Environ Qual **24:**543-552.

93. **Hellmann, B., L. Zelles, A. Palojarvi, and Q. Bai.** 1997. Emission of climate-relevant trace gases and succession of microbial communities during open-windrow composting. Appl Environ Microbiol **63:**1011-1018.

94. **Herrmann, R. F., and J. F. Shann.** 1997. Microbial community changes during the composting of municipal solid waste. Microb Ecol **33:**78-85.

95. **Hess, T. F., I. Grdzelishvili, H. Sheng, and C. J. Hovde.** 2004. Heat inactivation of *E. coli* during manure composting. Compost Sci & Util **12:**314-322.

96. **Higgins, A.** 2001. Foreverdung: At Mount Vernon, a restored barn and pit turn stable waste into black gold for the garden. http://gwpapers.virginia.edu/articles/news/dung.html.

97. **Hogg, D., J. Barth, E. Faviono, M. Centemero, V. Caimi, F. Amlinger, W. Devliegher, W. Brinton, and S. Antler.** 2002. Comparison of compost standards within the EU, North America, and Australia. Main report, the waste and resources action programme. Banbury, Oxon, U.K.

98. **Hoitink, H. A. J., and M. J. Boehm.** 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. Ann Rev Phytopath **37:**427-446.

99. **Hoitink, H. A. J., and P. C. Fahy.** 1986. Basis for the control of soil borne pathogens with composts. Ann Rev Phytopath **24:**93-114.

100. **Hoitink, H. A. J., A. G. Stone, and D. Y. Han.** 1997. Suppression of plant diseases by composts. HortScience **32:**184-187.

101. **Hora, R., K. Warriner, B. J. Shelp, and M. W. Griffiths.** 2005. Internalization of *Escherichia coli* O157:H7 following biological and mechanical disruption of growing spinach plants. J Food Prot **68:**2506-9.

102. **Howard, A., and Y. D. Wad.** 1931. The waste products of agriculture. Their utilization as humus, vol. Oxford University Press, London.

103. **Hunt, P. G., G. C. Smart, and C. F. Eno.** 1973. Sting nematode, *Belonolaimus longicaudatus*, immotility induced by extracts of composted municipal refuse. J Nematol **5:**60-63.

104. **Ibekwe, A. M., P. M. Watt, C. M. Grieve, V. K. Sharma, and S. R. Lyons.** 2002. Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater wetlands. Appl Environ Microbiol **68:**4853-62.

105. **Ingham, E.** 1998. Anaerobic bacteria and compost tea. BioCycle **39:**86.

106. **Ingham, E.** 1999. Making a high quality compost tea - Part II. Biocycle **40:**94.

107. **Ingham, E.** 1999. What is compost tea? Part I. Biocycle **40:**74-75.

108. **Ingham, E., and M. Alms.** 2001. The Compost Tea Brewing Manual, 2 ed, vol. 1. Soil Foodweb, Inc., Corvallis.

109. **Ingham, E. R.** 2000. Brewing compost tea. Kitchen Gardener **29:**16-19.

110. **Ingham, E. R.** 2005. November 2005 issue of E-Zine. Soil Food Web, Inc. http://www.soilfoodweb.com/04_news/documents/11_05SFIE-zine.pdf.

111. **Ingham, E. R., and M. Alms.** 1999. Compost tea manual, vol. Soil Food Web Inc., Corvallis, Oregon.

112. **Ingram, D. T., and P. D. Millner.** 2007. Factors affecting compost tea as a potential source of *Escherichia coli* and *Salmonella* on fresh produce. J Food Prot **70:**828-34.

113. **Islam, M., M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang.** 2004. Persistence of enterohemorrhagic *Escherichia coli* O157:H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. J Food Prot **67:**1365-70.

114. **Islam, M., J. Morgan, M. P. Doyle, S. C. Phatak, P. Millner, and X. Jiang.** 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Appl Environ Microbiol **70:**2497-502.

115. **Jablasone, J., K. Warriner, and M. Griffiths.** 2005. Interactions of *Escherichia coli* O157:H7, *Salmonella typhimurium* and *Listeria monocytogenes* plants cultivated in a gnotobiotic system. Int J Food Microbiol **99:**7-18.

116. **Jiang, X., J. Morgan, and M. P. Doyle.** 2003. Fate of *Escherichia coli* O157:H7 during composting of bovine manure in a laboratory-scale bioreactor. J Food Prot **66:**25-30.

117. **Jiang, X., J. Morgan, and M. P. Doyle.** 2003. Thermal inactivation of *Escherichia coli* O157:H7 in cow manure compost. J Food Prot **66:**1771-7.

118. **Johannessen, G. S., G. B. Bengtsson, B. T. Heier, S. Bredholt, Y. Wasteson, and L. M. Rorvik.** 2005. Potential uptake of *Escherichia coli* O157:H7 from organic manure into crisphead lettuce. Appl Environ Microbiol **71:**2221-5.

119. **Johnston, L. M., and L. A. Jaykus.** 2004. Antimicrobial resistance of *Enterococcus* species isolated from produce. Appl Environ Microbiol **70:**3133-7.

120. **Johnston, L. M., L. A. Jaykus, D. Moll, M. C. Martinez, J. Anciso, B. Mora, and C. L. Moe.** 2005. A field study of the microbiological quality of fresh produce. J Food Prot **68:**1840-7.

121. **Joklik, W. K., H. P. Willett, D. B. Amos, and C. M. Wilfert.** 1992. Zinsser Microbiology, 20th ed, vol. Appleton & Lange, Norwalk.

122. **Jurkevitch, E. J., and G. Shapira.** 2000. Structure and colonization dynamics of epiphytic bacterial communities and of selected component strains on tomato (*Lycopersicon esculentum*) leaves. Microb Ecol **40:**300-308.

123. **Kannangara, T., T. Forge, and B. Dang.** 2006. Effects of aeration, molasses, kelp, compost type, and carrot juice on the growth of *Escherichia coli* in compost teas. Compost Sci Util **14:**40-47.

124. **Karns, J. S., J. S. Van Kessel, B. J. McClusky, and M. L. Perdue.** 2007. Incidence of *Escherichia coli* O157:H7 and *E. coli* virulence factors in US bulk tank milk as determined by polymerase chain reaction. J Dairy Sci **90:**3212-9.

125. **Kato, K., and N. Miura.** 2008. Effect of matured compost as a bulking and inoculating agent on the microbial community and maturity of cattle manure compost. Biores Technol **99:**3372–3380.

126. **Kenney, S. J., G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat.** 2006. Migration of *Caenorhabditis elegans* to manure and manure compost and potential for transport of *Salmonella* newport to fruits and vegetables. Int J Food Microbiol **106:**61-8.

127. **Kenney, S. J., G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat.** 2005. Persistence of *Escherichia coli* O157:H7, *Salmonella newport*, and *Salmonella poona* in the gut of a free-living nematode, *Caenorhabditis elegans*, and transmission to progeny and uninfected nematodes. Int J Food Microbiol **101:**227-36.

128. **Ketterer, N., B. Fisher, and H. C. Weltzien.** 1992. Biological control of *Botrytis cinerea* on grapevine by compost extracts and their microorganisms in pure culture. Recent Advances in *Botrytis* Res. Proceedings 10th International *Botrytis* Symposium, Heraklion, Crete, Greece**..**179-186.

129. **Ketterer, N., and L. WSchwager.** 1992. Effect of compost extracts on the disease incidence and the phyllosphere flora of bush bean and tomato leaves. Med. van de Fac. Land. Univ. Gent **57:**411-421.

130. **Khaitsa, M. L., M. L. Bauer, G. P. Lardy, D. K. Doetkott, R. B. Kegode, and P. S. Gibbs.** 2006. Fecal shedding of *Escherichia coli* O157:H7 in North Dakota feedlot cattle in the fall and spring. J Food Prot **69:**1154-8.

131. **Kirchmann, H., and P. Widen.** 1994. Separately collected organic household wastes chemical composition and composting characteristics. Swedish J Agric Res **24:**3-12.

132. **Kmitta, J.** 2002. Something's brewing: The benefits of compost tea. Arbor Age **22:**36-37.

133. **Knewston, S. J. B.** 2009. Application of two microbial teas did not affect collard or spinach yield. HortScience **44:**73.

134. **Koepf, H. H.** 1992. Biodynamic farming: principles and practice, vol. 1. Anthro Press, New York.

135. **Kozdroj, J., and J. D. Van Elsas.** 2001. Structural diversity of microbial communities in arable soils of a heavily industrialized area determined by PCR-DGGE fingerprinting and FAME profiling. App Soil Ecol **17:**31-42.

136. **Krause, M. S., L. V. Madden, and H. A. J. Hoitink.** 2001. Effect of potting mix microbial carrying capacity on biological control of *Rhizoctonia* damping-off of radish and *Rhizoctonia* cron and root rot of poinsettia. Phytopath **91:**1116-1123.

137. **Kwok, O. C. H., P. C. Fahy, H. A. J. Hoitink, and G. A. Kuter.** 1987. Interactions between bacteria and *Trichoderma hamatum* in suppression of *Rhizoctonia* damping-off in bark compost media. Phytopath **77:**1206-1212.

138. **Larkin, R. P.** 2008. Relative effects of biological amendments and crop rotations on soil microbial communitites and soilborne diseases of potato. Soil Biol Biochem **40:**1341-1351.

139. **Larsen, H. E., B. Munch, and J. Schlundt.** 1994. Use of indicators for monitoring the reduction of pathogens in animal waste treated in biogas plants. Zentralbl Hyg Umweltmed **195:**544-55.

140. **Lazorovits, G.** 2001. Management of soil-borne plant pathogens with organic soil amendments: a disease control strategy salvaged from the past. Can J Plant Path **23:**1-7.

141. **LeChevallier, M. W., and G. A. McFeters.** 1985. Interactions between heterotrophic plate count bacteria and coliform organisms. Appl Environ Microbiol **49:**1338-41.

142. **Lemunier, M., C. Francou, S. Rousseaux, S. Houot, P. Dantigny, P. Piveteau, and J. Guzzo.** 2005. Long-term survival of pathogenic and sanitation indicator bacteria in experimental biowaste composts. Appl Environ Microbiol **71:**5779-86.

143. **Leverentz, B., W. S. Conway, W. Janisiewicz, M. Abadias, C. P. Kurtzman, and M. J. Camp.** 2006. Biocontrol of the food-borne pathogens *Listeria monocytogenes* and *Salmonella enterica* serovar Poona on fresh-cut apples with naturally occurring bacterial and yeast antagonists. Appl Environ Microbiol **72:**1135-40.

144. **Lin, S., H. Schraft, J. A. Odumeru, and M. W. Griffiths.** 1998. Identification of contamination sources of *Bacillus cereus* in pasteurized milk. Int J Food Microbiol **43:**159-71.

145. **Litterick, A. M., L. Harrier, P. Wallace, C. A. Watson, and M. Wood.** 2004. The Role of uncomposted materials, composts, manures, and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production. Crit Rev Plant Sci **23:**453-479.

146. **Little, M. D.** 1980. Agents of health significance: parasites, p. 47-58. *In* G. Bitton, B. L. Damron, G. T. Edds, and J. M. Davidson (ed.), Sludge - Health risks of land application. Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

147. **Loncaric, I., C. Donat, B. Antlinger, J. T. Oberlerchner, B. Heissenberger, and R. Moosbeckhofer.** 2008. Strain-specific detection of two *Aureobasidium pullulans* strains, fungal biocontrol agents of fire blight by new, developed multiplex-PCR. J Appl Microbiol **104:**1433-41.

148. **Lynch, M., J. Painter, R. Woodruff, and C. Braden.** 2006. Surveillance for foodborne-disease outbreaks--United States, 1998-2002. MMWR Surveill Summ **55:**1-42.

149. **Lynch, M. F., R. V. Tauxe, and C. W. Hedberg.** 2009. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiol Infect **137:**307-15.

150. **Malandraki, I., S. E. Tjamos, I. S. Pantelides, and E. J. Paplomatas.** 2008. Thermal inactivation of compost suppressiveness implicates possible biological factors in disease management. Biol Control **44:**180-187.

151. **Manser, A. G. R., and A. A. Keeling.** 1996. Practical book of processing and recycling municipal waste, vol. 1. CRC Press, Boca Raton, FL.

152. **Mari, I., C. Ehaliotis, M. Kotsou, I. Chatzipavlidis, and D. Goeorgakakais.** 2005. Use of sulfur to control pH in composts derived from olive processing by-products. Compost Sci Util **13:**281-87.

153. **Mays, D. A., G. L. Terman, and J. C. Duggan.** 1973. Municipal compost: effects on crop yields and soil properties. J Env Qual **2:**89-92.

154. **McCabe-Sellers, B. J., and S. E. Beattie.** 2004. Food safety: emerging trends in foodborne illness surveillance and prevention. J Am Diet Assoc **104:**1708-17.

155. **McGowan-Spicer, L. L., P. J. Fedorka-Cray, J. G. Frye, R. J. Meinersmann, J. B. Barrett, and C. R. Jackson.** 2008. Antimicrobial resistance and virulence of *Enterococcus faecalis* isolated from retail food. J Food Prot **71:**760-9.

156. **McGowan, L. L., C. R. Jackson, J. B. Barrett, L. M. Hiott, and P. J. Fedorka-Cray.** 2006. Prevalence and antimicrobial resistance of enterococci isolated from retail fruits, vegetables, and meats. J Food Prot **69:**2976-82.

157. **Merrill, R., and J. McKeon.** 2001. Apparatus design and experimental protocol for organic compost teas. Organic Farm Res Found Inform Bull 9:9-15.

158. **Michel, P., L. J. Martin, C. E. Tinga, and K. Dore.** 2006. Regional, seasonal, and antimicrobial resistance distributions of *Salmonella typhimurium* in Canada: a multi-provincial study. Can J Public Health **97:**470-4.

159. **Miller, F. C.** 1992. Composting as a process based on the control of ecologically selective factors, p. 515-544. *In* F. B. Metting, jr. (ed.), Soil Microbial Ecology, Applications in Agricultural and Environmental Management. Marcel Dekker, Inc., New York.

160. **Millner, P. D.** 2006. Control of strawberry black root rot with compost socks. Biocycle **47:**56-58.

161. **Misra, R. V., and R. N. Roy.** 2007. On farm composting methods. http://www.fao.org/ORGANICAG/doc/On_farm_comp_methods.pdf. Food and Agriculture Organization (FAO) of the United Nations, Rome.

162. **Mitchell, A. E., Y. J. Hong, E. Koh, D. M. Barrett, D. E. Bryant, R. F. Denison, and S. Kaffka.** 2007. Ten-year comparison of the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. J Agric Food Chem **55:**6154-6159.

163. **Moral, R., C. Paredes, M. A. Bustamante, F. Marhuenda-Egea, and M. P. Bernal.** 2009. Utilisation of manure composts by high-value crops: Safety and environmental challenges. Bioresource Technology **In-Press**.

164. **Mounir, R., A. Durieux, E. Bodo, C. Allard, J. P. Simon, E. H. Achbani, S. El-Jaafari, A. Douira, and M. H. Jijakli.** 2007. Production, formulation and antagonistic activity of the biocontrol like-yeast *Aureobasidium pullulans* against *Penicillium expansum*. Biotechnol Lett **29:**553-9.

165. **Mukherjee, A., D. Speh, E. Dyck, and F. Diez-Gonzalez.** 2004. Preharvest evaluation of coliforms, *Escherichia coli*, *Salmonella*, and *Escherichia coli* O157:H7 in organic and conventional produce grown by Minnesota farmers. J Food Prot **67:**894-900.

166. **Murray, P. R., K. S. Rosenthal, and M. A. Pfaller.** 2009. Medical microbiology, 6th ed, vol. Mosby/Elsevier, Philadelphia.

167. **Nakasaki, K., N. Uehara, M. Kataoka, and H. Kubota.** 1996. The use of *Bacillus licheniformis* HAI to accelerate composting of organic waste. Compost Sci Util **4:**47-51.

168. **Nakasaki, K., H. Yaguchi, M. Sasaki, and H. Kubota.** 1993. Effects of pH control on composting of garbage. Waste Manag Res **11:**117-125.

169. **Nell, J. H., A. G. Steer, and P. A. J. van Rensburg.** 1983. Hygienic quality of sewage sludge compost. Water Sci Tech **19:**181-194.

170. **North Carolina Agricultural Extension Service.** 1973. Dairy waste management alternatives. North Carolina State University Circular 568.

171. **NOSB.** 2004. Compost Tea Task Force Report. Agricultural Marketing Service, United States Department of Agriculture, Published Online: www.ams.usda.gov/nosb/meetings/compostteataskforcefinalreport.pdf

172. **Pain, I., and J. Pain.** 1980. The methods of Jean Pain: or another kind of garden, vol. 1. self-published.

173. **Papavizas, G. C., and R. D. Lumsden.** 1982. Improved medium for isolation of *Trichoderma* spp. from soil. Plant Dis **66:**1019-1020.

174. **Parsek, M. R., and C. Fuqua.** 2004. Biofilms 2003: emerging themes and challenges in studies of surface-associated microbial life. J Bacteriol **186:**4427-40.

175. **Parveen, S., N. C. Hodge, R. E. Stall, S. R. Farrah, and M. L. Tamplin.** 2001. Phenotypic and genotypic characterization of human and nonhuman *Escherichia coli*. Water Res **35:**379-86.

176. **Pascual, J. A., M. Ayuso, M. T. Hernandez, and C. Garcia.** 1998. Carbon mineralization in an arid soil amended with organic materials of varying degrees of stability. Comm Soil Sci Plant Anal **29:**835-846.

177. **Pascual, J. A., C. Garcia, and T. Hernandez.** 1999. Comparison of fresh and composted organic waste in their efficacy for the improvement of arid soil quality. Bioresource Technol **68**.

178. **Pell, A. N.** 1997. Manure and microbes: public and animal health problem? J Dairy Sci **80:**2673-81.

179. **Pereira-Neto, J. T., E. I. Stentiford, and D. D. Mara.** 1987. Low cost controlled composting of refuse and sewage sludge. Water Sci Technol **19:**839-845.

180. **Petersen, K. E., and W. O. James.** 1998. Agents, vehicles, and causal inference in bacterial foodborne disease outbreaks: 82 reports (1986-1995). J Am Vet Med Assoc **212:**1874-81.

181. **Peterson, G. L., K. L. Kosta, D. L. Glenn, and J. G. Phillips.** 2008. Utilization of soil solarization for eliminating viable *Tilletia indica* teliospores from Arizona wheat fields. Plant Dis **92:**1604-1610.

182. **Pharand, B., O. Carisse, and N. Benhamou.** 2002. Cytological aspects of compost-mediated induced resistance against *Fusarium* crown and root rot in tomato. Phytopath **92:**424-438.

183. **Piano, S., V. Neyrotti, Q. Migheli, and M. L. Gullino.** 1997. Biocontrol capability of *Metschnikowia pulcherrima* against *Botrytis* postharvest rot of apple. Postharvest Biol and Technol **11:**131-140.

184. **Pillai, S. D., K. W. Widmer, S. E. Dowd, and S. C. Ricke.** 1996. Occurrence of airborne bacteria and pathogen indicators during land application of sewage sludge. Appl Environ Microbiol **62:**296-9.

185. **Pogue, D. J., and R. Amer.** 1997. George Washington, the revolutionary farmer: America's first composter. www.cityfarmer.org/Washington.html.

186. **Poincelot, R. P.** 1974. A scientific examination of the principles and practice of composting. Compost Sci **15:**24-31.

187. **Prokkola, S., and P. Kivijarvi.** 2007. Effect of biological sprays on the incidence of grey mould fruit yield and fruit quality in organic strawberry production. Agric Food Sci **16:**25-33.

188. **Qadri, S. M., M. J. deSilva, S. G. Qadri, and A. Villarreal.** 1979. Presumptive identification of enterococci from other D streptococci by a rapid sodium chloride tolerance test. Med Microbiol Immunol **167:**197-203.

189. **Qadri, S. M., C. W. Nichols, and S. G. Qadri.** 1978. Rapid sodium chloride tolerance test for presumptive identification of enterococci. J Clin Microbiol **7:**238.

190. **Rasapoor, M., T. Nasrabadi, M. Kamali, and H. Hoveidi.** 2008. The effects of aeration rate on generated compost quality, using aerated static pile method. Waste Manag **29:**570-573.

191. **Raviv, M., Y. Oka, J. Katan, Y. Hadar, A. Yogev, S. Medina, A. Krasnovsky, and H. Ziadna.** 2005. High-nitrogen compost as a medium for organic container-grown crops. Bioresource Technol **96:**419-427.

192. **Reimers, R. S., D. B. McDonnell, M. D. Little, D. D. Bowman, A. J. Engleande, and W. D. Henriques.** 1986. Effectiveness of waste-water sludge treatment processes to inactivate parasites. Water Sci and Technol **18:**397-404.

193. **Reller, L. B., and S. Mirrett.** 1975. Motility-indole-lysine medium for presumptive identification of enteric pathogens of *Enterobacteriaceae*. J Clin Microbiol **2:**247-52.

194. **Riggle, D.** 1996. Compost teas in agriculture. Biocycle **37:**65-67.

195. **Rodale, J. I.** 1960. The complete book of composting, vol. 1. Rodale Books, Emmaus, PA.

196. **Ros, M., C. Garcia, and T. Hernandez.** 2001. The use of organic wastes in the control of erosion in a semiarid Mediterranean soil. Soil Use Manage **17:**292-293.

197. **Rotenberg, D., A. J. Wells, E. J. Chapman, A. E. Whitfield, R. M. Goodman, and L. R. Cooperband.** 2007. Soil properties associated with organic matter-mediated suppression of bean root rot in field soil amended with fresh and composted paper mill residuals. Soil Biol Biochem **39:**2936-2948.

198. **Rubin, A. R.** 1996. Presented at the 10th annual residuals and biosolids management conference: 10 years of progress and a look toward the future. Title: Is time-temperature really important?, Alexandria, VA.

199. **Russ, C. F., and W. A. Yanko.** 1981. Factors affecting salmonellae repopulation in composted sludges. Appl Environ Microbiol **41:**597-602.

200. **Ryan, M., D. Wilson, P. Hepperly, J. Travis, N. Halbrendt, and A. Wise.** 2005. More Consistency sought: Compost tea potential is still brewing. BioCycle **46:**30-32.

201. **Rynk, R., M. van de Kamp, G. B. Willson, M. E. Singley, T. L. Richard, J. J. Kolega, F. R. Gouin, L. Laliberty, D. Kay, D. W. Murphy, H. A. J. Hoitink, and W. F. Brinton.** 1992. On-Farm Composting Handbook, p. 186. vol. 54. USDA. Northeast Regional Agricultural Engineering Service - Cooperative Extension.

202. **Sackenheim, R., H. C. Weltzien, and W. K. Kast.** 1994. Effects of microflora composition in the phyllosphere on biological regulation of grapevine fungal diseases. Vitis **33:**235-240.

203. **Salter, C., and A. Cuyler.** 2003. Pathogen reduction in food residuals composting. Biocycle **44:**42-51.

204. **Samerski, C., and H. C. Weltzien.** 1988. Investigations on the mode of action of compost extracts in the host-parasite-system sugar beet-powdery mildew. J Plant Dis Protect **95:**176-181.

205. **Sanchez-Moreno, S., E. Alonso-Prados, J. L. Alonso-Prados, and J. M. Garcia-Baudin.** 2009. Multivariate analysis of toxicological and environmental properties of soil nematicides. Pest Manage Sci **65:**82-92.

206. **Saxton, A. M.** 1998. Presented at the Proceedings of the 23rd SAS Users Group International. A macro for converting mean separation output to letter groupings in Proc Mixed.

207. **Scheuerell, S.** 2003. Understanding how compost tea can control disease. Biocycle **44:**20-25.

208. **Scheuerell, S., and W. Mahaffee.** 2002. Compost tea: Principles and prospects for plant disease control. Compost Sci Util **10:**313-338.

209. **Scheuerell, S. J., and W. F. Mahaffee.** 2000. Assessing aerated and non-aerated watery fermented compost and Trichoderma harzianum T-22 for control of powdery mildew (*Spraerotheca pannosa* var. *rosae*) of rose in the Williamette Valley, Oregon (Abstract). Phytopathology 90:69.

210. **Scheuerell, S. J., and W. F. Mahaffee.** 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *Pythium ultimum*. Phytopath **v. 94, no. 11:**1156-1163.

211. **Scheuerell, S. J., and W. F. Mahaffee.** 2006. Variability associated with suppression of gray mold (*Botrytis cinerea*) on Geranium by foliar applications of bonaerated and aerated compost teas. Plant Dis **90:**1201-1208.

212. **Scheuerell, S. J., D. M. Sullivan, and W. F. Mahaffee.** 2005. Suppression of seedling damping-off caused by *Pythium ultimum, P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of Pacific Northwest compost sources. Phytopath **95:**306-315.

213. **Schuler, C., J. Biala, C. Bruns, R. Gottschall, S. Ahlers, and H. Vogtmann.** 1989. Suppression of root rot on peas, beans and beetroots caused by *Pythium ultimatum* and *Rhizoctonia solani* through the amendment of growing media with composted organic household waste. J Phytopath **127:**227-238.

214. **Serra-Wittling, C., S. Houot, and C. Alabouvette.** 1996. Increased soil suppressiveness to *Fusarium* wilt of flax after addition of municopal solid waste compost. Soil Biol Biochem **28:**1207-1214.

215. **Seurinck, S., E. Deschepper, B. Deboch, W. Verstraete, and S. Siciliano.** 2006. Characterization of *Escherichia coli* isolates from different fecal sources by means of classification tree analysis of fatty acid methyl ester (FAME) profiles. Environ Monit Assess **114:**433-45.

216. **Sharma, V. K., M. Canditelli, F. Fortuna, and G. Cornacchia.** 1997. Processing of urban and agro-industrial residues by aerobic composting: review. Energy Conver Manage **38:**453-478.

217. **Sharma, V. K., E. A. Dean-Nystrom, and T. A. Casey.** 1999. Semi-automated fluorogenic PCR assays (TaqMan) for rapid detection of *Escherichia coli* O157:H7 and other shiga toxigenic *E. coli*. Mol Cell Probes **13:**291-302.

218. **Shepherd, M. W., Jr., P. Liang, X. Jiang, M. P. Doyle, and M. C. Erickson.** 2007. Fate of *Escherichia coli* O157:H7 during on-farm dairy manure-based composting. J Food Prot **70:**2708-16.

219. **Shi, X., A. Namvar, M. Kostrzynska, R. Hora, and K. Warriner.** 2007. Persistence and growth of different *Salmonella* serovars on pre- and postharvest tomatoes. J Food Prot **70:**2725-31.

220. **Shigaki, F., A. Sharpley, and L. I. Prochnow.** 2007. Rainfall intensity and phosphorus source effects on phosphorus transport in surface runoff from soil trays. Sci Total Environ **373:**334-43.

221. **Shuvall, H., R. Jodice, M. Consiglio, G. Spaggiarri, and C. Spigoni.** 1991. Control of enteric microorganisms by aerobic-thermophilic co-composting of waste-water sludge and agro-industry wastes. Water Sci Technol **24:**401-405.

222. **Siddiqui, Y., S. Meon, R. Ismail, and M. Rahmani.** 2009. Bio-potential of compost tea from agro-waste to suppress *Choanephora cucurbitarum* L. the causal pathogen of wet rot of okra. Biological Control **49:**38-44.

223. **Sidhu, J., R. A. Gibbs, G. E. Ho, and I. Unkovich.** 2001. The role of indigenous microorganisms in suppression of *Salmonella* regrowth in composted biosolids. Water Res **35:**913-20.

224. **Singh, R., R. R. Sharma, S. Kumar, R. K. Gupta, and R. T. Patil.** 2008. Vermicompost substitution influences growth, physiological disorders, fruit yield and quality of strawberry (Fragaria x ananassa Duch.). Bioresour Technol **99:**8507-11.

225. **Sivapalasingam, S., E. Barrett, A. Kimura, S. Van Duyne, W. De Witt, M. Ying, A. Frisch, Q. Phan, E. Gould, P. Shillam, V. Reddy, T. Cooper, M. Hoekstra, C. Higgins, J. P. Sanders, R. V. Tauxe, and L. Slutsker.** 2003. A multistate outbreak of *Salmonella enterica* Serotype Newport infection linked to mango consumption: impact of water-dip disinfestation technology. Clin Infect Dis **37:**1585-90.

226. **Sivapalasingam, S., C. R. Friedman, L. Cohen, and R. V. Tauxe.** 2004. Fresh produce: a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. J Food Prot **67:**2342-53.

227. **Skanavis, C.** 1994. Evaluation of composted sewage sludge based on soil amendments for potential risks of salmonellosis. J Environ Health **56:**19-25.

228. **Smars, S., L. Gustafsson, B. Beck-Friis, and H. Johnsson.** 2002. Improvement of the composting time for household waste during an initial low pH phase by mesophilic temperature control. Bioresource Technol **84:**237-241.

229. **Smith, D. R., P. R. Owens, A. B. Leytem, and E. A. Warnemuende.** 2007. Nutrient losses from manure and fertilizer applications as impacted by time to first runoff event. Environ Pollut **147:**131-137.

230. **Smith, M., D. Friend, and H. Johnson.** 2007. Composting for the homeowner. http://web.extension.uiuc.edu/homecompost/history.html. University of Illinois Extension, College of Agricultural, Consumer and Environmental Sciences, Urbana-Champaign.

231. **Soares, H. M., B. Cardenas, D. Weir, and M. S. Switzenbaum.** 1995. Evaluating pathogen regrowth in biosolids compost. Biocycle **36:**70-76.

232. **Solomon, E. B., and K. R. Matthews.** 2005. Use of fluorescent microspheres as a tool to investigate bacterial interactions with growing plants. J Food Prot **68:**870-3.

233. **Solomon, E. B., C. J. Potenski, and K. R. Matthews.** 2002. Effect of irrigation method on transmission to and persistence of *Escherichia coli* O157:H7 on lettuce. J Food Prot **65:**673-676.

234. **Solomon, E. B., S. Yaron, and K. R. Matthews.** 2002. Transmission of *Escherichia coli* O157:H7 from contaminated manure and irrigation water to lettuce plant tissue and its subsequent internalization. Appl Environ Microbiol **68:**397-400.

235. **Spencer, R., and N. Goldstein.** 2006. Mixed MSW Composting is "Steady As She Goes". Biocycle **47:**18-22.

236. **Steele, M., A. Mahdi, and J. Odumeru.** 2005. Microbial assessment of irrigation water used for production of fruit and vegetables in Ontario, Canada. J Food Prot **68:**1388-92.

237. **Steger, K., A. Jarvis, S. Smars, and I. Sundh.** 2003. Comparison of signature lipid methods to determine microbial community structure in compost. J Microbiol Methods **55:**371-82.

238. **Stentiford, E. I.** 1996. Composting control: principles and practice, p. 224-252. *In* M. De Bertoldi, P. Sequi, B. Lemmes, and T. Papi (ed.), The science of composting, Part 1. Chapman and Hall, London.

239. **Stindt, A.** 1990. Untersuchungen zur Wirkung und zu den Wirkungsmechnismen von Kompostextrakten auf Botrytis cinerea Pers. ex Nocca & Balb an Erdbeeren, Kopfsalat und Buschbohnen. Dissertation, University of Bonn. 169 pp.

240. **Stine, S. W., I. Song, C. Y. Choi, and C. P. Gerba.** 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. J Food Prot **68:**913-918.

241. **Stoffella, P. J., and B. A. Kahn.** 2001. Compost utilization in horticultural cropping systems, vol. 1. Lewis, Boca Raton, Fl.

242. **Stone, A. G., S. J. Traina, and H. A. J. Hoitink.** 2001. Particulate organic matter composition oand *Pythium* damping-off of cucumber. Soil Sci Soci Amer J **65:**761-770.

243. **Strader, R., and G. S. Bromhal.** 1997. Evaluation of temperature fluctuations in a windrow composting operation. Proc 1997 Air & Waste Manag Assoc 90th Ann Meet Exhibit, June 8-13 1997, Toronto, Ontario. p.10.

244. **Tchobanoglous, G., h. Theisen, and S. Vigil.** 1993. Integrated solid waste management: Engineering principle and management issues, vol. 1. McGraw-Hill, New York.

245. **Temorshuizen, A. J., E. van Rijn, D. J. van der Gaag, C. Alabouvette, Y. Chen, J. Lagerlof, A. A. Malandrakis, E. J. Paplomatas, B. Ramert, J. Ryckeboer, C. Steinberg, and S. ZmoraNahum.** 2007. Suppressiveness of 18 composts against 7 pathosystems: Variability in pathogen response. Soil Biol Biochem **38:**2461-2477.

246. **Tiquia, S. M., T. L. Richard, and M. S. Honeyman.** 2002. Carbon, nutrient, and mass loss during composting. Nutr Cycl Agroecosys **10:**150-161.

247. **Tiquia, S. M., N. F. Y. Tam, and I. J. Hodgkiss.** 1996. Microbial activities during composting of spent pig-manure sawdust litter at different moisture contents. Bioresour Technol **55:**201-206.

248. **Todd, E. C., J. D. Greig, C. A. Bartleson, and B. S. Michaels.** 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 2. Description of outbreaks by size, severity, and settings. J Food Prot **70:**1975-93.

249. **Todd, E. C., J. D. Greig, C. A. Bartleson, and B. S. Michaels.** 2007. Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 3. Factors contributing to outbreaks and description of outbreak categories. J Food Prot **70:**2199-217.

250. **Tognetti, C., M. J. Mazzarino, and F. Laos.** 2007. Improving the quality of municipal organic waste compost. Bioresour Technol **98:**1067-1076.

251. **Tomenson, J. A., and G. A. Matthews.** 2009. Causes and types of health effects during the use of crop protection chemicals: data from a survey of over 6,300 smallholder applicators in 24 different countries. Int Arch Occupat Environ Heal **In Press:**1-15.

252. **Tonner-Klank, L., J. Moller, A. Forslund, and A. Dalsgaard.** 2007. Microbiological assessments of compost toilets: in situ measurements and laboratory studies on the survival of fecal microbial indicators using sentinel chambers. Waste Manag **27:**1144-54.

253. **Trankner, A.** 1992. Use of agricultural and municipal organic wastes to develop suppressiveness to plant pathogens. In Biological Control of Plant Diseases: Progress Challenges for the Future, Tjamos, E.S., G.C. Papavizas and R.J. Cook (ed), Plenum Press, New York. pp. 35-42.

254. **Trankner, A., and R. Kircher-Bierschenk.** 1988. Vorlaeufige Ergenisse bei der Bekaempfung des Apfelschorfes durch Extrakte aus kompostierten organischen Materialien. Med. Fac. Landbouww. Rijkuniv. Gent **53:**359-.

255. **Trankner, A., and R. Liesenfeld.** 1990. Unterdrueckung von *Pythium ultimum*, einer keimlingskrankheit der Erbse (*Pisum sativum*) durich Saatgutbehandlung mit Kompostextrakten. Mitt. Biol. Bundesanst. Land. Forstwirtsch. **266:**64.

256. **Tuitert, G., M. Szczach, and G. J. Bollen.** 1998. Suppression of *Rhizoctonia solani* in potting mixtures amended with compost made from organic household waste. Phytopath **88:**764-773.

257. **Turner, C.** 2002. The thermal inactivation of *E. coli* in straw and pig manure. Bioresour Technol **84:**57-61.

258. **Turner, C., A. Williams, R. White, and R. Tillett.** 2005. Inferring pathogen inactivation from the surface temperatures of compost heaps. Bioresour Technol **96:**521-529.

259. **United States. Bureau of the Census., United States. Agency for International Development., International Programs Center (U.S.), and United States. Agency for International Development. Office of Population.** 1986. World population profile, p. v. U.S. Dept. of Commerce For sale by the Supt. of Docs., U.S. G.P.O., [Washington, D.C.].

260. **Urban, J., and A. Trankner.** 1993. Control of gray mold (*Botrytis cinerea*) with fermented compost/water extracts. *In* N. J. Fokkema, J. Kohl, and Y. Elad (ed.), Biological control of foliar and post-harvest diseases: proceedings of a workshop. Bulletin of the International Organization for Biological and Integrated Control of Noxious Animals and Plants, West Palearctic Regional Section.

261. **Von Wintzingerode, F., F. A. Rainey, R. M. Kroppenstedt, and E. Stackenbrandt.** 1997. Identification of environmental strains of *Bacillus mycoides* by fatty acid analysis and species-specific 16S rDNA oligonucleotide probe. FEMS Microbiol Ecol **24:**201-209.

262. **Wachtel, M. R., L. C. Whitehand, and R. E. Mandrell.** 2002. Association of *Escherichia coli* O157:H7 with preharvest leaf lettuce upon exposure to contaminated irrigation water. J Food Prot **65:**18-25.

263. **Wachtel, M. R., L. C. Whitehand, and R. E. Mandrell.** 2002. Prevalence of *Escherichia coli* associated with a cabbage crop inadvertently irrigated with partially treated sewage wastewater. J Food Prot **65:**471-5.

264. **Wakase, S., H. Sasaki, K. Itoh, K. Otawa, O. Kitazume, J. Nonaka, M. Satoh, T. Sasaki, and Y. Nakai.** 2008. Investigation of the microbial community in a microbiological additive used in a manure composting process. Bioresour Technol **99:**2687-93.

265. **Wang, S., and S. Lin.** 2002. Composts as soil supplement enhanced plant growth and fruit quality of strawberry. J Plant Nutr **25:**2243–2259.

266. **Warriner, K., F. Ibrahim, M. Dickinson, C. Wright, and W. M. Waites.** 2003. Interaction of *Escherichia coli* with growing salad spinach plants. J Food Prot **66:**1790-7.

267. **Waterstripe, K. E., E. R. Ingham, and S. M. Robideaux.** 2005. Interactions of bacteria, fungi and *E. coli* in aerobically brewed compost teas. Applied Soil Ecology **(in press)**.

268. **Welke, S. E.** 2004. The effect of compost extract on the yield of strawberries and the severity of *Botrytis cinerea*. J Sustain Agricul **1:**57-68.

269. **Welke, S. E.** 2001. Effectiveness of compost extracts as disease suppressants in fresh market crops in BC. Project number 00-03; Research report submitted to: Organic Farming Research Foundation. www.ofrf.org Santa Cruz, CA.

270. **Welke, S. E.** 1999. Effectiveness of compost extracts as disease suppressants in fresh market crops in BC. Project number 99-31; Research report submitted to: Organic Farming Research Foundation. www.ofrf.org Santa Cruz, CA.

271. **Weltzien, H. C.** 1991. Biocontrol of foliar fungal disease with compost extracts, p. 430-450. *In* J. H. Andrews and S. S. Hirano (ed.), Microbial Ecology of Leaves. Springer-Verlag, New York.

272. **Weltzien, H. C.** 1988. Presented at the global perspectives on agroecology and sustainable agricultural systems (Proceeding of the sixth international conference of IFOAM). The effects of compost extracts on plant health, University of California-Santa Cruz, Santa Cruz, CA.

273. **Weltzien, H. C.** 1989. Some effects of composted organic materials on plant health. Agric. Ecosystems Environ. **27:**439-446.

274. **Weltzien, H. C.** 1990. The use of composted materials for leaf disease suppression in field crops. Monograph - British Crop Protection Council**:**115-120.

275. **Weltzien, H. C., K. Budde, N. Ketterer, C. Samerski, and A. Stindt.** 1990. Improved plant health through applications of composted organic material and composts extracts. Agricultural alternatives and nutritional self-sufficiency : for a sustainable agricultural system that respects man and his environment : proc of the IFOAM Seventh Internat Scientific Conference, Ouagadougou, January 2-5, 1989.**:**377-379.

276. **Weltzien, H. C., and N. Ketterer.** 1986. Control of downy mildew, *Plasmopara viticola* (de Bary) Berlese et de Toni, on grapevine leaves through water extracts from composted organic wastes. Phytopathol **116:**186-188.

277. **Weltzien, H. C., and N. Ketterer.** 1986. Control of *Phytophthora infestans* on tomato leaves and potato tubers through water extracts of composted organic wastes. Phytopathol **76:**1104.

278. **Winter, C. K., and S. F. Davis.** 2006. Organic Foods. Journal of Food Science **71:**117-124.

279. **Xi, B., H. Liu, W. Meng, H. Li, and Q. Wang.** 2001. [Composting process of municipal solid waste with high effective complex microbial community]. Huan Jing Ke Xue **22:**122-5.

280. **Xiong, L., and R. Shi.** 1994. Manganese toxicity to plants in autoclaved soil and the alleviation effect of vesicular arbuscular mycorrhizae. Acta Pedologica Sinica **31:**231-241.

281. **Yanko, W. A.** 1987. Occurrence of pathogens in distribution and marketing municipal sludges. Report No.: EPA/600.1-87/014. (NTIS: PB88-154273/AS). Springfield, VA: National Technical Information Service.

282. **Yao, S., I. A. Merwin, G. S. Abawi, and J. E. Thies.** 2006. Soil fumigation and compost amendment alter soil microbial community composition but do not improve tree growth or yield in an apple replant site. Soil Biol Biochem **38:**587-599.

283. **Yohalem, D. S., R. F. Harris, and J. H. Andrews.** 1994. Aqueous extracts of spent mushroom substrate for foliar disease control. Compost Sci Util **2:**67-74.

284. **Zhang, W., D. Y. Han, W. A. Dick, K. R. Davis, and H. A. J. Hoitink.** 1998. Compost and compost water extract-induced systematic acquired resistance in cucumber and Arabidopsis. Phytopathol **88:**450-455.

285. **Zhang, W., H. D. Y., D. W. A., D. K. R., and H. A. J. Hoitink.** 1998. Compost and compost water extract-induced systemic acquired resistance in cucumber and Arabidopsis. Phytopathology **88:**450-455.

286. **Zhang, X., L. Zhou, Q. Shen, L. Zong, X. Zhan, Q. Wu, X. Liao, and W. C. Wong.** 2002. [Changes of chemical and biological properties during composting sewage sludge by using forced aerated static pile model]. Ying Yong Sheng Tai Xue Bao **13:**467-70.

CURRICULUM VITAE

David Thomas Ingram
3504 Falling Green Road
Olney, MD 20832-1128

Cell (301) 938-2304
David.Ingram@ars.usda.gov

Education

Ph.D., Food Science, 2009.
University of Maryland, College Park, Maryland
Department of Nutrition and Food Science
Dissertation: Implications of Foodborne Pathogen Survival During Production and Pre-harvest Application of Compost and Compost Tea

M.S., Microbiology, 1999.
University of Maryland, College Park, Maryland.
Department of Molecular and Cellular Biology
Thesis: Development and Evaluation of the *E. coli* SELeCT™ System: A 24hr Method for the Isolation and Quantification of *E. coli* O157:H7 in Raw Meat and Poultry

B.S., Biology, 1994.
Dickinson College, Carlisle, Pennsylvania.

Research Experience

October 1998 – Present. Research Microbiologist (GS-9)
Environmental Microbial and Food Safety Laboratory (2008-present)
Environmental Microbial Safety Laboratory (2002-2007)
Animal Waste and Pathogen Laboratory (2001-2002)
Sustainable Agriculture Systems Laboratory (1998-2000)
USDA, ARS, ANRI
Building 201, Room 201A, BARC-EAST
10300 Baltimore Avenue
Beltsville, Maryland 20705-2350
Supervisors: Dr. Patricia Millner, Research Leader, 301-504-5631
Dr. Manan Sharma, Research Leader, 301-504-9198

Research activities involved a variety of activities that combined various techniques in both clinical and environmental microbiology. Research activities involved the study of human pathogens (mostly foodborne, but also bio-aerosol related) in various

farming practices that may contribute to the health and safety of the consumer and the food supply. Study highlights include: Surveyed fresh melon production plants for the prevalence and dissemination of antibiotic resistant Enterococci; Drafted national standards for the production and testing of foodborne pathogen in compost (United States Composting Council); Developed methods for on-farm rapid disinfection of foodborne pathogens in animal manures; Collected and analyzed Bio-aerosols from on-farm biosolids, manure and compost application events. Studied the fate of pathogenic bio-aerosols surrounding Combined Animal Feeding Operations (CAFO). Studied the dissemination of foodborne pathogens due to various farming practices (Conventional and Organic) that use contaminated manures, composts and compost teas as bio-control and/or fertilizer during production of fruits and vegetables; Developed methods to characterize the internalization potential of *E. coli* O157:H7 into the root and leaf tissues of baby spinach plants using hydroponic and soil growth systems.

June 1997 - October 1998. Research Technician.

Food Safety Laboratory
Department of Nutrition and Food Science
Rm.3420, Marie Mount Hall
University of Maryland at College Park
College Park, Maryland 20742-7521
Supervisor: Dr. Jianghong Meng, Assistant Professor, 301-405-1399.

Determined the capacity for growth and survival of enterohemorrhagic *E. coli* (EHEC) through the germination and storage of inoculated alfalfa sprouts, determined the prevalence and antibiogram (antibiotic resistance patterns) of *E. coli* and *Salmonella* isolates from retail meats, developed a novel molecular method (Strand Displacement Amplification) for the detection of EHEC in foods, evaluated several food additives in an attempt to control pathogens in ground beef, evaluated a rapid ELISA (O157 Dipstick, Kirkegaard and Perry Laboratories) for the detection of *E. coli* O157 in meats, determined the prevalence of *Salmonella* DT104 in clinical feline fecal samples from two Northern Virginia and two Maryland animal hospitals.

January - December 1995. Research Assistantship.

Pathogenic Microbiology Laboratory
Rm.1109, Microbiology Building
Department of Cellular Biology and Molecular Genetics
University of Maryland at College Park (UMCP)
College Park, Maryland 20742-4451
Supervisor: Dr. Sam W. Joseph, Professor, 301-405-5452

Funded by the United States Department of Agriculture, Food Safety Inspection Service (USDA-FSIS) to develop and evaluate methods for detecting and quantifying microbial foodborne pathogens in raw red meat and poultry.

June - August 1994. NIH Research Fellowship (MBCD,COP,DCT,NCI).
Molecular Breast Cancer Division; Clinical Oncology Program; Department of Cancer Treatment; National Cancer Institute; National Institutes of Health.
Building 10, Room 12C112
Bethesda, MD 20892
Supervisor(s): Dr. Jeffrey Moscow; Dr. Kenneth Cowan

Developed a PCR assay to determine the allelic frequency of a putative lung tumor suppressor gene (Human glutathione peroxidase 1 gene, *hgpx*1.)

June - August 1993. NIH Research Fellowship (MBCD,COP,DCT,NCI).
Supervisor(s): Dr. Jeffrey Moscow; Dr. Kenneth Cowan

Trained in molecular genetics techniques including: PCR, rt-PCR, Southern and Northern hybridizations, Western blots and DNA sequencing.

Teaching Experience

October 2006. Teaching Assistantship.
Pathogenic Microbiology (BSCI 424)
University of Maryland University College (UMUS) at Shady Grove.

August - December 1999. Teaching Assistantship.
Medical Microbiology (MICB 440)
University of Maryland University College (UMUC) at Shady Grove.

September - December 1996. Teaching Assistantship.
Pathogenic Microbiology (MICB 440) Laboratory
University of Maryland at College Park (UMCP).

June - August 1996. Teaching Assistantship.
Introduction to Microbiology (MICB 200) Laboratory, UMCP.

January - May 1996. Teaching Assistantship.
Introduction to Microbiology (MICB 200) Laboratory, UMCP.

September - December 1995. Teaching Assistantship.
Pathogenic Microbiology (MICB 440) Laboratory, UMCP.

Publications

Sharma, M., Ingram, D.T., Patel, J.R., Millner, P.D., Wang, X., Hull, A., Donnenberg, M. Internalization of *E. coli* O157:H7 in spinach cultivated in soil and hydroponic media, Journal of Food Protection (2009, In Press).

Ingram, D.T., Millner, P.D. Factors affecting compost tea as a potential source of *Escherichia coli* and *Salmonella* on fresh produce, Journal of Food Protection 70 (4):828-834, 2007

Harper, L.A., Weaver, L.H., Flesch, T.K., Wilson, J.D., Millner, P.D., Ingram, D.T. 2005. Nitrogen and other trace-gas emissions from swine production in the central great basin. Government Publication/Report for Agreement Number 58-6612-3-234. April 22, 2005.

Gagliardi, J.V., Millner, P.D., Lester, G., Ingram, D. On-Farm Postharvest Processing Sources of Bacterial Contamination to Melon Rinds, Journal of Food Protection, 66 (1):82-87, 2003.

Joseph, SW., Ingram, DT., Kaper, J. The Epidemiology, Pathogenicity and Microbiology of Foodborne *Escherichia coli* O157:H7**,** Reviews in Medical Microbiology, 13 (2):53-62, 2002.

Ingram, DT., Lamichhane, CM., Rollins, DM., Carr, LE., Mallinson, ET., Joseph, SW., Development of a Colony Lift immunoassay to Facilitate Rapid Detection and Quantification of *Escherichia coli* O157:H7 from Agar Plates and Filter Monitors, Clinical Diagnostic Laboratory Immunology, 5 (4), 1998.

Moscow, JA; Schmidt L; Ingram, DT; Gnarra J; Johnson B; Cowan, KH. Loss of Heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. Carcinogenesis, 15(12):2769-73, 1994.

Abstracts and Conference Proceedings

Ingram, D.T., Sharma, M., Mudd, C, Ferguson, S., 2009. Epiphytic Survival of Enterohemorrhagic and Avian Pathogenic *Escherichia coli* from Spinach Plants after Overhead Irrigation with (Currently Accepted) Contamination levels. International Association for Food Protection, July 2009, Grapevine Texas.

Ingram, D.T., Millner, P.D., Reynolds, S. 2009. Effects of Compost Tea and Compost Socks on Microbiological and Harvest Quality of Strawberry Fruits. International Association for Food Protection, July 2009, Grapevine Texas.

Nou, X, Millner, P., Patel, J., Sharma, M. and Ingram, D.T. Effect of fresh produce crop residue on the survival of *Escherichia coli* O157:H7 in soil. International Association for Food Protection, July 2009, Grapevine Texas.

Ingram, D.T., Patel, J., Millner, P.D. 2008. Prevalence of Shiga-Toxigenic *E. coli* and *Salmonella* in Commercially Available Compost. International Association for Food Protection , August 3-6, 2008, Columbus, Ohio.

Millner, P.D., Ingram, D.T., Reynolds, S., Shelton, D. and Patel, J. 2008. Characterization of Microbial Content of Organic and Conventional Produce in Maryland Relative to Production Practices and Inputs. International Association for Food Protection , August 3-6, 2008, Columbus, Ohio.

Ingram, D.T., Scheuerell, S.J., Millner, P.D. 2005. Evaluation of two compost tea brewing methods for the ability to propagate foodborne pathogens. American Society for Microbiology, June 5-9, 2005, Atlanta, GA.

Millner, P.D., Vanotti, M.B., Ingram, D.T., Ellison, A.Q., Hunt, P.G., Szogi, A.A. 2005. Microbial disinfection during multistage treatment of swine manure. 105th General Meeting of the American Society for Microbiology, Georgia World Congress Center, June 5-9, 2005, Atlanta, GA.

Ingram, D.T., Millner, P.D., Fecal Bacterial Pathogens and Indicators in Commercially Available Compost, International Association for Food Protection (IAFP, formerly IAMFES), P184, Program and Abstract Book p.111, Annual Meeting 2004.

Ingram, D.T., C. Lamichhane, S.Zhao, J. Meng, S. Joseph. Evaluation of a Dipstick-style ELISA for the detection of *E. coli* O157:H7 in raw ground beef. Presented, Annual Meeting of the American Society for Microbiology, 1999.

Ingram, D.T., Kantor, M.A., Meng, J. Prevalence and Survival of *E. coli* O157:H7 during the germination and storage of alfalfa sprouts. International Association for Food Protection, (IAFP, formerly IAMFES), Annual Meeting, August 1998. **Awarded third place in graduate student poster competition, IFT 1999.**

Gagliardi, J.V., Karns, J.S., Ingram, D.T., Survival of *E. coli* O157:H7 from Manure and Irrigation Water in Soil and on Cover Crops, Presented at the annual USDA, BARC Poster Day, December 1998.

Ingram, D.T., Mallinson, E.T., Rollins, D., Joseph, S.W., Comparison of a Rapid Diagnostic Assay (*E. coli* SELeCT ™) and MPN Methodologies for the Isolation, Quantification and Confirmation of *E. coli* O157:H7 in Raw Meat Products, Southern Poultry Science Society, Southern Conference on Avian Diseases, Atlanta GA., 1997**. (awarded first place in open poster competition)**.

Ingram, D.T., Rigakos, C.G., et. al., Development and Evaluation of a 24 hr Method (*E.coli* SELeCT™) for the Detection, Isolation and Identification of *Escherichia coli* O157:H7 in Raw Ground Meat and Poultry, Poster presentation at the VTEC '97 meeting in Baltimore, MD. June 22-28, 1997

Ingram, D.T., Rigakos C.G., Mallinson, E.T., et. al., Development of a 24 hr Assay (*E. coli* SELeCT™) for the isolation, quantification and positive identification of *E. coli* O157:H7 in Poultry and Red Meat, Abstracts, Annual Meeting of the American Society for Microbiology, 1996.

Technical Presentations

January 13, 2006, "The Latest on Compost Teas"; Future Harvest: Farming for Profit and Stewardship, 7th Annual Conference, Hagerstown, MD. www.futureharvestcasa.org/Final%20Conference%202006%20B.pdf

September 2005, "Compost Tea: An On-Farm Source of Foodborne Pathogens?" Mid-Atlantic Composting Association, Beltsville, MD. Semi-Annual Meeting 2005. www.midatlanticcompost.org/presentations.html

August 2004, "Growth of Foodborne Pathogens during Production of Compost Tea" International Association for Food Protection, Phoenix, AZ. Annual Meeting 2004. Program and Abstract Book T76, p. 157.

October 2004, 2003, 2001, 2000 (12th, 10th, 9th respectively) Annual Better Composting School, University of Maryland Cooperative Extension. Departments of Biological Resources Engineering, Natural Resource Sciences and Landscape Architecture, University of Maryland College Park. Discussed practices, research and goals of current composting projects at the USDA composting research facility in Beltsville, MD.

August 6 1998, ASM Student Chapter, Walter Reed Army Hospital. Rapid Diagnostic Methodologies for the detection of EHEC in foods.

June 17-18, 1997, Chesapeake Area Microbial Pathogenesis (CAMP '97), Baltimore, MD. Development and Evaluation of a 24hr Method for the Detection and Enumeration of *E. coli* O157:H7 in Spiked Ground Beef Samples.

Leadership and Volunteer Experiences

October 1, 2008 – Food Safety Camp. Led an educational microscopy class in this ARS-FSIS educational program for Elementary schools, GWCC, Beltsville, MD.

August 2005 – Present. Vice President of Education, Toastmasters International, Plant Industry Station, Club 2627, Area 44, Division D, District 36, Beltsville MD.

May 2000 - 2007. Volunteer, elementary school science fair judge; K-6.
Lanham Christian School, 8400 Good Luck Road, Lanham, MD 20706.
Greenbelt Elementary School. 66 Ridge Road Greenbelt, MD 20770

June 1999, 2000. Volunteer, Annual BARC Public Field Day.
10300 Baltimore Avenue, Beltsville, MD 20705.

1992 – 1997. Veterinary Medical Technician (Part-time).
Potomac Animal Hospital, Potomac, Maryland.
Responsible for the care and treatment of the animals and kennel staff coordinator. Weekend and holiday position.

1992 - 1994. Residence Hall Advisor (RA).
Responsible for the guidance of 40 first year men and women at Dickinson College (two years).

1994. Captain, Varsity Swim Team, Dickinson College.
Lettered in swimming for four years; Varsity lacrosse for two years.

1989 - 1991. Lifeguard/ Water Safety Instructor.
Country Glen Swim and Tennis Club, Potomac, Maryland. Taught standard Red Cross curriculum to students ages six to 46. Summer job (three years).

1989 and 1990. Camp Counselor.
Green Acres Day Camp, Rockville, Maryland. Responsible for twenty children ages four to six. Summer job (two years).

Membership in Professional Associations

American Society for Microbiology (ASM)
International Association for Food Protection (IAFP)
American Association for Food Hygiene Veterinarians (AAFHV)
Professional Association for Dive Instructors (PADI #9109212125; June 30 1991)
Toastmasters International, Plant Industry Station Club# 2627, Area 44, Division D, District 36

International Travel Experience

Antigua, Austria, Bermuda, Barbados, Costa Rica, Denmark, England, Finland, France, Germany, Italy, Japan, Russia, Spain.

Avocations

Triathlon, camping, skiing, guitar, chess, marksmanship, scuba

Proficiencies

Microsoft Office 2007, WordPerfect, Adobe Creative Suite 2, SAS for Windows 9.2, fluent in both Windows and Mac operating systems; Some knowledge of written and spoken Spanish.

Citizenship: United States of America

Date of Birth: February 12, 1972

April, 2009