Effects of Aeration, Molasses, Kelp, Compost Type, And Carrot Juice on the Growth of *Escherichia Coli* in Compost Teas

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Growth of a nonpathogenic *E. coli* strain (K12- MG1655, ATCC 700926) in aerated and nonaerated compost teas containing molasses, kelp and carrot juice was examined. Teas were prepared using four different compost types that had undetectable levels of indigenous *E. coli*. Three of the composts were produced by turn pile windrow composting method using dairy, swine and horse manure as feedstock, while the fourth, a vermicompost, was produced by feeding separated dairy solids to worms *Eisenia fetida*. Molasses and kelp enhanced the growth of *E. coli* in inoculated teas and the *E. coli* density was positively correlated with nutrient concentrations ranging from 0.1 to 8.0 g/L. Irrespective of the presence of molasses and kelp, *E. coli* was not detected in noninoculated teas. Even though *E. coli* is a facultative anaerobe, its growth was significantly higher in nonaerated teas than in aerated teas. Without aeration, dissolved oxygen in teas declined rapidly and fell below 0.1 mg/L within 20 h, whereas continuous aeration at 0.8 L/min maintained an aerobic condition (> 5 mg/L dissolved oxygen) in teas during the 48 h brewing period. The pH values of nonaerated teas were significantly lower than those of aerated teas and were always slightly acidic. *E. coli* growth in different compost types was significantly different. The density of *E. coli* was lowest in teas made with vermicompost and highest in teas made with swine manure compost. *E. coli* proliferations in both aerated and nonaerated swine manure compost teas were inhibited by carrot juice. Carrot juice lowered dissolved oxygen in aerated teas. The total bacterial densities in noninoculated compost teas were not reduced by carrot juice.

**Introduction**

Composts made from livestock manures are often used to prepare compost teas that are applied on plants to improve growth and suppress plant diseases (Weltzien 1991; Scheuerell and Mahaffee 2002). Even though composting kills pathogens (Larney et al. 2003; Lung et al. 2001) that may be present in manure (Pell 1997), there is a growing concern that compost teas are at risk for regrowth of any residual pathogenic strains of *E. coli* or other enteric pathogens remaining in compost. Addition of nutrients such as molasses or kelp to promote the growth of beneficial microbes has heightened concerns about regrowth. Reported effects of molasses on regrowth of *E. coli* in compost teas are contradictory. Bess et al. (2002) noted that addition of molasses to teas enhanced the growth of *E. coli*, while omission of molasses either reduced or eliminated *E. coli* in the starting compost. Duffy et al. (2004) observed a positive correlation between regrowth of virulent *E. coli* serotype O157:H7 and molasses at concentrations above 0.2% (v/v) in nonaerated compost teas. Waterstripe et al. (2005), however, noted that high concentrations (2.4% v/v) of molasses significantly reduced the growth of *E. coli* and observed that irrespective of the amount of molasses added, compost teas did not contain *E. coli* when made from composts devoid of any detectable levels of *E. coli*. Waterstripe et al. (2005) also observed that anaerobic conditions favoured the growth of *E. coli* and suggested that under aerobic condition *E. coli* cannot compete successfully with other bacteria, or fungi and protozoa in the tea. In addition to nutrients, compost type also appeared to affect the regrowth of pathogens. Duffy et al. (2004) observed a significantly greater regrowth of *Salmonella enterica* in teas made with chicken manure derived compost when compared to teas made with dairy manure derived compost. Furthermore, of the relatively few studies on *E. coli* in compost teas, only one study reported oxygen concentration which exceeded saturation levels. The present study was undertaken to examine the effects of aeration, additional nutrients (molasses, kelp), and compost type on the growth of *E. coli* in compost teas. In addition, carrot juice was investigated as a possible inhibitor of *E. coli* in compost teas since carrot tissue fluid inhibited the growth of pathogenic *E. coli* serotype O157:H7 (Abdul-Raouf et al. 1993). Cultured carrot cells are known
to secrete an antimicrobial phytoalexin 6-methoxymellein (Kurosaki and Nishi 1983). Kelp is often added to teas as a protein supplement along with other nutrients (Ingham and Alms 1999), but its effect on E. coli has not received much attention.

**Materials and Methods**

**Compost Characterization**

Four composts, dairy manure compost (DMC), swine manure compost (SMC) and horse manure compost (HMC) and a vermicompost (VC) were used in the study. DMC, SMC and HMC were prepared according to (Kannangara et al. 2000, 2004). Briefly DMC and SMC were prepared from soiled bedding and manure collected from barns bedded with wood shavings. Material was composted in 30 m x 3 m and 1.8 m high aerated concrete channels in an in-vessel composting for six weeks while turning the piles twice a week. HMC was prepared from wood shaving bedded horse stall waste. Material was composted on a roofed concrete pad using open windrow piles trapezoidal in section with top dimensions 1.21 m x 1.21 m and bottom dimensions 3.04 m x 3.04 m. Piles were turned twice a week for first four weeks and once a week for eight additional weeks. VC was obtained from Compost Science Biotechnologies Inc. Chilliwack, British Columbia where it was produced by feeding partially composted (composted for three weeks to remove readily produced heat energy) separated dairy solids to worms (Eisenia fetida) in 35 m x 2 m and 1.2 m high bins. All composts used in the experiment were minimum 6 months old following composting. All composts were characterized with respect to pH, electrical conductivity (EC), nitrate, ammonium, total bacterial and fungal counts and E. coli density. EC and pH were determined in a 1:1 (v:v) compost to deionised water mixture after continuous shaking for one hour. Nitrate and ammonium in the extracts were determined using a flow injector analyzer (Tecator FIA Star 5010A, Tecator, Sweden). In addition, nitrate and ammonium contents in the extracts were determined using a flow injector analyzer (Tecator FIA Star 5010A, Tecator, Sweden).

**Determination of Dissolved Oxygen, pH in Aerated and Nonaerated Noninoculated Dairy Manure Compost Teas**

To determine the effectiveness of aeration in maintaining an aerobic condition in teas, six 2 L glass jars of teas were prepared. DMC (26 g) was added to each jar containing 4 g molasses and 4 g kelp in 1L of preaerated water. Immediately following the addition of compost, three jars were aerated continuously at 0.8 L/min for 48 h using a single head oil-less Barnant diaphragm pump (Barnant Company, Illinois) and the remaining jars were left nonaerated. The oxygen concentrations in aerated and nonaerated teas were monitored at 0, 1, 2, 4, 6, 20 and 48 h using an oxygen meter (Orion, Model 850Aplus). The pH of the teas was determined at the end of 48 h.

**Preparation of E. coli Inoculum and Compost Tea**

Since none of the composts used in the study contained detectable levels of E. coli, a nonpathogenic E. coli strain (K12- MG1655, ATCC 700926) was inoculated into compost teas to compare the potential for growth of E. coli in experimental teas. E. coli inoculum was prepared by washing a 9 cm diameter petri dish of E. coli cultured on nutrient agar with 100 ml sterile water and then further diluting the washing 100 times with sterile water. Two mls of the diluted washings were inoculated into each jar of compost tea. The inoculum cell density was determined by plating 1 ml of
appropriately diluted inoculum in phosphate buffer (pH 7.2) on a 3M Petrifilm *E. coli* count plate. The *E. coli* density in the inoculants ranged from $1 \times 10^6$ to $1 \times 10^7$ CFU per ml. A new plate of *E. coli* was used for preparation of inoculants for each experiment. All compost teas used in the experiments were made by suspending 26 g of compost in 1 L of water in 2 L glass jars.

Effects of Molasses and Kelp on the Growth of *E. coli* In Aerated Dairy Manure Compost Tea

Compost teas made with DMC were amended with molasses or kelp at 0, 0.1, 1.0, 2.0, 4.0 or 8.0 g /L of water. All treatments were replicated thrice and each replicate was inoculated with 2 ml of *E. coli* inoculum as described previously. All the jars were aerated continuously at 0.8 L min$^{-1}$ for 48 h at room temperature (23°C). At the end of 48 h, 10ml aliquots were taken from each jar, diluted in sterile phosphate buffer and 1ml of appropriate dilutions were plated on 3M Petrifilm *E. coli* count plates to determine the *E. coli* densities. The effect of kelp on the growth of *E. coli* was determined using an identical experimental procedure using kelp at the same concentration in place of molasses.

Effect of Aeration and Nonaeration on the Growth of *E. coli* in Dairy Manure Compost Teas

The effects of aeration and nonaeration on the growth of *E. coli* were compared using DMC teas amended with molasses or kelp each at 4 g /L. Twelve jars of teas were prepared. Six jars were inoculated with *E. coli* as described previously and the remaining six jars were left noninoculated to serve as controls. Three jars from each of the inoculated and noninoculated treatments were aerated continuously at 0.8 L per min for 48 h and the rest were left nonaerated. At the end of 48 h, contents of the jars were stirred and 10 ml aliquots from each jar were withdrawn and *E. coli* densities were determined using 3M Petrifilm *E. coli* count plates as before. The pH, EC and oxygen concentration of the teas were determined at the end of 48 h.

Effects of Compost Type on the Growth of *E. coli* in Compost Teas

Compost teas were prepared using DMC, SMC and HMC and VC and amended with molasses and kelp each at 4 g /L. All teas were inoculated with *E. coli* as described previously. The teas for each compost type were replicated thrice and aerated continuously at 0.8 L per min for 48 h. At the end of 48 h, 10ml aliquots were withdrawn from each jar and *E. coli* densities were determined using 3M Petrifilm *E. coli* count plates as before. The pH, EC and oxygen concentration of the teas were determined at the end of 48 h.

In addition, to determine whether differences in *E. coli* growth that might be observed are due the differences in the mineral nutrients that get extracted into water, the aqueous phases obtained from unamended/noninoculated compost teas were analyzed for nutrients in a certified private laboratory (Norwest).

Effect of Carrot Juice on the Growth of *E. coli* In Aerated and Nonaerated Swine Manure Compost Teas

Twelve jars of kelp and molasses amended SMC teas were prepared. Six of the jars contained 100 ml of carrot juice included in the final volume of 1L. Carrot juice was prepared by homogenizing 800 g of carrot (*Daucus carota* var. *sativus*) root in 2.5 L water in a glass jar Waring blender. The homogenate was filtered through two layers of cheese cloth and centrifuged at 4000 g for 15 min and the supernatant decanted and used in teas. All the jars were inoculated with *E. coli* as described previously. Half of the jars with, and half without carrot juice were aerated continuously at 0.8 L per min for 48 h and the remainder left nonaerated. At the end of 48 h *E. coli* densities of the teas were determined using 3M Petrifilm *E. coli* count plate as described previously. The pH, EC and oxygen concentration of the teas at 48 h were determined.

Effect of Carrot Juice on Total Culturable Bacterial Density in Dairy and Swine Manure Compost Teas

Two sets of six jars of kelp and molasses-amended compost teas were prepared. One set contained DMC while the other contained SMC. In addition, 100 ml each of carrot juice was included in three jars from each set in the final volume of 1L. Carrot juice was prepared as described previously. All the jars were aerated continuously at 0.8 L per min for 48 h. At the end of 48 h, 10 ml aliquots were withdrawn from each tea and ten fold serial dilutions were prepared in sterile water. Each dilution was plated on nutrient agar at 0.1 ml per plate and incubated at 25°C for 72h. Bacterial colonies were counted and the bacterial densities in teas were determined.

All the data were analysed by ANOVA using SigmaStat (SPSS Inc, Chicago, Illinois) software package and the means were compared using Tukey’s test.

Results and Discussion

The C: N ratio was the least in VC and highest in HMC (Table 1) in solid composts. The available car-
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TABLE 1.
Carbon, C:N ratio and mineral contents of composts and compost teas (unamended and noninoculated aerobic).
DMC, SMC and HMC are dairy, swine and horse manure composts respectively. VC is vermicompost.

<table>
<thead>
<tr>
<th>Compost Type Characteristic</th>
<th>Solid Compost</th>
<th>Unamended/Noninoculated Compost Tea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DMC</td>
<td>SMC</td>
</tr>
<tr>
<td>C:N</td>
<td>14.1</td>
<td>17.0</td>
</tr>
<tr>
<td>Total C%</td>
<td>36.5</td>
<td>33.40</td>
</tr>
<tr>
<td>Available C%</td>
<td>10.5</td>
<td>6.65</td>
</tr>
<tr>
<td>% Major Elements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2.59</td>
<td>1.96</td>
</tr>
<tr>
<td>P</td>
<td>1.00</td>
<td>2.19</td>
</tr>
<tr>
<td>K</td>
<td>3.07</td>
<td>1.97</td>
</tr>
<tr>
<td>Ca</td>
<td>2.62</td>
<td>1.938</td>
</tr>
<tr>
<td>Mg</td>
<td>0.654</td>
<td>1.096</td>
</tr>
<tr>
<td>S</td>
<td>0.48</td>
<td>0.60</td>
</tr>
<tr>
<td>Na</td>
<td>0.404</td>
<td>1.096</td>
</tr>
<tr>
<td>ppm Minor Elements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe</td>
<td>2340</td>
<td>3350</td>
</tr>
<tr>
<td>Mn</td>
<td>440</td>
<td>397</td>
</tr>
<tr>
<td>Zn</td>
<td>205</td>
<td>542</td>
</tr>
<tr>
<td>Cu</td>
<td>47.1</td>
<td>122</td>
</tr>
<tr>
<td>B</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>Mo</td>
<td>4.8</td>
<td>4.6</td>
</tr>
</tbody>
</table>

**TABLE 2.**
Chemical and microbial characteristics of the composts used in the experiment

<table>
<thead>
<tr>
<th>Compost Type</th>
<th>DMC</th>
<th>SMC</th>
<th>VC</th>
<th>HMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₃⁻ (mg/g dry wt)</td>
<td>0.22 ± 0.02e</td>
<td>0.88 ± 0.01</td>
<td>0.2 ± 0.01</td>
<td>n.d.4</td>
</tr>
<tr>
<td>NH₄⁺ (mg/g dry wt)</td>
<td>0.11 ± 0.01</td>
<td>0.38 ± 0.003</td>
<td>0.91 ± 0.04</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>pH</td>
<td>7.7 ± 0.01</td>
<td>8.1 ± 0.02</td>
<td>6.4 ± 0.02</td>
<td>6.2 ± 0.01</td>
</tr>
<tr>
<td>EC (mS/cm²/g)</td>
<td>5.2 ± 0.04</td>
<td>9.0 ± 0.01</td>
<td>3.5 ± 0.01</td>
<td>2.1 ± 0.02</td>
</tr>
<tr>
<td>Bacterial cfu² (per g/dry wt)</td>
<td>3 x 10⁷</td>
<td>1 x 10⁷</td>
<td>2 x 10⁷</td>
<td>9 x 10⁶</td>
</tr>
<tr>
<td>Fungal cfu² (per g/dry wt)</td>
<td>7 x 10³</td>
<td>2.3 x 10³</td>
<td>1.5 x 10⁴</td>
<td>1.2 x 10⁴</td>
</tr>
<tr>
<td>E. coli</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

aDairy manure compost; bSwine manure compost; cVermicompost; dHorse manure compost; e±S.E., n=3; fnot detected; miliSiemens per centimeter; Colony forming units

bon was the least in VC and highest in DMC. Major elements were higher in DMC and SMC compared to VC and HMC in solid composts. The minor elements Mn, Zn, B, and Cu were higher in DMC and SMC compared to VC and HMC. However, both VC and HMC had higher Fe than either of DMC or SMC. In unamended and noninoculated compost teas SMC had higher N and P compared to DMC, VC and HMC compost teas. The elements Ca, Mg and S did not differ substantially in any of the compost teas. More Na was found in SMC compared to other teas. Minor elements did not differ in composts teas except for Zn where tea made with SMC had substantially higher levels than the others. Since the agitation that accompanies the aeration process encourages the dissolution of nutrients into water compared to the unagitated anaerobic teas, it was decided to analyze only the aerobic teas for comparison of the compost teas in terms of nutrients.

Nitrate was highest in SMC and was not detectable in HMC (Table 2). The nitrates of VC and DMC were similar. The highest level of ammonium was found in VC followed by SMC. The ammonium content of HMC and DMC were similar. The EC was highest in SMC and lowest in HMC. The bacterial density was highest in HMC and lowest in SMC. The fungal densities were higher in VC and HMC compared to DMC and SMC. E. coli were not detected in 10% suspension of any of the compost either by 3M Petrifilm or by the MPN method. The composts were therefore considered to be devoid of any detectable levels of E. coli.
The oxygen concentrations in the teas aerated continuously at 0.8 L / min stayed at or above 6 mg / L during 48 h brewing period (Figure 1). This represents 69% of saturation level of dissolved oxygen in water at 23°C at atmospheric pressure (Weiss 1970). Generally 5 mg/L dissolved oxygen represents the minimum aerobic condition needed to support a diverse population of aquatic organisms (Davis 1975). Dissolved oxygen in nonaerated teas depleted rapidly, and within 20 h fell to less than 0.05 mg / L. Thus in contrast to aerated tea, the oxygen concentration in nonaerated tea fell to 0.6% of the saturation level within 20h. The depletion of oxygen is most likely due to the consumption by microbes and not due to oxygen scavenging chemicals in the compost. The pH (5.67 ± 0.02) of nonaerated tea at 48h was significantly different from the pH (8.06 ± 0.01) of aerated tea. The lower pH in nonaerated tea is most likely due to organic acids since organic acid production by microbes under anaerobic condition is well documented (Lynch 1977; Brinton 1998). EC of the nonaerated teas differed significantly from that of aerated teas, even though the difference was small compared to difference in pH values.

None of the composts contained detectable levels of E. coli in 10% suspension of compost when tested by the 3M Petrifilm method and by the MPN method. Both methods detect strains of E. coli that secrete enzyme β glucuronidase. This suggested that little or no E. coli came from any of the four composts when teas were made using 2.6% suspension of compost in water. E. coli was not detected in either of the noninoculated aerated or noninoculated nonaerated DMC teas (Figure 3A).

Molasses and kelp stimulated the growth of non-pathogenic E. coli strain (K12: MG1655), Figure 2 (A, B). E. coli density in the tea increased as the concentration of molasses and kelp increased. As noted by Duffy et al. (2004) and Waterstripe et al. (2005) very little growth of E. coli was detected in the absence of molasses in the tea. Molasses contains approximately 50% sugars (Miller and Churchill 1986) that are available to support the growth of a variety of microorganisms including E. coli. Waterstripe et al. (2005) observed that E. coli growth in 2.4% molasses containing teas was significantly less than growth in 0.6% molasses containing teas. Since the highest concentration of molasses used in the present study (8 g / L) also produced the highest density of E. coli, it is difficult to corroborate their findings. Bess et al. (2002) reported molasses increased E. coli growth under very high (> 10 mg / L) dissolved oxygen concentrations when teas were made with composts containing low levels of naturally occurring E. coli. Our findings agree with Bess et al. (2002) that molasses is more effective than kelp in enhancing E. coli growth in teas.

Even though E. coli is a facultative anaerobe, its growth was significantly and consistently higher under anaerobic than aerobic conditions (Figure 3A). These findings agree with the Waterstripe et al. (2005) assertion that anaerobic conditions favour the growth of E. coli in compost teats. Since E. coli resides in a rela-
tively anaerobic environment within animal digestive tract, it may favour anaerobic environment over aerobic environment. Aeration is considered detrimental to the pathogen load in manure. For example, in the United Kingdom it is recommended that prior to field application manure slurries be aerated to reduce pathogen load (Jones 1999). Considering the rapid decline of oxygen in nonaerated teas in the present study, the observation by Duffy et al. (2004) that E. coli increased with the increase of molasses most likely have occurred under anaerobic conditions, since latter used relatively low compost to water ratio (1:9) and made no attempt to aerate except shaking flasks vigorously. Furthermore, Duffy et al. (2004) did not report either the dissolved oxygen or pH of the teas. In the present study the pH and oxygen concentration of nonaerated teas were significantly lower than that of aerated teas (Figure 3B). The EC values of aerated teas in the present study were slightly but significantly lower than unaerated DMC teas (Figure 3B).

Teas made from VC were least conducive to E. coli growth while those made from SMC were most conducive (Figure 4 A, B). Duffy et al. (2004) noted a difference in the growth of Salmonella but not E. coli in the teas made with two composts prepared from feed stock that contain dairy and poultry manure and speculated differences of nutrients in the composts could have affected pathogen growth. The nitrate and the ammonium concentration in VC are higher than DMC, yet the E. coli growth in VC tea was lower than DMC tea. Thus mineral nutrient content alone may not explain the difference in E. coli growth in the two teas. The fact that the increase in molasses concentration in teas while keeping the compost to water ratio the same result in a significant increase in E. coli growth indicates that readily available carbon source is a limiting factor to growth. Furthermore the agitation that accompanies aeration, is conducive to enhanced solubility of minerals in water, does not result in an increase in the E. coli growth compared to unagitated anaerobic teas is further indication that minerals released from compost may play a limited role in E. coli growth. Whether the lack of growth of E. coli in VC teas is due to organic constituents secreted by worms that are inhibitory to E. coli is yet to be determined. No E. coli was detected in VC obtained following feeding of worms with E. coli containing feed stock (Kannangara unpublished data). Vermicomposting reduced human
pathogens in biosolids (Eastman et al. 2001). E. coli growth was less in HMC compared to DMC. The pH values and oxygen concentrations were similar in teas of all four compost types (Figure 4C). EC was highest in teas made with SMC followed by teas made with DMC. Both SMC and DMC had higher EC values than VC and HMC (Table 1.)

Carrot juice significantly reduced the growth of E. coli in both aerated and nonaerated SMC teas (Figure 5A) where nonaerated teas showed a more drastic reduction compared to aerobic teas. Even though E. coli density in aerated and nonaerated teas without carrot juice was significantly different, they were similar in the aerated and nonaerated teas with carrot juice. Despite continuous aeration, carrot juice also reduced the dissolved oxygen concentration in aerated teas (Figure 5B). The pH and EC of carrot juice were 6.2 and 2.37 (mS/cm) respectively. Unlike the oxygen concentration, the pH and EC of the teas were not affected by carrot juice. The addition of carrot juice to DMC and SMC teas did not reduce the overall bacterial density (Figure 6). In SMC teas, carrot juice resulted in significantly greater total bacterial counts.

**Conclusions**

Compost teas are used by organic and conventional growers to suppress plant diseases and provide plant-available nutrients. Since microbial density in teas is assumed to reflect tea quality (Ingham and Alms 1999), teas are supplemented with additional nutrients to promote the growth of compost microbes. However, these nutrients are not selective and are also accessible to undesirable microorganisms that may be present in the compost due to inadequate composting and improper compost handling. In the present study, E. coli was not detected in teas made with composts that had no detectable levels of E. coli in a 10% compost suspension. This suggests that compost quality plays a significant role in pathogen regrowth. Nutrient addition may not be detrimental if the compost is relatively free of pathogens. Both molasses and kelp enhanced growth of E. coli under aerobic and anaerobic conditions. Even though E. coli is a facultative anaerobe, the growth of added E. coli strain (K-12 MG1655 ATCC 700926) in teas was consistently and significantly greater under anaerobic than under aerobic conditions. Compost type had a significant influence on pathogen growth. The teas made with VC were least conducive while those made with SMC were most conducive to growth of E. coli. Enhanced growth response by E. coli to the addition of molasses indicates that readily available carbon sources may be a limiting factor than minerals on the growth of E. coli in compost teas. Addition of carrot juice to teas significantly reduced the growth of E. coli under aerobic and anaerobic conditions. Carrot juice did not reduce the overall microbial density in DMC and SMC teas. Since aerobic and anaerobic teas are both effective in controlling plant diseases (Scheuerell and Mahaffee 2002), any negative impact of anaerobic condition in enhancing E. coli growth can be mitigated by the use of carrot juice.
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