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Persistence of *Escherichia coli* in batch and continuous vermicomposting systems

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ABSTRACT

Vermicomposting is a biooxidation process in which epigeicearthworms act in synergy with microbial populations to degrade organic matter. Vermicomposting does not go through a thermophilic stage as required by North American legislations for pathogen eradication. We examined the survival of a Green Fluorescent Protein (GFP) labeled *Escherichia coli* MG1655 as a model for the survival of pathogenic bacteria in both small-scale batch and medium-scale continuously-operated systems to discern the influence of the earthworm *Eisenia fetida*, nutrient content and the indigenous vermicompost microbial community on pathogen abundance. In batch systems, the microbial community had the greatest influence on the rapid decline of *E. coli* populations, and the effect of earthworms was only visible in micro bially-impoverishedvermicomposts. No significant earthworm density-dependent relationship was observed on *E. coli* survival under continuous operation. *E. coli* numbers decreased below the US EPA compost sanitation guidelines of 10^3 Colony Forming Units (CFU)/g (dry weight) within 18–21 days for both the small-scale batch and medium-scale continuous systems, but it took up to 51 days without earthworms and with an impoverished microbial community to reach the legal limit. Nutrient replenishment (i.e. organic carbon) provided by continuous feed input did not appear to extend *E. coli* survival. In fact, longer survival of *E. coli* was noticed in treatments where less total and labile sugars were available, suggesting that sugars may support potentially antagonist bacteria in the vermicompost. Total N, pH and humidity did not appear to affect *E. coli* survival. Several opportunistic human pathogens may be found in vermicompost, and their populations are likely kept in check by antagonists.

1. Introduction

Organic waste comprises 30–50% of residential waste (Recyc-Québec, 2009, 2014). Due to their environmental impact upon landfilling (i.e. greenhouse gas emissions, leachate), the government of Quebec wishes to ban organic waste landfilling by 2020 (MDDEP, 2011). Treatment methods have been hierarchically prioritized by the Ministry of sustainable development, environment and parks (MDDEP, 2010): (1) reduce waste (food reuse), (2) upcycle waste (feed systems), (3) recycle waste (composting and biomethanisation), and finally (4) energetic valorization (thermal treatments).Vermicomposting can be considered an organic waste upcycling method, allowing the production of proteins destined for food (Sabine, 1983) or feed (Edwards, 1985; Edwards and Arancon, 2004), making it a preferred valorization method compared to the more widespread nutrient recycling focused composting (MDDEP (Ministère du développement durable de l'environnement et des parcs), 2010). Upcycling organic waste with earthworms, or other invertebrates such as insects, is strongly recommended to alleviate the environmental impacts of agriculture, waste management, and to sustain food production for a rapidly growing global population (van Huis, 2013). Finally, domestic vermicomposting further reduces the environmental impact of organic waste management by eliminating transport-related nuisances (Lleó et al., 2013).

The fastest growing valorization methods in the province, i.e., centralized composting and biomethanisation, has strict governmental guidelines, requiring, among other things, thermal

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Abbreviations: GFP, Green Fluorescent Protein; CFU, Colony Forming Units; PBS, phosphate buffered saline; OC, organic carbon; N, total nitrogen.

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sanitation to prevent pathogen survival (55 °C for 3 days) (MDDEP, 2012a,b). However, vermicomposting is a mesophilic composting method that relies on epigeic earthworms to stabilize the organic waste (Domínguez and Edwards, 1997; Mustin, 1987). Using the same initial substrate, composts and vermicompost have been shown to host different microbial communities (Neher et al., 2013) and vermicompost may lead to higher plant yields when used as a fertilizer (Arancon et al., 2004a,b; Atiyeh et al., 2000).

The main bottleneck for the wider application of upcycling technologies such as vermicomposting is the absence of a thermal sanitation phase, which would make it a recognized process to significantly or further reduce pathogens (CCME, 2005; EPA, 1994). Source-separated organic waste can be contaminated with potential human pathogens (Droffner et al., 1995). Various pathogens may be found in organic waste (fungi, virus, parasites such as helminth egg (Lalander et al., 2013)) but we focus here on bacteria. Despite low fecal pathogen counts, domestic compost is not recognized as exempt from pathogenic risks, even though few people are aware of personal protection measures required to manipulate potentially contaminated residual organic matter (Hébert, 2015). Despite the evident interest in studying the fate of several bacterial pathogens in upcycling technologies (i.e. *Staphylococcus aureus*, *Clostridium perfringens*, *Enterococcus* spp. and *Listeria monocytogenes*, *Streptococcus* sp.), composting regulations currently focus on a restricted set of pathogenic or indicator enterobacteriales. In Europe (EC (European Commission), 2006, Lasaridi et al., 2006) and North America (CCME, 2005; EPA, 1994; Hébert, 2015), bacterial sanitation targets *Salmonella* and *E. coli*, the latter being the focus of the current article. Where raw or improperly composted manures were used on farms (Beuchat, 2002; Islam et al., 2005) outbreaks of pathogenic *E. coli* O157:H7 may arise (Jiang et al., 2002). Earthworms promote movement of faecal indicators in soils (Artz et al., 2005; Joergensen et al., 1998) and vermicompost (Williams et al., 2006), and potentially favour their short-term, but not long-term, survival (Williams et al., 2006). The decline of bacterial pathogens indicators during vermicomposting has been reported for coliforms in biosolids (Eastman et al., 2001) a study which may contain methodological flaws (Bowman et al., 2006; Hill et al., 2013), horse manure (Murry and Hinckley, 1992), biosolids and cow manure (Contreras-Ramos et al., 2005), pig slurry (Monroy et al., 2009) with significant reduction observed only in low dose vermireactors, and even human excreta from vermicomposting toilets (Hill and Baldwin, 2012) though thermotolerant coliforms may not be sufficiently reduced for use of the end product without an additional sanitation step. However, because of physico-chemical and biological differences in the substrates, pathogen reduction through vermicomposting may differ in domestic source-separated organics compared to previously studied manures and biosolids. End-product quantification of *E. Coli*, sometimes satisfying regulatory criteria (Lleó et al., 2013) or not (Grantina-Ievina et al., 2013), and they are insufficient to address the human pathogen reduction potential of vermicomposting. Furthermore, bacterial counts in mature compost samples are unlikely to be conducted in domestic settings, and regulatory requirements of such tests to minimize risks for biosolids or municipal source-separated organics (Hébert, 2005) appear of minimal interest in domestic settings. Despite numerous studies conducted on *E. coli* survival in vermicompost (Contreras-Ramos et al., 2005; Eastman et al., 2001; Hill et al., 2013; Monroy et al., 2009), a better understanding of the mechanisms involved is still necessary. Understanding the mechanisms governing the reduction of pathogens in small-scale vermicomposting systems using fruits and vegetables is thus necessary. The use of non-pathogenic bacterial models can help understanding the behavior of potentially pathogenic bacteria.

Distinguishing the impact of earthworms from that of vermicompost bacteria is a promising approach in the elucidation of this mechanism. Short-term indirect effects of the earthworms on bacterial communities have been suggested as a potential mechanism for pathogen reduction in vermicompost (Monroy et al., 2009). Earthworms affect soil microbes directly or indirectly by (1) comminution (fractionating), burrowing and casting, (2) grazing, and (3) dispersal (Brown, 1995; Lussenhop, 1992; Visser, 1985). The fate of bacteria during gut transit is variable (Aira et al., 2002; Brown, 1995; Wolter and Scheu, 1999) depending on bacterial or earthworm species, bacterial numbers, metabolic state (active or spores), substrate and feed type (Brown and Doube, 2004; Brown, 1995; Pedersen and Hendriksen, 1993). Though *E. coli* numbers generally decreases in earthworms pharynx and/or crop (Pedersen and Hendriksen, 1993), serovar O157:H7 may survive gut passage (Williams et al., 2006). Nevertheless, non-pathogenic strains of *E. coli* may be used to model the survival of pathogenic bacteria in vermicompost (Ogden et al., 2001). Earthworms synthesize and secrete various immunoprotective proteins, peptides and fluids with antimicrobial properties that can mediate lytic reactions against a variety of microorganisms (Bilej et al., 2000; Cooper et al., 2002; Cooper et al., 2004; Lassegues et al., 1989; Pedersen and Hendriksen, 1993; Satchell, 1983; Valembois et al., 1991; Wang et al., 2003).

Competition with indigenous microorganisms can be brought about by either pre-emption of resources or direct competition. It has been shown to cause a reduction of various pathogens during composting (Ryckeboer et al., 2003). Indigenous microflora can pre-empt space and resources (carbon, nitrogen, and minerals), such that pathogens have difficulty in establishing themselves (Said et al., 2006). In vermicomposting, pathogenic microorganisms may be less efficient competitors for the nutrients present in earthworm castings (Eastman et al., 2001; Panikkar et al., 2004). The fluctuation of physico-chemical parameters during vermicomposting has been shown to influence the bacterial communities of the vermicompost, in a way that differs in continuously fed systems or without feed input (Hénault-Ethier et al., 2015). Furthermore, small-scale and medium-scale vermicomposting systems may lead to differences in nutrient cycling (Shalabi, 2006). Further characterizing the physico-chemical variation during model pathogen reduction monitoring in different scale systems could thus help to elucidate the underlying mechanism.

The goal of the present study is to understand how model pathogenic bacteria can be regulated during the vermicomposting of source-separated organic waste. More specifically, we assessed how earthworms and indigenous microbes affected the survival of green-fluorescent protein (GFP) labeled *E. coli* monitored on ampicillin enriched LB media during batch and continuous vermicomposting, based on the hypothesis that both must have a significant impact in limiting *E. coli* survival through direct and indirect interactions. Variations in the chemical composition of the vermicompost (organic C, total N, total and labile sugars, pH) were also studied in relation to *E. coli* survival.

2. Methods

2.1. Preparation of the E. coli inoculum

Commensal *E. coli* strains are considered good models for the survival of pathogenic *E. coli* O157 strains in soil studies (Ogden et al., 2001). The strain MG1655 (ATCC 70092) was chosen because it is a non-pathogenic wild-type strain whose handling and containment requirements are less intensive than for the pathogenic strains. To facilitate enumeration in the microbial-rich community, *E. coli* MG1655 was transformed with the plasmid pGFPuv