Science of the Total Environment 468–469 (2014) 302–305

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Short Communication

Clay mineral type effect on bacterial enteropathogen survival in soil

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HIGHLIGHTS

- Clay mineral types differentially alter physicochemical parameters in soil.
- Different clay mineral types have differential effects on enteropathogen survival.
- The effect of clay type on pathogen survival in soil is enteropathogen specific.

ARTICLE INFO

Article history:
Received 4 April 2013
Received in revised form 13 August 2013
Accepted 13 August 2013
Available online 12 September 2013

Editor: Charlotte Poschenrieder

Keywords:
Clay mineral
Pathogen survival
Salmonella
Listeria
Escherichia coli

ABSTRACT

Enteropathogens released into the environment can represent a serious risk to public health. Soil clay content has long been known to have an important effect on enteropathogen survival in soil, generally enhancing survival. However, clay mineral composition in soils varies, and different clay minerals have specific physiochemical properties that would be expected to impact differentially on survival. This work investigated the effect of clay materials, with a predominance of a particular mineral type (montmorillonite, kaolinite, or illite), on the survival in soil microcosms over 96 days of *Listeria monocytogenes*, *Salmonella* Dublin, and *Escherichia coli* O157. Clay mineral addition was found to alter a number of physicochemical parameters in soil, including cation exchange capacity and surface area, and this was specific to the mineral type. Clay mineral addition enhanced enteropathogen survival in soil. The type of clay mineral was found to differentially affect enteropathogen survival and the effect was enteropathogen-specific.

1. Introduction

Enteropathogens released into the environment can represent a serious risk to public health. A wide range of sources exist, including grazing and domestic animals, environmental reservoirs, wildlife, and land-spreading of animal manures/slurries and sludge (Brennan et al., 2010; Gerba and Smith, 2005). Soil, in particular, can act as a reservoir for, or a mitigator against, further contamination of disease vectors such as water and food sources. A wide range of physical, chemical and biological factors are known to impact on the fate and transport of enteropathogens within soil (Crane and Moore, 1984; Jamieson et al., 2002; van Elsas et al., 2011). In the particle size sense, clay content has long been known to have an important effect on enteropathogen survival in soil, generally enhancing survival (Garcia and McKay, 1970; Santamaría and Toranzos, 2003). However, the clay size fraction of different soils can consist of drastically different clay mineral types each type having its own specific physiochemical and mineralogical properties (particle size, shape, surface area, cation exchange capacity (CEC), moisture absorption, swelling potential, elasticity, and provision of mineral nutrients among many others) that may be anticipated to impact differentially on enteropathogen survival (England et al., 1993; Höper et al., 1995).

Kaolinite, montmorillonite and illite represent some of the main groups of clay minerals found within soils (Marshall, 1975; Soil Science Society of America, 1989). Montmorillonite and illite are 2:1 (3 layer) clays, while kaolinite is a 1:1 clay (2 layer) with a smaller expansion and absorption capacity. While a number of studies have investigated the impact of clay type on survival of bacteria such as *Rhizobium*, *Agrobacterium* and *Pseudomonas* sp. (Heijnen et al., 1993; Heynen et al., 1988; Stutz et al., 1989) in soil, there is limited knowledge on the nature or extent of any effect on enteropathogen survival. This is a surprising omission as the behaviour of bacteria within soil is known to be

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http://dx.doi.org/10.1016/j.scitotenv.2013.08.037

species- and even strain-specific (Brennan et al., 2013; Dong et al., 2002; Fontes et al., 1991). Knowledge of the impact of clay minerals on enteropathogen survival is important for the development of risk assessments and management strategies aimed at reducing public health risks from activities such as land application of wastes containing human pathogens.

2. Materials and methods

Microcosms comprising of soil only or soil with a clay mineral amendment were established. A sandy, brown podzolic, grassland soil (N 52 17 40.75, W 06 29 48.91) sampled to a depth of 0.10 m and sieved to 4 mm, was amended with three clay materials with a predominance of the clay minerals montmorillonite (bentonite, Sigma 285234), kaolinite (Sigma 03584) and illite (in the form of so called French green clay, Argila Verde, Portugal) respectively. Prior to use, the minerals were washed in deionised water within dialysis sacks (Sigma) to reduce salinity effects, which would affect adsorption (Gordon and Millero, 1984). When the electrical conductivity (WTW Microprocessor Conductivity Meter LF196) reached 40 μS/cm the minerals were washed in the inoculation matrix (1/100 strength phosphate buffered saline (~160 μS/cm); used as it was found to approximate the electrical conductivity of the pore water of the soil), dried, and ground (Retsch mechanical grinder). For clay-amended treatments, clay minerals were added (hand mixed and shaken) to soil at a concentration of 10% dry weight. Properties of the soil, clay minerals and the experimental mixes are shown in Table 1. Quantitative mineralogical analysis (Table 2) was carried out on the soil and clay minerals by X-ray powder diffraction (XRPD) to determine their actual mineralogical composition (Omotoso et al., 2006).

Enteropathogens, *Listeria monocytogenes* (#1778), *Salmonella* Dublin (NCTC 9676), and *Escherichia coli* O157 (#3704) were incubated (35 °C *Listeria*; 37 °C others) overnight in Luria–Bertani broth, spun down and washed (×3) prior to re-suspension in the inoculation matrix. Final inoculum loadings added for individual pathogen microcosms were 2×10^7, 1.7×10^8 and 4×10^7 CFU (colony forming units) for *E. coli*, *S.* Dublin and *L. monocytogenes*, respectively. Soil, or amended soil, microcosms of 10 g dry weight had their moisture contents adjusted so that on inoculum addition they were at 65% water holding capacity. Microcosms were held at 15 °C throughout the incubation period. Replicate (n = 3) microcosms for each treatment and pathogen were destructively sampled on days 0, 3, 6, 12, 24, 48 and 96. Enteropathogen extraction was carried out by suspending the soil in 50 ml of the inoculation matrix, vortexing briefly, and shaking on an end-over-end shaker for 30 min. Following serial dilution, suspensions were then plated onto Sorbitol MacConkey, XLD or Oxford agars (Oxoid) for *E. coli*, *S.* Dublin and *L. monocytogenes*, respectively for enumeration. There were no detectable background enteropathogen populations in the soils prior to inoculation. Mean extraction efficiencies across treatments (based on extraction at time 0) were 70 ± 9% for *E. coli*, 82 ± 10% for *S.* Dublin and 51 ± 9% for *L. monocytogenes*. The interactions between pathogen, treatment and time were analysed as a 3 × 4 × 7 factorial design using Anova (Genstat 14). All assumptions of the analyses were met. Mean CFU values were used in Statistica (V10) to calculate the death rate (k-value) using a first order decay function (Moynihan et al., 2013).

Table 2
Quantitative XRPD results for the un-amended soil and the washed clay minerals used for amendment, indicating actual mineralogical compositions.

Sample ID	Quartz	Feldspars	Montmorillonite	Kaolinite	Illite/mica[a]	Others[b]
Soil	79	8	–	2	10	1
Montmorillonite	4	5	85	3	–	3
Kaolinite	5	10	–	78	7	1
Illite	10	3	–	8	29	50

[a] Includes mixed-layer Illite-smectite.
[b] Illite contains substantial calcite.

3. Results and discussion

The addition of the clay mineral to the soil was found to alter a number of physicochemical parameters in soil (Table 1). This included cation exchange capacity and surface area, and was specific to the mineral type. The survival of the 3 enteropathogens for the 4 treatments is shown in Fig. 1 and the decay rates are shown in Table 3. Overall statistical results indicated that the pathogen, treatment and time interaction factor was significant ($p < 0.001$). Bonferroni posthoc analysis indicated that all elements within the pathogen and treatment groupings were significantly different from each other ($\alpha = 0.05$). In general, the addition of clay minerals to the soil enhanced survival; however, the effect was enteropathogen-specific and varied temporarily. It is not well understood how different clay types influence bacterial survival but it is known that they influence many physicochemical properties of soil (Filip, 1973; Höper et al., 1995). Factors believed to have a role in enhanced survival effect of clay minerals include an increased buffering capacity, moisture retention and nutrient availability, and a decrease in particle size (with concomitant increase in surface area) resulting in protection from predators, parasites, desiccation, UV, toxins and antibiotics (Bitton et al., 1972; England et al., 1993; Filip, 1973; Heijnen et al., 1993; Heynen et al., 1988; Höper et al., 1995; Johnson et al., 2006;

Table 1
Properties of soil, clay and soil clay mixes.

	pH	TC %	TN %	C:N ratio	LOI %	CEC cmol + kg^{-1}	TON mg/l	NH_4–N mg/l	Zeta potential mV	Surface area sq. m/g	Particle size		
											Sand (20–2000 μm)	Silt (2–20 μm)	Clay (0.2–2 μm)
Soil	6.3	3.6	0.3	12.0	6.3	15.9	12.7	2.9	n/d	1.5	76	23	2
Kaolinite (post-washing)	8.1	BLD	BLD	–	2.5	7.1	0.5	3.3	−57.2	8.3	9	80	11
Montmorillonite (post-washing)	8.9	0.3	BLD	–	1.1	98.3	1.1	4.9	−39.0	50.0	12	51	37
Illite (post-washing)	7.8	0.4	BLD	–	1.6	63.9	0.7	3.2	−34.7	32.7	12	64	24
Soil kaolinite mix	7.4	3.0	0.2	15.0	6.1	17.1	14.2	2.8	n/d	2.3	65	31	3
Soil montmorillonite mix	7.5	3.0	0.3	10.6	6.0	25.1	16.0	2.8	n/d	3.7	74	24	3
Soil illite mix	7.3	3.4	0.2	15.1	6.1	69.3	16.5	2.8	n/d	3.5	69	28	4

BLD = below level of detection. TC (total organic carbon) and TN (total nitrogen) was determined by an automated Dumas combustion procedure using a Flash EA 1112 Elemental Analyser (Thermo Finnigan, Italy). LOI (loss on ignition) was calculated after overnight ignition at 450 °C. CEC was calculated as a sum of exchangeable cations (displacement using 1 M ammonium acetate and quantification using inductively coupled plasma atomic emission spectroscopy) and exchangeable acidity (titrimetrically determined using barium hydroxide solution following displacement in 1 M barium acetate). Note that the CECs of the illite and soil illite mix are erroneous due to the presence of calcite in the illite. TON (total oxidised nitrogen) and NH_4^+ was analysed on an Aquakem 600 Discrete Analyser after extraction with 2 M KCL. Zeta potential was analysed using Malvern Zeter Sizer Nano using the Smoluchowski model. Surface area was measured by Nitrogen gas adsorption using a Coulter SA 3100 and calculated according to the BET equation. Particle size samples were dispersed in di-ionised water and sodium hexametaphosphate/sodium carbonate solution by end-over-end shaking and ultrasonication prior to analysis using laser diffractometry (Malvern Mastersizer 2000).