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Investigating the bacterial community and amoebae population in rural domestic wastewater reclamation for irrigation

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ABSTRACT

Reclamation of domestic wastewater for agricultural irrigation is viewed as a sustainable option to create an alternative water source and address water scarcity. Free-living amoebae (FLA), which are amphizoic protozoa, are widely distributed in various environmental sources. The FLA could cause considerable environmental and health risks. However, little information is available on the risk of these protozoa. In this study, we evaluated the feasibility using rural domestic wastewater for agricultural irrigation, and analyzed dynamic changes of the microbial community structure and FLA populations in raw and treated wastewater, as well as the phyllosphere and rhizosphere of lettuce production sites that were irrigated with different water sources. The bacterial community dynamics were analyzed by terminal restriction fragment length polymorphism (T-RFLP). The bacterial community structures in the influent were similar to that in the effluent, while in some cases relative abundances varied significantly. The populations of *Acanthamoeba* spp. and *Hartmannella vermiformis* in the anaerobically treated wastewater were significantly higher than in the raw wastewater. The vegetables could harbor diverse amoebae, and the abundances of *Acanthamoeba* spp. and *H. vermiformis* in the rhizosphere were significantly higher than in the phyllosphere. Accordingly, our studies show insight into the distribution and dissemination of amoebae in wastewater treatment and irrigation practices.

Introduction

Domestic wastewater is used as an alternative water source for agricultural irrigation, aquaculture production and industrial processes in water shortage and dry regions over the world. Its environmental and health risks need serious consideration. The untreated domestic wastewater not only contains numerous hazardous agents, but also harbors a range of pathogenic bacteria and amoebae, which pose a potential health risk. Irrigation with wastewater for agriculture may introduce and accumulate chemical and biological contaminants in soils and crops. Many studies have identified the transfer of waterborne pathogens to the phyllosphere and rhizosphere from wastewater and the environment (Al-Lahham et al., 2003; Warriner et al., 2003; Cooley et al., 2003; Heaton and Jones, 2008; Orlofsky et al., 2011; Yang et al., 2015).

Free-living amoebae (FLA) are amphizoic protozoa that can be found commonly in various environments, such as wastewater,

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swimming pools, surface water and soils (Tsvetkova et al., 2004; Rodriguez-Zaragoza, 1994). *Acanthamoeba* spp., *Hartmannella vermiformis*, *Naegleria fowleri*, *Balamuthia mandrillaris* and *Sappinia diploidea* are known as causative agents that have pathogenicity, mainly in immunocompromised groups (Visvesvara et al., 2007; Marciano-Cabral and Cabral, 2003; Schuster and Visvesvara, 2004; Grün et al., 2014; Qvarnstrom et al., 2013; Khan, 2009). The amoebae genera *Acanthamoeba* and *Hartmannella* were determined to be potential carriers of pathogenic bacteria, especially *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Mycobacterium* spp., *Vibrio cholera*, *Listeria monocytogenes* and *Escherichia coli*, which are widely distributed in water systems (Loret and Greub, 2010; Berger et al., 2006; Thom et al., 1992; Lorenzo-Morales et al., 2007). Additionally, scientists found that *Acanthamoeba* genotypes and *Hartmannella* showed great thermotolerance and osmotolerance, and their cysts had high resistance to disinfection practices (Lu et al., 2015). In a previous study, the FLA population was determined in the wastewater samples from five Spanish wastewater treatment plants. It was observed that treatment with sodium hypochlorite showed no significant reduction in the number of amoebae at concentrations of 0–100 mg/L (García et al., 2011). The FLA and amoebae-resisting bacteria were investigated at various stages of a drinking water plant fed with river water. Amoebae were identified positively with a quantitative method, though not isolated after chlorination (Thomas et al., 2008). Another concern with FLA is their colonization in water systems that allows the survival of waterborne pathogenic bacteria, as they have the ability to recolonize in crops after wastewater reclaimation. Most of the published studies on biological contamination and health risks, as well as wastewater reuse, have been based on certain indicator bacteria, such as *E. coli*, *Salmonella* sp., *Staphylococcus aureus* and Coliform (Zhang et al., 2015). However, few investigations have reported the occurrence of pathogenic amoebae, even though they are widely present in near all ecosystems. Wastewater that is commonly used for agricultural irrigation contains a variety of bacteria and protists, while the transfer of pathogenic microorganisms from wastewater to the phyllosphere and rhizosphere has seldom been discussed. The treated effluent may pose potential health risks by disseminating pathogenicity when discharged into the receiving rivers or reused for agricultural irrigation. Considering the potential environmental and health risks, the pathogenic amoebae community and population in not only wastewater, but also on leafy greens and root zones, need to be investigated.

This study aims to characterize dynamic changes in the bacterial community and amoebae population in raw and treated domestic wastewater from an on-site anaerobic biofilm reactor treatment process. The treated wastewater was intended for use in local agricultural irrigation. We also investigated the amoebae population on the phyllosphere and rhizosphere of lettuce irrigated with domestic wastewater. The sedimentation and culture methods were used for counting helminth eggs. A culture-independent real-time PCR was used for detection of the protozoan parasites. This study highlighted the occurrence of two amoebae in raw and treated wastewater, and on the phyllosphere and rhizosphere of lettuces. Moreover, further research is needed to elucidate the microecological behavior of amoebae and amoebae-associated pathogens in both sewage systems and agricultural fields, which may better assess the potential health risks, as some FLA are pathogenic and involved in the dissemination of pathogenic bacteria.

1. Materials and methods

1.1. Design and operation of pilot anaerobic biofilm bioreactor process

The pilot-scale domestic wastewater treatment reactor in this study was operated with three anaerobic biofilm bioreactor processes. The bioreactor was made of polyethylene materials and had a working volume of 360 L. The reactor temperature was maintained at 20–28°C, and had a 72 hr. hydraulic retention time (HRT). As sketched in Fig. 1, the pilot plant was installed with a wastewater pooling tank and a set of anaerobic biofilm processes, followed by an effluent storage tank. No disinfection facility was set up with the pilot plant.

The plot experiment was performed in a vegetable garden located in Huairou District, Beijing, China. The lettuce (*Lactuca sativa* L.) was used as a model vegetable to estimate microbial contamination on crops irrigated with the different water sources in this study. The lettuce seeds, purchased from Chinese Academy of Agricultural Sciences (CAAS.), were pretreated before seeding as described previously (Quilliam et al., 2012). Briefly, seeds were surface sterilized using 3% sodium hypochlorite solution for 15 min, followed by several rinses with sterile distilled water. The lettuce seeds were planted in 2 × 2 m plots in an open local field. Experimental sites were designed and operated with 3 irrigation patterns using raw wastewater (RW), treated effluent (TE), and potable water (PW) as a controlled trial. Each operation site was applied in four replicates with a randomized block design.

1.2. Samples collection and DNA extraction Q4

Water samples were collected in June–October at monthly intervals. All samples were pooled in 1 L sterile polyethylene bottles, and transported immediately to the laboratory for physicochemical analysis and molecular assays. The pH and electrical conductivity were tested on site using a portable multi-parameter meter (HACH HQ40d, USA). For analyzing microbial communities and pathogens, 100 mL of each water sample was filtered with 0.22 μm mixed cellulose membranes (47 mm diameter, Millipore, USA) to obtain microbial cells in a centrifuge tube, and then stored at −80°C until required.

Lettuces were harvested at the mature stage (about 8 weeks), and the leaf samples were collected aseptically using sterilized scissors and placed in homogeneous bags. Microbiological pellets were obtained as described previously (Zhang et al., 2009). Rhizosphere soil samples were collected after shaking off soil loosely adhered to the roots, and sieved and homogenized through a 0.9 mm sieve after vacuum freeze drying for further analysis.

Genomic DNA of the aforementioned samples was extracted in Lysing Matrix tubes using the FastDNA SPIN Kit for Soil (MP Biomedicals, USA) according to the manufacturer's instructions, except for the following: the tubes were shaken in a FastPrep® Instrument for 45 sec at a speed of 5.0 m/sec, and DNA

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