Bioresource Technology 211 (2016) 610–617

Contents lists available at ScienceDirect

Bioresource Technology

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

A regulating method for the distribution of phosphorus fractions based on environmental parameters related to the key phosphate-solubilizing bacteria during composting

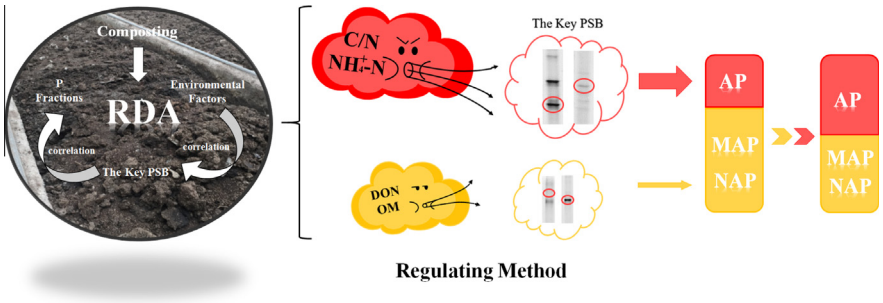
Yuquan Wei [a], Zimin Wei [a,*], Zhenyu Cao [a], Yue Zhao [a], Xinyu Zhao [b], Qian Lu [a], Xueqin Wang [a], Xu Zhang [a]

[a] College of Life Science, Northeast Agricultural University, Harbin 150030, China
[b] Laboratory of Water Environmental System Engineering, Chinese Research Academy of Environmental Science, Beijing 100012, China

HIGHLIGHTS

- Assess the abundance and incidence of culturable PSB during different composting.
- Analyze the structure and succession of culturable PSB community during composting.
- Identify the correlation between PSB and P fractions and environmental factors.
- An adjusting method for the distribution of P fractions of composts was proposed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form 25 March 2016
Accepted 27 March 2016
Available online 29 March 2016

Keywords:
Composting
Phosphate-solubilizing bacteria (PSB)
Organic wastes
Denaturing gradient gel electrophoresis (DGGE)
Redundancy analysis (RDA)

ABSTRACT

This study was conducted to assess the abundance, incidence and diversity of the culturable phosphate-solubilizing bacteria (PSB) community during different organic wastes composting. The key PSB affecting different phosphorus (P) fractions and their relationship with environmental variables were analyzed by redundancy analysis (RDA). The results showed that there were distinct differences in amounts, incidence and community composition of PSB for the composts from different sources. Regression analysis demonstrated significant corrections between the density and incidence of PSB and pH, temperature, OM and DOC/DON. Most of culturable PSB showed high percentages of identity with the phyla of *Firmicutes* and *Proteobacteria*. There were thirteen key PSB correlated closely ($p < 0.05$) with different P fractions variation. Conclusively, we suggested a process control method to regulate the distribution of P fractions during composting based on the relationship between the key PSB and P fractions as well as environmental parameters.

1. Introduction

Composting is the widely used method to stabilize different organic solid waste materials through the degradation of biodegradable components by microbial communities under controlled conditions, and the compost obtained is suitable as an amendment for soils, thereby recycling waste materials (de Guardia et al., 2010). Throughout the stages of composting there is a succession of different dominant microbial groups, including bacteria, actinobacteria and fungi, which play a key role both in the composting process and in compost properties (Pepe et al., 2013; Lopez-Gonzalez et al., 2015). Therefore, knowledge of the microbial structure and microbial biomass in composts is important to predict its potential impact on soil.

* Corresponding author.
E-mail addresses: weizimin@neau.edu.cn, weizm691120@163.com (Z. Wei).

http://dx.doi.org/10.1016/j.biortech.2016.03.141
0960-8524/

Phosphorus (P) is an essential mineral nutrient that often limits plant growth and is applied to soil in the form of phosphatic fertilizers (Wei et al., 2015; Chang and Yang, 2009). However, only 5–30% of the fertilizer P applied to soils is recovered by plants as chemical fertilizer is immobilized rapidly and becomes unavailable to plants (Mander et al., 2012). Phosphate-solubilizing bacteria (PSB) play fundamental roles in biogeochemical phosphorus cycling in natural and agricultural ecosystems, which are able to transform the insoluble phosphorus to plant-available forms by acidification, chelation, exchange reactions, and polymeric substances formation (Chang and Yang, 2009). To date, there are numerous studies on PSB and it has been reported that a substantial number of bacterial species exhibits P solubilization capacity (Vassilev et al., 2014; Mander et al., 2012; Oliveira et al., 2009). In addition to *Pseudomonas* and *Bacillus*, other genera of PSB include *Arthrobacter*, *Enterobacter*, *Klebsiella*, *Proteus*, and *Serratia*, etc. (Acevedo et al., 2014). Despite PSB may provide agronomic benefits, their abundance in soil is not always sufficient to compete with other microorganisms, which constitute 1–50% of the total bacterial population (Sharma et al., 2013). To overcome this problem, the use of PSB-based bio-fertilizers may be useful for improving the absorption of insoluble P.

Given that composting ecosystem exhibited rich bacteria biomass and diversity with different enzymatic capabilities, numerous studies have been conducted to screen and identify PSB from composts by culture on selective media containing insoluble forms of inorganic P (Jurado et al., 2014; Chang and Yang, 2009). However, the composition of phosphate-solubilizing bacterial community may be influenced by the complex environmental conditions of composting (Lopez-Gonzalez et al., 2015). At the same time, differences in composting processes and materials influenced the amounts and diversity of PSB in composts (Chang and Yang, 2009), but few available reports was about the succession and distribution of phosphate-solubilizing bacterial diversity within various raw materials during composting using culture-dependent methods and molecular techniques.

Direct multivariate analyses, such as redundancy analysis (RDA) and canonical correspondence analysis (CCA), were used to consider the correlation between changes in microbial community composition and environmental variables, moreover, they were widely applied to multifaceted environmental samples especially from composting (Wang et al., 2015; Zhang et al., 2011). Nowadays, these techniques have been increasingly used to identify the primary factors influencing microbial communities with special biological capacity, such as methanogenic communities (Lin et al., 2012), denitrifying bacteria (Chen et al., 2014), ammonia-oxidizing archaea and bacteria (Liu et al., 2015). Considering that it is unknown whether phosphate-solubilizing is intrinsic ability of PSB, or a reaction by-product connected with other processes, the teleological argument advanced the trait that the abundance and composition of PSB may be linked to underlying selective condition in the environment (Mander et al., 2012). Nevertheless, there is little consideration whether the changes of PSB community compositions could be associated with physico-chemical parameters in the compost originates from diverse sources.

The aims of this study were to: (1) compare the phosphate-solubilizing bacterial density and community structure of different compost materials, (2) study the relationship between different P fractions and the PSB community during composting, and (3) identify the main environmental factors affecting the key culturable PSB species from diverse composts. This research will provide useful information about regulating the distribution of P fractions by the key PSB and may help to guide future studies about composting bioaugmentation during different composting.

2. Materials and methods

2.1. Sample collection and storage

Seven trapezoidal piles of pig manure (PM), chicken manure (CM), municipal solid waste (MSW), kitchen waste (KW), green waste (GW), straw (SW), fruits and vegetables waste (FVW) were prepared by Shanghai Songjiang Composting Plant. Composting was considered finished when the temperature of the pile became stable and the germination index approached 80%. Approximately 2 kg of each compost were collected on different days. One part of compost samples were freshly processed for bacterial counts and the isolation of phosphate-solubilizing bacteria (PSB). Some samples were stored at 4 °C for analysis of P fractions and the others were used for physical–chemical analysis. Details of composting and analytical procedures employed can be accessed in other studies on the environmental parameters aspects and P fractions properties of the composting assays described that have been recently published (Wei et al., 2014, 2015). Relevant P fractions properties included total phosphorus (TP), inorganic phosphorus (IP), organic phosphorus (OP), water-soluble phosphorus (WSP), citric acid phosphorous (CAP), Olsen phosphorus (Olsen P), microbial biomass phosphorus (MBP), available P (AP), moderately available and non-available P (MAP&NAP) and ten involved environmental parameters were the ratio of carbon to nitrogen (C/N), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), organic matter (OM), ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), moisture, temperature, pH and germination index (GI).

2.2. Isolation and enumeration of bacteria and PSB

The cultivable bacteria were estimated using a standard dilution-plating procedure. Fresh samples (10 g) of composts were suspended in 90 mL of sterile saline solution (NaCl, 0.9% w/v) and shaken (150 rpm) for 30 min at room temperature. Ten-fold dilutions were made in sterile saline solution and 0.1 mL was spread out in appropriate medium. Bacteria were cultured in standard Nutrient Agar (Cultimed, Spain) for 48 h at 30 °C. PSB was cultivated in National Botanical Research Institute's phosphate growth medium devoid of yeast extract (NBRIY) medium supplemented with 1.5% Bacto-agar (Difco Laboratories, Detroit, MI, USA) contained L^{-1}: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $(NH_4)_2SO_4$, 0.5 g; NaCl, 0.2 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; KCl, 0.2 g; $MnSO_4{\cdot}H_2O$, 0.002 g; $FeSO_4{\cdot}7H_2O$, 0.002 g and Bacto-agar, 15 g (Nautiyal, 1999). The PSB was defined as a transparent zone cleared of precipitated phosphate around the edge of the colony. Cell densities were expressed as number of colony-forming units (CFU) of a given microbe per gram of composting material (wet weight). The incidence of PSB (%) was calculated as the proportion of PSB in bacteria.

2.3. DNA extraction and PCR–DGGE analysis

All PSB were selected out from the NBRIY medium and cultured together in LB (Luria–Bertani) media at 30 °C overnight. Total phosphate-solubilizing bacterial DNA was extracted using TIANamp bacteria DNA kit (TIANGEN BIOTECH (BEIJING) CO., LTD). After purified using the commercial DNA purification kit (Bioteke, Beijing), the extracted DNA was dissolved in 100 μL of TE buffer (10 Mm Tris–HCl, 1 Mm EDTA, pH = 8) and stored at −20 °C before use.

To analyze the DNA of the PSB community, 16S rRNA genes were amplified using the prokaryotic primers 341F/534R (Muyzer et al., 1993). A GC clamp was attached to forward primers to prevent complete separation of the strands during DGGE. Polymerase chain reaction (PCR) conditions for each 50-μL reaction