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Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation

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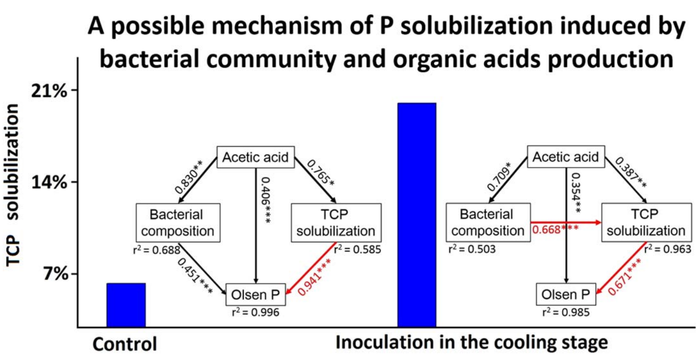
GRAPHICAL ABSTRACT



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ABSTRACT

Enriched phosphate-solubilizing bacteria (PSB) agent were acquired by domesticated cultivation, and inoculated into kitchen waste composting in different stages. The effect of different treatments on organic acids production, tricalcium phosphate (TCP) solubilization and their relationship with bacterial community were investigated during composting. Our results pointed out that inoculation affected pH, total acidity and the production of oxalic, lactic, citric, succinic, acetic and formic acids. We also found a strong advantage in the solubilization of TCP and phosphorus (P) availability for PSB inoculation especially in the cooling stage. Redundancy analysis and structural equation models demonstrated inoculation by different methods changed the correlation of the bacterial community composition with P fractions as well as organic acids, and strengthened the cooperative function related to P transformation among species during composting. Finally, we proposed a possible mechanism of P solubilization with enriched PSB inoculation, which was induced by bacterial community and organic acids production.

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1. Introduction

Although the total amount of phosphorus (P) is high in many lands, P deficiency is a constraint to plant growth worldwide due to the low availability in soil (Wei et al., 2016b). Therefore, P-fertilizers is frequently applied, particularly in conventional intensive agricultural soils to overcome P-limitation. Unfortunately, most of chemical P-fertilizers can be immobilized by Ca^{2+}, Fe^{3+} and Al^{3+}, which lead to the rapid formation of poorly available P for plants after applied (Malik et al., 2012). Given that demand for improving the availability of P-fertilizers continues to increase and phosphate-solubilizing bacteria (PSB) has a key role in the mobilization and transformation of soil P, more and more attention was paid on the understanding of the mechanisms for solubilizing of mineral phosphates and how to improve the efficiency of PSB application.

Many studies have shown that PSB can transform the insoluble P to soluble forms by acidification, chelation, exchange reactions, and polymeric substances formation, which may be related to the production of organic acids, the release of protons (H^+), the redox-active metals, etc. (Delvasto et al., 2006). Among them, the mechanism that phosphate solubilizing ability of PSB is associated with the release of low molecular weight organic acids is generally accepted. Low molecular weight organic acids may play an important role the detoxification of pollutants, the bioavailability of heavy metals and even microbial activity in soil conditions (Onireti et al., 2017). On the other hand, these organic acids (e.g., oxalic acid, citric acid, acetic acid, succinic acid, etc.) could also lower pH, chelate the cations (mainly calcium) bound to phosphate through their hydroxyl and carboxyl groups or compete with phosphate for adsorption sites, leading to the increased solubility and availability of mineral phosphates (Mander et al., 2012; Khan et al., 2007). However, the predominant organic acids produced by different phosphate-solubilizing microorganisms were diverse distinctly (Panhwar et al., 2014; Nakasaki et al., 2013). Moreover, the process of P-solubilization is a complex phenomenon, which are severely affected by many factors. The impacts of environmental factors, such as temperature, pH, oxygen concentration, moisture, etc. on microbial P-solubilizing capability have been widely investigated (Zhu et al., 2012; Chen et al., 2006), but there is no studies specifically on the influence of the interaction of microbial communities and PSB on solubilizing insoluble phosphate and production of organic acids.

Composting is a controlled biological decomposition process of organic matter, accompanying with a dynamic activity of various microbial populations (Xi et al., 2016; Zhao et al., 2016a; He et al., 2014). Given that composting can affect the distribution of P fractions, a large number of studies has been oriented to the addition of mineral phosphates with poor availability and inoculation of phosphate solubilizing microbes during composting to develop P-enriched compost (Wei et al., 2016b; Chang and Yang, 2009). Many microorganisms have been utilized as solubilizing insoluble P inoculants in composts. However, considering that the inoculation during composting may have different performances due to the influence of the source of microbial inoculum, the scale of composting, and the stage of inoculation etc. (Zhao et al., 2016b), we hypothesized that: (1) compared to uninoculated compost, changes in the time of inoculation of phosphate solubilizing microbes during composting may cause different biological microenvironment and bacterial community structure due to the competition or collaborative symbiosis between inoculants and indigenous bacteria, (2) compared to uninoculated compost, inoculation of PSB in different stages will stimulate microbial activity related to organic acids production more and therefore lead to more solubilization of precipitated inorganic P, and (3) different bacterial community relationship may change the type and amount of organic acid involved in the P-solubilization process.

Given that tricalcium phosphate (TCP) is mainly used as a model material as they represent the vast majority of precipitated inorganic P salts and kitchen waste (KW) has a relatively low P content (Wei et al., 2016b; Chen et al., 2006), this study was essentially focused to the effect of bacterial community and different organic acids on TCP-solubilization during KW composting. Besides, different stages for inoculation were employed. Direct multivariate analyses, such as redundancy analysis (RDA), could be used as a preliminary estimate for the correlation between changes in microbial community and environmental variables, and structural equation modeling (SEM) could further construct complex relationships among composting biotic and abiotic factors (Liang et al., 2017; Flores-Renteria et al., 2016; Wei et al., 2016a). Nevertheless, there is little consideration whether changes of bacterial community and organic acids production could be associated with P-solubilization during composting by these relationship model.

Our intent was to evaluate our hypothesis by RDA and SEM to link bacterial community, organic acids production and TCP-solubilization in different inoculation treatments, which may provide a better understanding of the P-solubilization process of phosphate-solubilizing inoculant during composting and lead to new perspectives on strategies to improve P availability of composts with precipitated inorganic P.

2. Materials and methods

2.1. Preparation of phosphate-solubilizing inoculant

The enriched PSB was obtained from different solid waste composting samples as described by Wei et al. (2016a). The enrichment process was similar to the method described as Zhang et al. (2016), but the enriched medium was sterile National Botanical Research Institute's phosphate growth medium (NBRIP) broth (Wei et al., 2016a). The liquids with enriched PSB was cultivated for 3 days at 40 °C until the population up to 10^8 CFU mL^{-1} to measure the phosphate-solubilizing activity in broth culture including water soluble phosphorus (WSP) and microbial biomass phosphorus (MBP) according to Panhwar et al. (2014), and pH of the medium was recorded with a pH meter. The phosphate-solubilizing content of the PSB agent increased after the process of domestication culture for 10 cycles, and the 10th cycle of phosphate-solubilizing inoculant with high P-solubilization capacity was centrifuged and suspended in sterile water, then sprayed on the composting mixture. The concentrations of the strain were 1×10^8 CFU mL^{-1}.

2.2. Composting experiment design

KW was prepared as the main material, which was collected from the cafeteria in Northeast Agricultural University (Harbin, China), mainly containing the cooked food residues, such as steamed rice, noodles, steamed bread, and cooked vegetable, meat, fish. The indigestible materials, such as plastics, bones, and egg shell, were selected before the KW were crushed and homogenized. Sawdust was added to adjust the C/N radio of 25, which was obtained from a timber mill in Harbin, China. Details of raw materials were described in Table 1. The initial moisture was adjusted to 60%. Five lab-scale composting experiments were carried out for 40 days in the special compost reactor (the working volume was 12.5 L) as described by Zhao et al. (2016b) and various treatments with three replicates each were applied as shown in Table 2. Considering the phosphate-solubilizing inoculant was enriched at the temperature below 50 °C, it was not inoculated at high-temperature stage of composting. The KW with a relatively low P content was chosen as the major raw material and therefore allowed the impact of enriched phosphate-solubilizing inoculant with different stages inoculation on the solubilization of TCP in composting to be readily observed. During composting, moisture was maintained at 50–60% and the ventilation rate was 0.5 L min^{-1} kg^{-1} in order to maintain oxygen concentration inside the composts above 10%. Composting materials were turned over manually every four days to ensure homogenized. Representative compost samples of each treatment were