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Impact of anti-acidification microbial consortium on carbohydrate metabolism of key microbes during food waste composting

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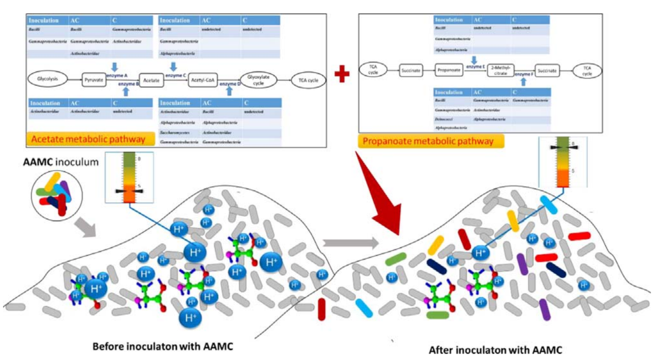
GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the effect of anti-acidification microbial consortium (AAMC), which act synergistically for rapid bioconversion of organic acids on carbohydrate metabolism of key microbes in the course of food waste (FW) composting by metaproteomics. AAMC was inoculated to the composting mass and compared with treatment with alkaline compounds and the control without any amendment. Inoculating AAMC could effectively accelerate carbohydrate degradation process and improve composting efficiency. Carbohydrate metabolic network profiles showed the inoculation with AAMC could increase significantly the types of enzymes catalysing the degradation of lignin, cellulose and hemicellulose. Furthermore, AAMC inoculum could increase not only diversities of microbes producing key enzymes in metabolism pathways of acetic and propionic acids, but also the amounts of these key enzymes. The increase of diversities of microbes could disperse the pressure from acidic adversity on microorganisms which were capable to degrade acetic and propionic acids.

1. Introduction

Food waste (FW) is the largest component of municipal solid waste by weight and constitutes about 45% and 55% in Europe and developing countries, respectively (Cerda et al., 2017). Until a few years ago, landfill was still the most commonly used disposition of FW in China

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(Wang et al., 2016; Zhou et al., 2016, 2018). This not only reduces the life span of landfills, but also create environmental problems like leachate, odour and greenhouse gas emission (Chan et al., 2016; Mu et al., 2017). Composting is a simple and low-cost technology for FW stabilization that converts organic compounds into value-added products (Kopčić et al., 2014; Wei et al., 2014, 2016; Zhao et al., 2017). However, the rapid degradation of the readily available organic matter leads to accumulations of low molecular weight organic acids during the initial stage of FW composting, which eventually inhibits the microbes and deteriorates the composting performance (Plachá et al., 2013; Wang et al., 2013; Chan et al., 2016).

In order to cope with the problems caused by low pH during composting of FW, many researchers have suggested that chemical substances, such as $Ca(OH)_2$, CH_3COONa, coal ash, lime and MgO and K_2HPO_4, could be added to prevent the decrease in pH (Bergersen et al., 2009; Yu and Huang, 2009; An et al., 2012; Wang et al., 2013; Wang et al., 2016; Wang et al., 2018). Ferric nitration addition also could mitigate the inhibition of organic acids by stimulating denitrification which consumed protons and organic acids (Shou et al., 2017). Maintaining pH within a proper range by dosing the basic salts is an effective approach to increase the dissociated/undissociated ratio of low molecular weight organic acids, alleviating their inhibition during FW composting. However, this method also often leads to other problems, such as high salinity in the compost product and high nitrogen loss during FW composting process. There have been many attempts to solve these problems. Struvite formation during composting through supplementation Mg and P salts could conserved nitrogen but in the same time increased the electrical conductivity (EC) of the FW compost. Wang et al. (2016) showed that addition of both lime and struvite salts synergistically provide advantages to buffer the pH, reduce ammonia emission and salinity, and accelerate FW composting. Chan et al. (2016) used zeolite to control the EC under struvite composting of FW and exhibited that co-amendment with 10% zeolite effectively reduced the EC and improved the humification degree of FW compost.

In addition to supplementing chemical substances, an alternative approach to prevent the drop of pH and thereby improve FW composting efficiency is microbial inoculation into the raw compost material (Nakasaki et al., 2013). Choi and Park (1998) reported that *Kluyveromyces marxianus* could not only reduce acidity of FW compost but also increase the quantity of indigenous thermophilic bacteria in composting substrate. Nevertheless, whether the increase of thermophilic bacteria was influenced by the inoculation of *Kluyveromyces marxianus* could not be determined. Nakasaki et al. (2013) showed that short-chain organic acids could be rapidly degraded by inoculated *Pichia kudriavzevii* RB1.The initial lag phase seen in the growth of indigenous microorganism was also eliminated, the composting period was reduced by 2 days. However, because high temperature was lethal for the yeast, it died in early stages of composting and did not contribute in thermophilic phase of composting. In order to avoid the accumulation of organic acids after the yeast dies, Nakasaki and Hirai (2017) tried to prevent the death of the inoculated yeast by maintaining the temperature at 40 °C for 2 days during the heating stage in the initial phase of composting and exhibited that controlling the composting temperature, in addition to inoculating the yeast, was effective in accelerating the composting process. As was well known, the production of organic acids depended strongly on the amount of easily degradable carbonaceous materials in raw compost materials. Thus, the time of temperature being maintained at mesophilic temperature depended on the composition of the compost raw material. Additionally, the lactic acid bacterium *Pediococcus acidilactici* TM14 could avoid the accumulation of organic acids by an indirect way (Tran et al., 2015). That is, it stimulated the QH1 (a fungus that was indigenous in the compost raw material), which in turn degraded all the organic acids and modified the pH and the environmental conditions for the other microorganisms to operate. The magnitude of effect of the lactic acid bacterium on the indigenous microorganisms may be dependent on composting raw materials.

Being different from aforementioned single microbial strain inoculum, in this study, in order to improve survival rate of inoculated microbes in the course of competing with indigenous microbes for nutrients and further accelerate organic matter degradation, anti-acidification microbial consortium, which act synergistically for rapid bioconversion of organic acids was developed. It originated from the initial phase of FW composting when the pH of composting material maintained at a range of 4–5 for a long time, so theoretically, the microbial consortium was well adapted to the conditions in FW compost and degraded organic acids strongly. In our anterior study, inoculation with the anti-acidification microbial consortium (AAMC) could effectively eliminate the negative influence of the accumulation of organic acids on organic matter degradation in the course of FW composting and improved the variation of total bacteria. Proteins reflect the actual functionality with respect to metabolic reactions and regulatory cascades, and give more direct information about microbial activity than functional genes and even the corresponding messenger RNAs (Liu et al., 2015). In order to further reveal mechanisms of action of AAMC and optimize inoculation technique, the effects of inoculation with AAMC on carbohydrate metabolic networks as well as ecological interactions of key microbes during FW composting were unraveled and characterized by metaproteomics.

2. Materials and methods

2.1. Composting materials

The FW was obtained from the dining hall of Chinese Research Academy of Environmental Science (Beijing, China). The refractory materials, such as napkin, bone were manually separated and discarded. Residual samples were minced into pieces with diameter less than 5 mm and mixed well before the composting experiments began. Wheat bran as bulking agent was added to adjust the ratio of carbon/nitrogen (C/N) and moisture of composting substrate. It was acquired from the Chinese Research Academy of Environmental Science. The chemical characteristics of the FW were as follows: a C/N ratio of 23.3, an organic matter of 79.9% and a moisture of 78.7%. Some basic characteristics of wheat bran were: C/N ratio, 32.4; organic matter content, 89.3%; moisture, 10.2%.

2.2. Composting reactor

The composting process was carried out in the reactor, the core part of which was a cylinder 330 mm in diameter and 400 mm in height, and the volume of the cylinder was approximately 34 L. A 6 mm trachea was connected to the top of the reactor, and a perforated metal plate was used at the bottom to hold the composting substrates and to distribute air equally. The composting system was connected to a blower that provided the reactor with air from the bottom. The wall of the cylinder was equipped with a detector for recording the temperature of composting substrates.

2.3. Source, construction and composition of anti-acidification microbial consortium (AAMC)

The microbial consortium originated from the initial phase of FW composting when the pH of composting material maintained at a range of 4–5 for one month. A 5-g sample was used as the source of the microorganisms, transferred into 50 ml of sterile water, and incubated at a room temperature for 1 h in an orbital shaker incubator with the revolving speed of 150 rpm. After standing, 1 ml of the suspension was added into each of 12 autoclaved flasks (each containing 50 ml of Luria–Bertani medium (Sigma™)) In order to simulate pH conditions of the initial phase of FW composting, pH of Luria–Bertani medium was adjusted to 5.3 by mixed acids which were composed of acetic,