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Inoculation of lactic acid bacterium accelerates organic matter degradation during composting

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ABSTRACT

In this study, the impact of microbial inoculation upon indigenous microorganisms leading to the acceleration of food waste composting was investigated. The lactic acid bacterium *Pediococcus acidilactici* TM14 was inoculated into compost raw material composed of rabbit food with the addition of organic acids that simulate food waste. The strain TM14 produced a high concentration of lactic acid in the early stages of composting, whereas composting without inoculation of TM14 accumulated high levels of acetic acid, up to 22 mg g^{-1} dry solid of compost, which is detrimental to indigenous microorganisms. The growth of TM14 enhanced the proliferation of fungi having the ability to degrade organic acids, and thus the organic acids contained in the compost material were completely decomposed. Levels of TM14 and the fungi diminished immediately after the increase in composting temperature. However, the fungi modified the environmental conditions allowing for the activity of thermophilic bacteria, which play important roles in composting. In consequence, organic matter degradation in the composting was accelerated.

1. Introduction

Treatment of food waste is an urgent issue since food waste putrefies easily and produces an offensive odor, which is harmful to environmental quality. Composting converts the putrescible organic materials into a stabilized form acceptable for use as soil conditioner without the risk of phytotoxicity (Haug, 1993). Thus, the reclamation of compost produced from food waste for agricultural use has attracted considerable interest (Lee et al., 2004; Li et al., 2013). Food waste composting often encounters the problem of low pH levels, which delays composting as low pH inhibits the activity of microorganisms (Cheung et al., 2010). Indeed, low pH conditions alter the charge interaction of amine and carboxyl groups within amino acids, and change the conformations of proteins. This in turn, inhibits the enzymatic activities of microorganisms and damages the microorganisms themselves, causing them to die (Haug, 1993; Srivastava and Srivastava, 2003).

Composting can proceed simply with the action of the microorganisms indigenous to the raw material, and does not require an inoculation of any foreign microorganism. However, such composting may have a low efficiency or be delayed if the indigenous microorganisms are not adequate or are inhibited by certain environmental limitations. In such cases, it was found that microbial inoculants are effective in improving the composting process (Gray et al., 1971). Some studies on food waste composting have demonstrated the advantages of microbial inoculants in enhancing composting efficiency in terms of (i) improvement in the maturity of compost by using *Thermoactinomyces vulgaris* A31 (Ke et al., 2010); and (ii) increase of total bacterial count resulting in high biodegradability by inoculating with amylolytic and cellulolytic thermophilic bacteria related to *Geobacillus* species (Sarkar et al., 2010).

The benefit of using an inoculant in the composting of simulated food waste with low pH was previously observed (Nakasaki et al., 2013). A newly isolated yeast, *Pichia kudriavzevii* RB1, can degrade organic acids and thus increase the pH value of composting material beyond the neutral pH. Hence, the proliferation of bacteria is promoted and vigorous degradation of organic matter consequently attained. That paper aimed to elucidate the effectiveness of RB1 in accelerating composting. However, an interesting phenomenon was observed at the same time, which is that the composting eventually started even if RB1 was not inoculated. Additionally, in composting without RB1 inoculation, two characteristic lactic acid bacteria, closely related to *Pediococcus acidilactici*

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and *Weissella paramesenteroides*, were observed. It appeared these lactic acid bacteria contributed to the changes in environmental conditions allowing for the activity of microorganisms that play important roles in composting. In this study, the detailed mechanisms for the acceleration of aerobic composting by the inoculation of the lactic acid bacteria were tried to elucidate.

Lactic acid bacteria often adhere to food waste and become the dominant bacteria during storage (Wang et al., 2001). The effect of lactic acid bacteria on anaerobic composting was addressed in the studies of Hemmi et al. (2004) and Asano et al. (2010). They studied the presence of lactic acid bacteria throughout the acidulocomposting process, which was maintained at a low pH and high temperature and in anaerobic conditions, to treat garbage. This system achieved high stabilization due to the presence of the lactic acid bacteria, which maintained the microbial community by repressing the growth of other microorganisms and preventing the putrefaction of the garbage. However, it seems that no previous study has reported on the mechanism of the effect of lactic acid bacteria on the acceleration of aerobic composting.

2. Materials and methods

2.1. Composting material

Commercial rabbit food with the trade name Rabbit Food Timothy™ (Easter Co. Ltd., Tatsuno, Japan) was used as a representative model of food waste (Nakasaki et al., 2013). The carbon and nitrogen content of the dry weight rabbit food base were 44.0% and 2.43%, respectively, as determined by elemental analysis. Hence the C/N ratio was 18.1. To determine the pH of the rabbit food, a suspension was prepared by homogenizing rabbit food in water at a ratio of 1:9 (w/w) using an ACE homogenizer (Nihonseiki Kaisha Ltd., Tokyo, Japan). Subsequently the pH was then determined to be 5.7, using a pH meter (Model D-51; Horiba Co., Ltd., Tokyo, Japan). The rabbit food was then mixed with sawdust as a bulking agent and commercial seeding material (Alles G™; Matsumoto Laboratory of Microorganism Co. Ltd., Matsumoto, Japan) at a ratio of 10:9:1 to create the compost raw material. The cell density of mesophilic fungi, mesophilic bacteria, and thermophilic bacteria contained in the commercial seeding material were 4.12×10^4, 1.56×10^6, and 7.18×10^5 colony forming unit (CFU) g^{-1} dry solid material (DS), respectively. The pH of the seeding material was 7.8. Food waste itself is usually acidic because of the presence of organic acids formed mainly during storage. Sundberg et al. (2011) demonstrated that four types of organic acid were characteristic of food waste: lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA). These four organic acids were added into the compost raw material to obtain concentrations of 12.45, 2.90, 3.02, 2.43 mg g^{-1} DS, respectively (Nakasaki et al., 2013). The pH of the raw compost mixture was approximately 5.2 after the addition of organic acids. The initial moisture content of compost material was adjusted to 60% (w/w).

Two characteristic lactic acid bacteria closely related to *Pediococcus acidilactici* and *Weissella paramesenteroides* were isolated from the compost produced without inoculation of RB1 shown in the previous paper (Nakasaki et al., 2013). Two strains isolated on a deMan-Rogosa-Sharpe (MRS) agar plate (Difco™ Lactobacilli MRS broth supplemented with agar) were designated as *P. acidilactici* TM14 and *W. paramesenteroides* TA15 and stored in a −80 °C freezer before use. As a first attempt to elucidate the effect of lactic acid bacteria on the acceleration of composting, only TM14 was used as an inoculant in this study.

Two types of composting experiments were performed: Run A was the control run without inoculation; Run B was inoculated with strain TM14 at a cell density of 10^8 CFU g^{-1} DS. To prepare the inoculum, the TM14 was precultured in the MRS medium twice at 37 °C for 2 days under shaking conditions of 150 rpm. The TM14 in the liquid medium was washed with distilled water and then suspended in distilled water.

2.2. Composting operation

The composting experiments were carried out in a bench-scale reactor (diameter: 300 mm, depth: 400 mm). This reactor was made of thermoresistant polyvinyl chloride resin insulated by polystyrene Styrofoam to prevent heat loss from the reactor.

The composting was started by introducing approximately 3000 g of compost raw material into the reactor. Air was supplied from the bottom of the reactor at a rate of 45 l h^{-1}. Temperature was increased by self-heating due to heat liberation from microbial activity until the set temperature of 60 °C, which is the optimum temperature for the composting process (Nakasaki et al., 1985), was reached. After this point, temperature was controlled by regulating the air feed rate. However, at the later stages of the composting process, the heat generation was lower than the heat dissipation due to the exhaustion of easily degradable organic matter. Heat was therefore supplied by an electrical ribbon heater surrounding the reactor in order to keep the set temperature. The exhausted gas was passed through an H_2SO_4 solution to eliminate the NH_3 prior to the determination of CO_2 percentage using an infrared analyzer (Ohtaki et al., 1998).

The organic matter degradation was expressed as the CO_2 evolution rate and the conversion of carbon, calculations for which were detailed in a previous paper (Nakasaki et al., 1998). The CO_2 evolution rate is the rate of organic matter degradation, while the conversion of carbon is the degree of mineralization of organic matter. During the composting process, the compost material was turned and sampled once daily. Moisture content was maintained at 60% (w/w) by adding distilled water if required. The composting was operated for 15 days. It has been reported that experiments conducted in this composting system are highly reproducible (Ohtaki et al., 1998).

2.3. Physicochemical analysis

The compost samples collected were measured for moisture content, pH, and concentration of organic acids. The moisture content was determined by measuring in triplicate the loss of weight after drying at 105 °C for 24 h in a dry oven (Model DS600; Yamato Scientific Co., Ltd., Tokyo, Japan). The pH of compost sample was determined as mentioned in Section 2.1. The concentration of organic acids was measured using a high-pressure liquid chromatography (HPLC) system equipped with an UV-2075 detector (JASCO Corp., Tokyo, Japan) and a SUGAR SH 1011 column (Shodex, Tokyo, Japan). To prepare the liquid sample for HPLC measurement, the compost suspension was filtered through a 0.2-μm cellulose acetate membrane filter. Conditions used for HPLC measurement were according to the manufacturer's instructions; the mobile phase consisted of 5 mM sulfuric acid at the flow rate of 1 ml min^{-1} and the temperature was set at 50 °C.

2.4. Microbial cell density

A dilution plating technique was applied to measure the cell density of the three types of microorganisms, i.e., mesophilic fungi; mesophilic bacteria, including lactic acid bacteria; and thermophilic bacteria. The growth of mesophilic bacteria and thermophilic bacteria was observed on the trypticase soy agar medium (pH = 7.3; trypticase peptone, 17 g l^{-1}; phytone peptone, 3 g l^{-1}; NaCl, 5 g l^{-1}; K_2HPO_4, 2.5 g l^{-1}; glucose, 2.5 g l^{-1} and agar, 20 g l^{-1}). Mesophilic