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Simultaneous production of L-lactic acid with high optical activity and a soil amendment with food waste that demonstrates plant growth promoting activity

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A unique method to produce highly optically-active L-lactic acid and soil amendments that promote plant growth from food waste was proposed. Three *Bacillus* strains *Bacillus subtilis* KBKU21, *B. subtilis* N3-9 and *Bacillus coagulans* T27, were used. Strain KBKU21 accumulated 36.9 g/L L-lactic acid with 95.7% optical activity and 98.2% L-lactic acid selectivity when fermented at 43°C for 84 h in a model kitchen refuse (MKR) medium. Residual precipitate fraction (anaerobically-fermented MKR (AFM) compost) analysis revealed 4.60%, 0.70% and 0.75% of nitrogen (as N), phosphorous (as P_2O_5), and potassium (as K_2O), respectively. Additionally, the carbon to nitrogen ratio decreased from 13.3 to 10.6. AFM compost with KBKU21 promoted plant growth parameters, including leaf length, plant height and fresh weight of *Brassica rapa* (Komatsuna), than that by chemical fertilizers or commercial compost. The concept provides an incentive for the complete recycling of food waste, contributing towards a sustainable production system.

[**Key words:** Optically active L-lactic acid; Organic soil amendment; Plant growth promoting bacteria; Food waste biomass]

According to a report published online by EC-FAO Food Security Programme (1), food security remains a fundamental issue for mankind with respect to the physical availability and economical accessibility of foods, though global quality of life has gradually improved. Increased sustainable agricultural production and reduction of food waste generation are two identified methods of ensuring food security (2). These can be achieved by managing land and water utilization, minimizing soil erosion, and increasing the efficiency of agrochemical use, as well as recycling food waste. Proper utilization of fertilizers is also important. According to another report published online by WRAP on household food and drink waste in the United Kingdom (3), food waste is defined as food with the potential to be eaten, together with any unavoidable waste that is lost from the human food supply chain. The recycling of organic-rich food waste, particularly kitchen refuse and yard wastes, however, is limited compared to that of inorganic wastes, such as plastic- and metal-based materials (4). Therefore, recycling organic waste biomass is an important research imperative that can enrich unfertilized agricultural land in a sustainable manner via soil amendment, and also reduce greenhouse gas emissions from landfills.

The utilization of food waste as compost fertilizer is one solution to recycle waste biomass. Since traditional composting of organic waste generates carbon dioxide via oxidative degradation of organic carbon, the productions such as lactic acid and methane from the anaerobic fermentation of waste biomass has recently attracted much attention in terms of greenhouse gas mitigation (5–7). Purified L-lactic acid and D-lactic acid with high optical activity are important monomers that serve as the feedstock of poly L-lactic acid (PLLA) and poly D-lactic acid (PDLA), respectively, which are recyclable material used to make biodegradable plastic (8–10). It was also found that a variety of thermotolerant bacterial species of the genus *Bacillus* can selectively accumulate optically active L-lactic acid from model kitchen refuse (MKR) (11). On the other hand, certain thermotolerant *Bacillus* strains demonstrating *in vitro* plant growth promoting activities, such as P-solubilization, N-fixation, and the production of auxin as well as ammonia, improved and enhanced the growth of maize plants under arid soil conditions (12). One promising plant growth promoting bacteria (PGPB) was isolated and identified as *Bacillus subtilis* KBKU21 (12). It is hypothesized that *B. subtilis* KBKU21 could serve multi-functional roles as L-lactic acid producer and a PGPB simultaneously in the fermentative transformation of food waste (Fig. S1).

In the present study, three thermotolerant *Bacillus* strains including *B. subtilis* KBKU21, were used for MKR fermentation. The content of L-lactic acid in terms of optical activity, as well as

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selectivity, in the fermented supernatant of MKR medium was evaluated. Then the residual precipitate, described as anaerobically fermented MKR (AFM) compost, was tested for its plant growth promotion activity by incorporating it into planting medium as soil amendment material. Since screening test on the growth performance of *Zea mays* (monocotyledon, C4 plant) was previously performed, we further investigate plant growth promoting effect of strain KBKU21 on *Brassica rapa* (dicotyledon, C3 plant) based on the fact that it is widely used in evaluating growth performance respond under different fertilizer rates and fertilizer types (13–18).

MATERIALS AND METHODS

Microorganisms and growth conditions A possible PGPB strain, *B. subtilis* KBKU21 previously isolated from Kanchanaburi research station area, Thailand was used in this study (12). Two lactic acid producing *Bacillus* strains, *B. subtilis* N3-9 and *Bacillus coagulans* T27, previously isolated from food waste compost and farmland soil, respectively, was used as comparison strains for L-lactic acid fermentation efficiency (11). In addition, *B. subtilis* N3-9 was also used to validate that not all *B. subtilis* but strain KBKU21 is capable in performing both L-lactic acid fermentation and production of functional compost that have plant growth promoting activity. In a preliminary study, we investigated the thermotolerance of *Bacillus* strains KBKU21, N3-9, and T27, and found their optimal growth temperatures were 43°C, 45°C, and 50°C, respectively. All culture stocks were reactivated in glucose yeast peptone (GYP) medium (20 g glucose, 5 g yeast extract, and 5 g polypeptone in 1 L of deionized water) by incubating at their optimal growth temperatures for 12 h under static conditions prior to L-lactic acid production screening.

Fermentation of MKR for L-lactic acid production and preparation of AFM compost Standard MKR medium was prepared as described in the previous report (11), by homogenized the following ingredients per liter of tap water: vegetables (66.7 g of carrot peel, 66.7 g of cabbage, and 66.7 g of potato peel), fruit (50 g of banana peel, 50 g of apple peel, and 50 g of orange peel), 70 g of baked fish, 50 g of rice, and 30 g of used tea leaves. Saccharification was performed at 50°C for 2 h at pH 5.8 using industrial glucoamylase (Glucozyme 20,000, Nagase ChemteX, Osaka, Japan) at the concentration of 300 ppm. After saccharification, pH of the MKR medium was adjusted to 7.0 with 10% ammonia solution. Simultaneous production of L-lactic acid and functional compost from MKR medium was carried out as follows: a loopful of bacterial colony was taken from individual slant culture of *Bacillus* strains KBKU21, N3-9 or T27, and inoculated into an 18 mL test tube each containing 5 mL of GYP broth. Incubation was carried out at each optimized growth temperature for 24 h, under constant shaking at 140 rpm. A 0.3 mL aliquot of individual seed culture was then inoculated in 30 mL of standard MKR medium and incubated statically for another 24 h. These cultures were entirely used to inoculate 1 L MKR medium, which were further incubated statically at the appropriate optimal growth temperature. Aliquots of fermenting broth were sampled at 12 h intervals (0–120 h) for pH and organic acid content analysis. The pH of the fermenting MKR broth was adjusted to 7.0 every 24 h using 10% aqueous ammonia solution to avoid inhibition caused by high levels of undissociated organic acid in the medium (19). Fermented MKR medium was harvested after 120 h, and centrifuged at 10,000 ×*g* for 20 min at 4°C to obtain organic acid-rich supernatant and the solid organic matter-rich precipitate fraction. AFM composts were prepared by drying the precipitate fractions at room temperature for one week.

Analytical methods Organic acids, including lactic acid, formic acid, acetic acid, and propionic acid, and the optical activity of lactic acid were analyzed using high performance liquid chromatography (HPLC), as described previously (20). Selectivity of lactic acid production was defined as the percentage lactic acid (g) of the total organic acid (g) content. Optical activity of L-lactic acid was defined as $([L] - [D]) \times 100/([L] + [D])$, where [L] and [D] denotes the concentrations of L-lactic acid and D-lactic acid, respectively. Total soluble sugar (g/L) was determined by the phenol-sulfuric acid method (21). The water content of AFM compost was measured by heating at 105°C for 48 h. An elemental analysis for carbon (C) and nitrogen (N) was performed using a C/N Corder (Macro Corder JM1000 CN, J-Science Lab, Kyoto, Japan), while potassium (K) content was analyzed using an atomic absorption spectrometer (Hitachi, Tokyo, Japan) and available phosphate (P) content was determined by spectrometry using the molybdenum blue method (22).

Cultivation of *Br. rapa* Plant growth promotion by AFM composts was tested with *Br. rapa* (Japanese name, Komatsuna) using a complete randomized design with a sample size of 7. The experiment was performed in triplicate. Three dependent variables investigated were plant biomass, plant height, and leaf length. Two independent variables investigated were the type of bacteria (*Bacillus* strains KBKU21, N3-9, and T27) used in the MKR fermentation, and the N, P and K mineral content (basal or triple rate) amended in the planting soil (Table 1, R1–R12). The controlled variables were the type of plant used, the soil type, watering conditions, and pot size. Briefly, Neubauer pots (15 cm dial) were filled with black soil and separately amended with AFM compost carrying strains KBKU21, N3-9 or T27. Chemical fertilizer (Table 1, R13 and R14) and commercial compost fertilizer (Table 1, R15 and R16) served as positive controls, while unamended soil served as a negative control (Table 1, R17). Also, we investigated the effect of P and K application rate by adjusting the nutrient contents (N:P:K) in the fermented MKR compost to a final ratio of 50:50:50 (Table 1, R7–9 and R15) and 150:150:150 (Table 1, R10–12 and R16) using chemical fertilizers and commercial compost. The planting medium was homogenized and left undisturbed for 7 days in nursery at 25°C before sowing with *Br. rapa* (20 seeds per pot). Seed germination process is defined as completed when the radicle has grown out of the covering seed layers (23). Germination rate was recorded as a percentage at 7 days after sowing (DAS), and sprouts were thinned out to 7 seedlings at 14 DAS. Plant height (mm), leaf length (mm), and fresh body weight (g) were determined at 28 DAS.

Statistical analysis Data are presented as the mean ± standard deviation (SD), and compared using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Lactic acid fermentation of MKR An initial drop in pH to below 6 after a 12 h incubation were recorded for all *Bacillus* strains KBKU21, N3-9, and T27 fermenting MKR medium, indicating an accumulation of organic acids due to the metabolism of sugars (Fig. 1). Total sugar content declined from 70 to 75 g/L to 27–32 g/L after 120 h of fermentation. In all cases, lactic acid accumulated predominantly in the liquid fraction of the fermented MKR. Strain KBKU21 rapidly accumulated 36.9 g/L

TABLE 1. Experimental design for cultivation of Komatsuna.

Run	Abbreviation	MKR compost	N (ammonium sulfate)	P (calcium biphosphate)	K (potassium chloride)	Commercial compost	Fertilizer component (mg/pot)		
							N	K_2O	P_2O_5
R1	KBKU21	+	−	−	−	−	50	9.82	7.59
R2	T27	+	−	−	−	−	50	9.35	7.36
R3	N3-9	+	−	−	−	−	50	9.97	9.41
R4	KBKU21 (3×)	+	−	−	−	−	150	29.5	22.8
R5	T27 (3×)	+	−	−	−	−	150	28.1	22.1
R6	N3-9 (3×)	+	−	−	−	−	150	29.9	28.2
R7	KBKU21 + PK	+	−	+	+	−	50	50	50
R8	T27 + PK	+	−	+	+	−	50	50	50
R9	N3-9 + PK	+	−	+	+	−	50	50	50
R10	KBKU21 + PK (3×)	+	−	+	+	−	150	150	150
R11	T27 + PK (3×)	+	−	+	+	−	150	150	150
R12	N3-9 + PK (3×)	+	−	+	+	−	150	150	150
R13	NPK_{KBKU21}	−	+	+	+	−	50	9.82	7.59
R14	NPK	−	+	+	+	−	50	50	50
R15	Commercial compost	−	−	−	−	+	50	50	50
R16	Commercial compost (3×)	−	−	−	−	+	150	150	150
R17	No amendment	−	−	−	−	−	0	0	0

Footnotes: +, with; −, without; 3×, triple rate.