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

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24 promoting bacteria; Food waste biomass]

26 **Abbreviations:** AFM, anaerobically fermented model kitchen refuse; DAS, days after
27 sowing; GYP, glucose yeast peptone; LS, liquid supernatant; MKR, model kitchen
28 refuse; PGPB, plant growth promoting bacteria; PDLA, poly D-lactic acid; PLLA, poly
29 L-lactic acid; and SP, solid precipitate

ABSTRACT

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 purity 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 P₂O₅), and potassium (as K₂O), 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.

INTRODUCTION

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 pure 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 purity, as well as 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).

MATERIAL 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 *B. 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 typical strain KBKU21 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 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 20000, 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 a 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 to 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 feedback inhibition caused by high levels of organic acid in the medium (19). Fermented MKR medium was harvested after 120 h, and centrifuged at $10,000 \times g$ 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 *Brassica rapa*

Plant growth promotion by AFM composts was tested with *Brassica rapa* (Japanese name, Komatsuna) using a complete randomized design with a sample size of seven. 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, runs 1–12 (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 Run 15) 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 seven days in nursery at 25°C before sowing with *B. 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 seven days after sowing (DAS), and sprouts were thinned out to seven 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 $\pm$ 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 70-75 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 lactic acid in the medium at 84 h. Total lactic acid production for strain T27 and N3-9 at 120 h were 45.3 g/L and 37.8 g/L, respectively. Results revealed that *B. subtilis* KBKU21 have comparable level of lactic acid concentration with the control *B. coagulans* T27 and *B. subtilis* N3-9 when fermented at their respective optimal growth temperatures.

Table 2 summarizes the fermentation results, showing maximum L-lactic acid production, selectivity, and optical purity. Even though lactic acid selectivity for KBKU21 (98.2%) was slightly lower than that by T27 (99.2%) and N3-9 (98.5%), the optical purity of L-lactic acid by KBKU21 (95.7%) was higher than that of T27 and N3-9 (87.8% and 92.8%, respectively). As soluble sugar still remained in the fermented broths, further optimization would be expected to increase L-lactic acid yield and concentration. We have previously reported that many species of thermotolerant and thermophilic bacteria in the Bacillaceae can produce L-lactic acid with high optical activity from food waste biomass (11,24). Similarly, this study found that *B. subtilis* strain KBKU21, originally isolated from soil as a PGPB, selectively accumulated a significant amount of L-lactic acid with high optical purity (95.7%), as the main product from MKR medium. High optical purity is useful in producing polylactic acids with high crystallinity, which is a key determinant for the polymer practical melting point of 180°C or lower (25). Purified L-lactic acid granules can be utilized in biopolymer industries to produce the biopolymer polylactide or biodegradable plastic products, whose properties are comparable to fossil-petroleum-based polymers but are biodegradable and more environment friendly (8,26).

Major nutrient contents in fermented MKR compost

The mineral contents of the L-lactic acid rich liquid supernatant (LS) fraction and solid precipitate (SP) fraction obtained from centrifugation of MKR medium fermented with each strain are given in Table 3. In general, the C:N ratio in the MKR medium decreased after fermentation with *Bacillus* strain KBKU21, N3-9, and T27, from 15.7–16.7 to 5.3–6.0 in the LS, and from 13.3–13.9 to 10.1–10.9 in the SP after a

120 h fermentation period. The decrease in organic C in the AFM composts could be primarily attributed to the solubilization of organic matters to produce organic acids, in addition to the liberation of carbon dioxide during cell respiration. Increase of N-content through addition of ammonia as a neutralizing agent in the MKR medium every 12 h also drastically lowered the C:N, especially in the LS. Meanwhile, a slight increase in organic C-content in the LS fraction with *Bacillus* strains KBKU21 and T27 was recorded, which might be resulted from dissolved L-lactic acid and other organic acids present in the LS fraction. On the other hand, a slight decrease was recorded in the available P-content in LS, and this was accompanied by an increase in the SP fractions. When microbial biomass built up in the MKR medium after 120 h of fermentation, soluble P in the orthophosphate ions form (H_2PO_4^- , HPO_4^{2-} , PO_4^{3-}) could have been utilized to form new building blocks in nucleic acids (27). P-fixation could also have occurred through a reaction between anion phosphates and cation earth minerals, such as calcium (Ca^{2+}), to form the insoluble calcium phosphate [$\text{Ca}(\text{PO}_4)_2$] or rock phosphate. K-content recorded a mild increase at 120 h post-fermentation in SP, relative to their initial content.

Effect of AFM compost on the germination of *Brassica rapa*

Germination rate of *B. rapa* seeds in soil amended with MKR compost fermented by *Bacillus* strain KBKU21 or strain T27 was comparable to that of soil amended with commercial compost, chemical fertilizers, or control soil (Fig. 2). However, seed germination in soil amended with MKR compost fermented by *B. subtilis* N3-9 was significantly reduced ($p > 0.05$). Reduction in seed germination with strain N3-9 could possibly be caused by one or several metabolites in the anionic

fractions produced by *B. subtilis*, as reported by (28), as well as by the plant hormone, abscisic acid, as demonstrated by (29). Although auxin might be one of the plant hormones produced by the three *Bacillus* strains used in this study, the Komatsuna seed germination percentage did not exhibit any significant differences. As reviewed by (30), auxin is not a necessary hormone for seed germination, but is necessary for the growth of young seedlings through embryogenesis, vascular tissue development, and cell elongation (31). Since interactions between PGPB and crops in terms of germination rate and growth parameters have been shown to be either beneficial, deleterious or neutral (32), our results suggest that compost prepared using *B. subtilis* KBKU21 or *B. coagulans* T27 are non-phytotoxic and are suitable for agricultural application.

Growth promotion of AFM compost on the cultivation of *Brassica rapa*

Nitrogen is of utmost importance primary nutrient needed by plant for optimal growth, and its application rate could significantly affect the yield and growth performance of *Brassica* species (13–15). As the main N source in this study was in organic N form originally from the fermented MKR compost, and to some extent from the 10% aqueous ammonia solution used during pH adjustment in L-lactic acid fermentation, nitrogen availability for plant root uptake might dependent on microbial deaminase rate and net influx rates per root surface area unit (16). For this reason, we investigated the effect of triple application rate on Komatsuna growth by multiply the AFM compost with *Bacillus* strain KBKU21 (R4 and R10), T27 (R5 and R11) or N3-9 (R6 and R12). However, results revealed that higher application rates and nutrient contents did not bring positive effects. Similar results were also observed in Komatsuna amended with commercial compost under single and triple application rates of N:P:K

(R15 and R16). This showed rapid response on plant growth towards compost fertilizer might not be instant as seen in many inorganic N-fertilizer studies (13,17–18).

As described in Table 4, plant growth parameters, including fresh weight, plant height, and leaf length, were promoted using AFM compost with *B. subtilis* KBKU21 (R1, R4, R7, and R10), *B. coagulans* T27 (R2, R5, R8, and R11), or *B. subtilis* N3-9 (R3, R6, R9, and R12) as soil amendment in Komatsuna cultivation, with or without PK adjustment. Generally, AFM compost with KBKU21 was more effective in promoting Komatsuna growth than that with T27 (R2) or N3-9 (R3) (Fig. S2). When compared to NPK_{KBKU21} (R13) under same nutrient conditions, AFM compost with KBKU21 significantly ($p < 0.05$) promoted not only increased plant height (207%), but also increased leaf length (153%) and wet weight (149%). Results also revealed growth promotion effects of KBKU21-AFM compost with P and K adjustment under basal application rate (R7) were superb in comparison to control runs with chemical fertilizers (R14, by 120%) or commercial compost (R15, by 129%). However, no significant different was found between Komatsuna fresh weight obtained from P and K adjusted run (R7, 0.48 ± 0.16 g) or unadjusted run (R1, 0.46 ± 0.17 g) of KBKU21-AFM compost. This implies adjustment of P and K minerals is important for obtaining good Komatsuna yield in AFM compost with strain N3-9 and strain T27, but less important when PGPB strain KBKU21 is used. Its potential in reducing fertilizer usage and farming cost without causing yield lost but rather biomass gain is coincides with previous study on maize crop grown under arid agricultural land (12).

There have been reports of various kinds of PGPB, including *Acinetobacter*, *Azobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Serratia*, *Streptococcus* and *Streptomyces* spp., and some have been used

as bio-fertilizer and bio-pesticide inoculants (33–36). Growth parameters of Komatsuna amended with *B. subtilis* KBKU21-fermented AFM compost were greater among the runs. Several biochemical mechanisms unique to the selected *Bacillus* strains, such as N-fixation, P-solubilization, and indole-acetic acid (IAA) and siderophore production, may have contributed to the vigorous growth of Komatsuna in this study.

Strain KBKU21 was able to produce 260.6 µg/mL of ammonia when grown in peptone broth, as well as 6.4 µg/mL of auxin when 500 µg/mL tryptophan was added to the broth (12). A model explaining the mechanism of bacterial auxin uptake by plants has been illustrated, where the amino acid tryptophan exuded by plant roots was taken up by PGPB and converted into auxin before being released back to the rhizosphere (37). The L-lactic acid could have possibly assisted in the 'slow release' mechanism, and aided in the solubilization of inorganic minerals fixed in soil colloids for Komatsuna uptake, as reported in a previous study (38). This would be likely, since the AFM compost with no P and K adjustment (R1) promote Komatsuna growth comparably to AFM compost with adjusted P and K (R7), and perform even better than chemical fertilizer (R14). Hence, in addition to direct effect exerted from biological systems such as P-solubilization and N-fixation, it is believed that the breakdown of macromolecules, such as proteins, lipids and polysaccharides, as well as mineral nutrients previously fixed in the bacterial cell biomass, could have enriched the soil organic colloids. Although strain KBKU21 lacks of siderophores for iron chelation, it is believed that by excreting organic acid in the rhizosphere, iron can become more soluble and available for plant and bacterial uptake (39).

This study revealed AFM compost not only can act as the 'sink' for moisture, as organic matter, and as bacterial proliferation medium, but also can act as a 'source' for

slow-releasing minerals, such as N, P, K, and highly optically pure L-lactic acid, that could synergistically contributing to the vigorous growth of Komatsuna. Even though strain KBKU21 did not produce highest L-lactic acid in term of selectivity and yield in this study, it remains the only strain with dual functional capabilities in turning food waste to two value-added end products. Utilizing this novel PGPB *B. subtilis* KBKU21 in fermenting food waste could thus be regarded as a useful method for producing both highly optically pure L-lactic acid for the biodegradable plastic industry and a new type of anaerobically fermented bio-compost that is capable of promoting plant growth under low fertilizer inputs. This could be useful as a kind of functional compost or soil conditioner. Compared to traditional composting, in which easily-degradable organic carbon is oxidized to produce carbon dioxide, anaerobic organic acid production via microbial fermentation can trap carbon inside the useful chemical material. Thus, the process could recognize as a greenhouse gas-less process.

In conclusion, this study revealed the potential of *B. subtilis* KBKU21 in converting kitchen refuse to two useful end products: raw material for biodegradable plastic, and functional compost. KBKU21 is proposed as a novel PGPB strain that is capable of producing highly optically pure L-lactic acid from food waste using non-sterilizing fermentation techniques. We further demonstrated that the growth parameters of Komatsuna amended with basal rate of KBKU21-AFM compost, without P and K adjustments, were comparable, and better than that with chemical fertilizers and commercial compost. We believe that the concept demonstrated in this study provides a strong driving force for the complete recycling of food waste, and will contribute towards the establishment of sustainable society.

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2 **FIG. 1.** Time course of MKR compost fermentation to L-lactic acid by (A) *B. subtilis* KBKU21 (43°C), (B) *B. coagulans* T27 (50°C) and
3 (C) *B. subtilis* (N3-9) (45°C) under intermittent pH adjustment at 7.0 at 12 h interval. Symbols: *filled circle*, lactic acid; *filled triangle*, total
4 sugar; *filled square*, formic acid; *open circle*, acetic acid; *open square*, pH.

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FIG. 2. Effect of MKR compost on germination of Komatsuna seed. Error bars show 95% confidence intervals of the mean germination.

1 **TABLE 1.** Experimental design for cultivation of Komatsuna.

Run	Abbreviation	MKR compost	N	P	K	Commercial compost	Fertilizer component (mg/pot)		
			(Ammonium sulfate)	(Calcium biphosphate)	(Potassium chloride)		N	K ₂ O	P ₂ O 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 (3x)	+	-	-	-	-	150	29.5	22.8
R5	T27 (3x)	+	-	-	-	-	150	28.1	22.1
R6	N3-9 (3x)	+	-	-	-	-	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 (3x)	+	-	+	+	-	150	150	150
R11	T27 + PK (3x)	+	-	+	+	-	150	150	150
R12	N3-9 + PK (3x)	+	-	+	+	-	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 (3x)	-	-	-	-	+	150	150	150
R17	No amendment	-	-	-	-	-	0	0	0

2 Footnotes: +, with; -, without; 3x, triple rate.

3 **TABLE 2.** Summary of fermentation of MKR medium.

<i>Bacillus</i> strain	Maximum accumulated lactic acid (g/L)	Lactic acid selectivity (w/w %)	Optical purity (%) ^a
KBKU21	36.9 (84 h)	98.2	95.7
T27	47.6 (108 h)	99.2	87.8
N3-9	39.8 (96 h)	98.5	92.8

4 ^a Data at 120 h

5 **TABLE 3.** Major nutrient contents in the solid precipitate and liquid supernatant of
6 MKR before (0 h) and after (120 h) fermentation.

		C/N		C (%)		N (%)		P ₂ O ₅ (%)		K ₂ O (%)	
		0 h	120 h	0 h	120 h	0 h	120 h	0 h	120 h	0 h	120 h
Solid	KBKU21	13.3	10.6	55.6	49.1	4.20	4.60	0.50	0.70	0.78	0.75
precipitate	T27	13.8	10.1	54.0	52.5	3.90	5.20	0.59	0.76	0.76	0.80
(SP)	N3-9	13.9	10.9	55.3	51.7	4.00	4.80	0.50	0.89	0.69	0.79
Liquid	KBKU21	16.3	4.92	40.9	42.3	2.50	8.60	0.63	0.54	2.60	3.50
supernatant	T27	16.7	5.93	41.2	42.1	2.50	7.10	0.73	0.28	2.60	2.80
(LS)	N3-9	15.7	5.35	43.2	41.2	2.80	7.70	0.73	0.49	2.60	3.10

8 **TABLE 4.** Plant growth promoting effect of MKR compost for Komatsuna plant
9 cultivation compare with control.

Run	Abbreviation	Fresh weight (g)	Plant height (mm)	Leaf length (mm)
R1	KBKU21	0.46 ± 0.17 (115)	74.0 ± 11.0 (112)	32.3 ± 6.4 (113)
R2	T27	0.34 ± 0.14 (85)	65.8 ± 8.7 (100)	26.9 ± 4.9 (94)
R3	N3-9	0.34 ± 0.13 (85)	63.5 ± 12.3 (96)	27.0 ± 5.9 (94)
R4	KBKU21 (3x)	0.43 ± 0.16 (108)	70.7 ± 12.5 (107)	30.4 ± 5.9 (106)
R5	T27(3x)	0.33 ± 0.10 (83)	64.7 ± 9.3 (98)	25.4 ± 7.0 (89)
R6	N3-9 (3x)	0.31 ± 0.11 (78)	62.7 ± 10.0 (95)	26.6 ± 5.3 (94)
R7	KBKU21 + PK	0.48 ± 0.16 (120)	71.8 ± 10.0 (109)	32.7 ± 5.4 (114)
R8	T27 + PK	0.39 ± 0.13 (98)	68.5 ± 8.4 (104)	29.1 ± 4.9 (101)
R9	N3-9 + PK	0.35 ± 0.17 (88)	62.8 ± 14.7 (95)	27.5 ± 7.5 (96)
R10	KBKU21 + PK (3x)	0.42 ± 0.15 (105)	68.2 ± 11.2 (104)	30.7 ± 6.1 (107)
R11	T27 + PK (3x)	0.42 ± 0.16 (105)	68.6 ± 11.3 (104)	30.4 ± 5.1 (106)
R12	N3-9 + PK (3x)	0.35 ± 0.14 (88)	61.5 ± 12.2 (93)	27.3 ± 6.8 (95)
R13	NPK _{KBKU21}	0.21 ± 0.05 (53)	51.9 ± 5.3 (79)	20.4 ± 3.1 (71)
R14	NPK	0.40 ± 0.21 (100)	65.9 ± 13.3 (100)	28.7 ± 6.8 (100)
R15	Commercial Compost	0.37 ± 0.15 (93)	64.4 ± 12.1 (98)	28.6 ± 6.7 (100)
R16	Commercial Compost (3x)	0.41 ± 0.14 (103)	63.1 ± 15.0 (96)	29.6 ± 5.8 (103)
R17	No amendment	0.22 ± 0.07 (55)	49.4 ± 7.0 (75)	20.2 ± 3.9 (70)

10 KBKU21, N3-9 and T27 were applied as soil amendment. Plant were harvested after 3
11 weeks for various growth measurements (n = 7). All data presented were mean value $\pm$
12 SE. Data presented in the parenthesis were percentage of plant growth promoting effect
13 relative to control, R14.