PHILIPP AGRIC SCIENTIST
Vol. 101 No. 1, 60–69
March 2018

ISSN 0031-7454

Biofertilizer and Liquid Organic Fertilizer Production by *Klebsiella* sp. and *Bacillus* sp.

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The quality and quantity of organic fertilizers were mainly affected by suitable carriers and technological limitations. Experiments were conducted to determine the potential of *Bacillus* sp. in enhancing the fermentation process of daily waste substrates and to evaluate the survival of *Klebsiella* sp. in various carriers. The test for antagonistic activity between *Bacillus* sp. and *Klebsiella* sp. showed that they were not competitive against each other. Fruit-bacteria (fruit waste substrate in combination with *Bacillus* sp.) was the best treatment which had the highest amount of microorganisms on day 15 and on day 30 of the fermentation process. The survival of *Klebsiella* sp. was monitored over a period of 2 mo in dry inoculation as biofertilizer and in fresh inoculation as liquid organic fertilizer. Compared with bagasse and corn husk, rice straw harbored the highest number of bacteria. In fresh microbial inoculants, molasses was better than diluted distillery slop solution by nearly maintaining the number of *Klebsiella* sp. at 1.2 × 10^7 CFU g^{-1}, which decreased at day 60. The number of *Klebsiella* sp. in fermented fruit waste was also highest at day 15 and gradually decreased towards day 60. Although the number of *Klebsiella* sp. fell sharply during incubation time, it was higher in biofertilizer than in liquid organic fertilizer.

Key Words: *Bacillus* sp., biofertilizer, *Klebsiella* sp., liquid organic fertilizer

Abbreviations: CFU – colony-forming unit, CMC – carboxymethyl cellulose, EC – electrical conductivity, NBRIP – National Botanical Research Institute's phosphate, PGPR – plant-growth-promoting rhizobacteria

INTRODUCTION

Plant-growth-promoting rhizobacteria (PGPR) have a vital role in agricultural systems, especially as a biofertilizer. 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and P solubilization are properties of PGPR that promote forage corn growth (Piromyou et al. 2011). Rhizobacteria also improve the productivity of the crop via excretion of plant-growth-promoting substances such as vitamins, gibberellins and kinetin (Karthikeyan et al. 2008). In addition, the mixed biofertilizer with diazotrophic or N_2-fixing bacteria, which increases phosphorus and potassium in the soil, can be used as an alternative for NPK fertilizer (Andrade et al. 2013).

PGPRs are classified on the basis of their function and characteristics. *Klebsiella* sp. and *Bacillus* sp. are useful PGPRs that have been used to produce solid and liquid biofertilizers. *Klebsiella* sp. can fix nitrogen and are classified as associative nitrogen fixers, or diazotrophs (Pedersen et al. 1978; Mahl et al. 1965). Results from the study of Sachdev et al. (2009) showed that the shoot height and root length of inoculated wheat seedlings were significantly increased in all six *Klebsiella*-producing strains compared with the control treatment. Moreover, *Klebsiella* species SBP-8 protected the plants against adverse effects of salt and temperature stress (Singh et al. 2015). In addition, *Bacillus* sp. belong to cellulolytic bacteria (Koeck et al. 2014) which have enzymes to digest cellulose and hemicellulose to smaller units, thereby improving the fermentation process of daily waste substrates. In an earlier study, Zainudin et al. (2013) found that the isolated *Bacillus* sp. is helpful in rapid composting and degrading of lignocellulosic oil palm from an empty fruit bunch within 40 d.

Both chemical fertilizers and the rhizosphere that contains PGPR can increase crop yield. However, the use of chemical fertilizers is not eco-friendly for sustainable crop development. It can destroy the original properties

of the soil and can be harmful to microorganisms as well as plant growth (Kumar et al. 2012). Hence, the use of chemical fertilizers should be reduced. Biofertilizers may serve as a good alternative based on their remarkable characteristics. According to Muraleedharan et al. (2010), biofertilizers are products that contain different types of living microorganisms which can live symbiotically or non-symbiotically in the rhizosphere and plant root by colonization, and thus promote plant growth when applied to plant surface, soil or seed.

Actually, biofertilizers do not provide nutrients for plants as chemical fertilizers do but can stimulate plants to access available nutrients in the environment and to provide direct or indirect gains in crop yield (Figueiredo et al. 2010). Compared with solid biofertilizer, liquid biofertilizer can be an ideal resource for improving the shelf-life of a biofertilizer because of its potential in providing a sufficient amount of nutrients, enhancement of cell/spore/cyst formation and cell protection, and high-temperature tolerance. Thus, the successful commercialization of less expensive liquid biofertilizers is a challenge to researchers, considering that improving the shelf-life of such products is still a major concern (Brar et al. 2012).

To the best knowledge of the authors, there have been many researches about rhizobacteria in biofertilizer, but very few publications are available in the literature that discuss the issue of cellulolytic bacteria *Bacillus* sp. and rhizobacteria *Klebsiella* sp. in liquid and solid biofertilizers. The objectives of this study were (1) to determine the potential of *Bacillus* sp. in enhancing the fermentation process of daily waste substrates and (2) to evaluate the survival capability of *Klebsiella* sp. strain in producing solid and liquid organic biofertilizer.

MATERIALS AND METHODS

The study was conducted from January to March 2015 in the Microbial Fertilizer Laboratory, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand. The ambient temperature ranged from 28 to 37 °C.

Klebsiella sp. and *Bacillus* sp. isolates were obtained from the Microbial Fertilizer Laboratory group, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand. The *Klebsiella* sp. was selected based on its multiple roles in nitrogen fixation, phosphate solubilization, potassium solubilization, ACC deaminase activities, ammonium production, indoleacetic acid (IAA) and gibberellic acid production, whereas *Bacillus* sp. was selected based on its high cellulase activity (Nhu and Riddech 2016). The fruit waste substrate was fermented in combination with *Bacillus* sp. whereas biofertilizer production was determined by the number of *Klebsiella* sp.

Antagonistic Activity Test

Bacillus sp. and *Klebsiella* sp. were used as microbial inocula in the fermentation process. The antagonistic activity between *Bacillus* sp. and *Klebsiella* sp. was tested on nutrient agar using the perpendicular method (Mittal et al. 2008). Survival of *Klebsiella* sp. in the liquid organic fertilizer was determined using the nutrient agar culture and checked with gram staining of random samples of colonies.

Fermentation of Vegetable and Fruit Waste Substrates using *Bacillus* sp.

Experimental design. Vegetable and fruit waste substrates in combination with molasses (10 times diluted) were used to produce liquid organic fertilizer. The ratio of substrates: molasses: distilled water was 3:1:1 (w/v). The fermentation process was done in plastic buckets at room temperature. Molasses were used as a supplement for the culture medium of bacteria. *Bacillus* sp. at 10^8 colony-forming unit (CFU) mL^{-1} and 10% of inoculum size (v/v) was used in this process. Completely randomized design was used on six treatments with three replications. Treatment 1 (fruit) was the combination of fresh fruit waste substrate, molasses and water, and addition of *Bacillus* sp. in treatment 2 (fruit-bacteria). Treatment 3 (vegetable) was fresh vegetable waste substrate, molasses and water while treatment 4 (vegetable-bacteria) contained fresh vegetable waste substrate, molasses and water with addition of *Bacillus* sp. The control (control-fruit and control-vegetable) treatment consisted of substrates and water only.

Microbial analysis

Total nitrogen fixer in nitrogen-free medium, total potassium-solubilizing bacteria, total phosphate-solubilizing, proteolytic, amylolytic and cellulolytic bacteria, pH and electrical conductivity (EC) were measured at 5, 10, 15 and 30 d after fermentation.

Total phosphorus and potassium were analyzed using the method of Ramanathan and Ting (2015) while organic matter and total nitrogen were detected by using the method of Schumacher (2002) and Campins-Falco et al. (2008), respectively.

Total nitrogen fixer in nitrogen-free medium

Total nitrogen fixer was determined in the nitrogen-free medium (Dobereiner and Day 1976) by spread plate technique. Bacteria were screened in the nitrogen-free medium composed of mannitol (20 g), dipotassium

phosphate (0.2 g), magnesium sulfate (0.2 g), sodium chloride (0.2 g), potassium sulphate (0.1 g), and calcium carbonate (5 g). Plates were incubated at 28 °C for 48 h.

Total potassium-solubilizing bacteria

Aleksandrov agar medium consisting of 1% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPO_4$ and 0.5% potassium aluminum silicate, and agar (1.5%, pH–4.5) was used for the detection of potassium solubilization bacteria (Sugumaran and Janartham 2007). After seeding the samples, plates were incubated at 28 ± 1 °C for 3–5 d and observed for a clear zone formed around the colonies due to solubilization of inorganic potassium.

Total phosphate-solubilizing bacteria

Total phosphate-solubilizing bacteria were identified as the colonies of isolates that formed a clear halo zone on National Botanical Research Institute's phosphate (NBRIP) which consisted of NBRIP medium containing glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $MgCl_2{\cdot}6H_2O$, 5 g; $MgSO_4{\cdot}7H_2O$, 0.25 g; KCl, 0.2 g; $(NH_4)_2SO_4$, 0.1 g; and agar (1.5%, pH 7.0) (Nautiyal 1999).

Total cellulase-producing bacteria

Cellulase-producing bacteria were identified on carboxymethyl cellulose (CMC) medium (Kasana et al. 2008), which consisted of 0.2% $NaNO_3$, 0.1% K_2HPO_4, 0.05% $MgSO_4$, 0.05% KCl, 0.2% CMC sodium salt, 0.02% peptone, and 1.5% agar. The plates were incubated at 28°C for 48 h and flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 mL distilled water) for 3–5 min; a clear zone indicates positive cellulose production.

Total protease-producing bacteria

Protease-producing bacteria were screened using skim milk agar medium that contained 2% (w/v) skim milk, 1% (w/v) tryptone and 7% (w/v) NaCl (Xiong et al. 2007). The plates were incubated at 37°C for 24 h and colonies having clear halo zones were identified as positive producers of protease.

Total amylase-producing bacteria

Bacterial isolates were screened on starch agar plate which was composed of beef extract, 3 g; soluble starch, 10 g; and 1.5% agar in 1 L of distilled water (Alariya et al. 2013). After incubation at 37°C for 24 h, plates were flooded with Gram's iodine (2.0 g KI and 1.0 g iodine in 300 mL distilled water) for 3–5 min to observe the clear halo zone around colonies as indicator of positive amylase production.

Production of Microbial Inoculum as Biofertilizer and Liquid Organic Fertilizer

Fresh inoculation as liquid organic fertilizer production

The fermentation products of fruit waste in combination with *Bacillus* sp., molasses [diluted 1:100 (v/v) by sterilized distilled water] and distillery slop solution [diluted 1:100 (v/v) by sterilized distilled water] were used for fresh microbial inoculants as liquid organic fertilizer. Liquid organic fertilizer was determined as the amount of total nitrogen, phosphorus, potassium and organic matter. The solution was autoclaved for 60 min at 121°C, inoculated with *Klebsiella* sp. at a cell density of 10^8 CFU mL^{-1}, 10% (v/v) of inoculum size, and then incubated at room temperature for 2 mo. The percentage survival of *Klebsiella* sp. cell was determined on nutrient agar by spread plate technique at day 15, 30, 45 and 60. The pH and electrical conductivity (EC) were determined at day 15, 30, 45 and 60.

Carrier preparation

Rice straw, bagasse and corn husk were used as carrier for preservation of *Klebsiella* sp. Carriers were ground to small pieces (0.1–0.5 mm size) by blender, then 500 g of each carrier was packed in polyethylene bags, carefully covered by button and autoclaved three times for 60 min at 121 °C, in three replications (modification by El-Fattah et al. 2013). The package bag was then dried for 12 h at 60 °C in a hot air oven (Wang et al. 2015). The composition of total nitrogen, total phosphorus, potassium and organic matter of the carrier was analyzed (Campins-Falco et al. 2008). Dry microbial inoculants were prepared by immobilizing isolates on agricultural carriers and incubated at room temperature for 2 mo.

Dry inoculation as biofertilizer production

The 40% (v/w) broth culture of *Klebsiella* sp. at a cell density 10^8 CFU mL^{-1} was mixed thoroughly with the carrier and added with sterilized water to attain a moisture content of 70%. The percentage survival of *Klebsiella* sp. in the carrier was determined by viable count on agar plate after it was kept at 15, 30, 45 and 60 d. The pH, EC, and moisture content of the carrier were also determined.

Data Analysis

Data were analyzed using IBM SPSS Statistics 19 software. Least significant difference test (LSD) at 5% level was used to compare means of the treatments with three replications by completely randomized design. Microsoft Office Excel 2013 was used for the data presentation.

Fig. 1. Antagonistic activity between *Bacillus* sp. and *Klebsiella* sp. (Rows: *Klebsiella* sp. and a column: *Bacillus* sp.)

RESULTS

Antagonistic Activity Test

Bacillus sp. and *Klebsiella* sp. were used as microbial inocula. The antagonistic activity between *Bacillus* sp. and *Klebsiella* sp. was tested using the perpendicular method. Results showed that they were not competitive against each other (Fig. 1), suggesting that *Bacillus* sp. can be used for fermentation of the fruit waste substrate, and that the product can be used to produce liquid organic fertilizer by adding *Klebsiella* sp.

Fermentation of Vegetable and Fruit Waste Substrates with Bacillus sp.

Changes in plant-growth-promoting bacteria, proteolytic and amylolytic bacteria during fermentation

Figure 2 shows the number of nitrogen-fixing and potassium- or phosphate-solubilizing bacteria in liquid fertilizer with or without addition of *Bacillus* sp. In general, the number of bacteria in the fruit waste treatment rapidly increased from day 0 to day 5, peaked at day 15 of culture, and later on, decreased at day 30. In the nitrogen-free medium at day 5, the significantly higher number of bacteria was 6.96 and 6.95 log CFU mL^{-1} in the vegetable and vegetable-bacterial treatments, respectively. However, this number in the fruit and control-fruit treatments was rapidly increased and reached the highest number at day 15 (7.46 and 7.53 log CFU mL^{-1}, respectively). The amount of nitrogen-fixing bacteria at day 30 was around 5.5 log CFU mL^{-1}.

Phosphate solubilization bacteria were higher in number in the vegetable substrate at day 5 (7.15 log CFU mL^{-1} in the control-vegetable and 7.13 log CFU mL^{-1} in the vegetable-bacteria substrate) and then decreased at day 30. While phosphate solubilization bacteria in the fruit-bacteria culture had a slow increase during the first 5 d of inoculation, there was a rapid increase at day 15 (8.26 log CFU mL^{-1}) and the highest number of phosphate solubilization bacteria remained until day 30 (6.73 log CFU mL^{-1}) (Fig. 2).

Among all fruit waste treatments, the number of bacteria in the potassium solubilization media was also significantly higher in the fruit-bacteria treatments at day 10 and day 15 (7.48 and 7.92 log CFU mL^{-1}, respectively) (Fig. 2). At day 30, this number was 10^6 CFU mL^{-1} in the fruit waste treatments and 10^5 in the vegetable treatments.

Comparison of the fruit waste treatments showed that the number of nitrogen-fixing bacteria and of potassium-solubilizing bacteria was higher in the vegetable waste treatments in the first 5 d and did not change much during incubation time. Moreover, the total number of phosphate solubilization bacteria was highest compared with total nitrogen-fixing and potassium solubilization bacteria.

The number of bacteria in the vegetable substrate was highest in cellulase, protease and amylase agar at day 5. The vegetable-bacteria substrate had the highest amount of cellulose-producing bacteria (6.75 log CFU mL^{-1}). The control-vegetable treatment with 6.88 log CFU mL^{-1} bacteria could produce protease enzyme. The number of amylase enzyme-producing bacteria in the vegetable substrate was significantly higher in the fruit treatments (6.99 log CFU mL^{-1}).

The number of protease and amylase bacteria in the control-fruit treatment was 7.27 and 7.31 log CFU mL^{-1}, respectively, at day 10. However, the highest number of bacteria in skim milk, starch and CMC media was in the fruit treatment (7.29, 7.32, and 7.30 log CFU mL^{-1}, respectively), followed by the fruit-bacteria treatment in which the number of amylolytic bacteria was 7.31 log CFU mL^{-1} in starch medium (Fig. 3).

The number of bacteria in all treatments was approximately 6 log CFU mL^{-1} at day 15. Significantly, fruit waste substrate in combination with *Bacillus* sp. (fruit-bacteria treatment) was the best treatment which had the highest number of bacteria in skim milk and starch and CMC media (Fig. 3).

The number of bacteria in the vegetable substrate was higher than that in the fruit waste substrate at day 5, while the number of bacteria in the fruit waste substrate was higher than that in the vegetable substrate at day 10. Compared with day 15, the number of bacteria at day 30 decreased in nitrogen-fixing, NBRIP, Aleksandrov and starch media. At day 30, the number of bacteria in the fruit waste substrate treatments was higher than that in the vegetable waste substrate. The fruit-bacteria treatment had the highest number of bacteria in five media.

Changes in pH and electrical conductivity (EC) during fermentation

Compared with the fruit waste (control-fruit, fruit, and fruit-bacteria) supplemented treatments, the vegetable supplemented (control-vegetable, vegetable, and vegetable-bacteria) treatments had higher pH at every time point examined, and the pH increased with time. The average pH in the fruit waste treatments (control-

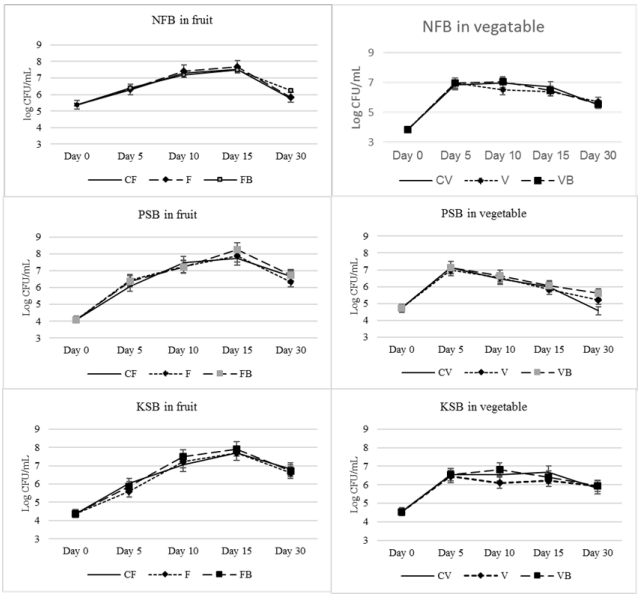

Fig. 2. Number of nitrogen-fixing (NFB), phosphate solubilizing (PSB) and potassium solubilizing (KSB) bacteria in fruit and vegetable fermentation. (CF: Control Fruit, F: Fruit, FB: Fruit and bacteria, CV: Control vegetable, V: vegetable, VB: Vegetable and bacteria in fruit (F)- or vegetable (V)-supplemented medium)

fruit, fruit, and fruit-bacteria) increased from 3.47 on day 5 to 5.41 on day 30, while the pH of the vegetable waste (control-vegetable, vegetable, and vegetable-bacteria) treatments increased from 4.78 on day 5 to 7.29 on day 30 (Fig. 4).

EC of the vegetable substrate was nearly twice higher than that of the fruit waste substrate and did not change much for 30 d. The control-fruit had the lowest EC with only 5.10 μS cm^{-1} at day 5 which increased to 5.3 μS cm^{-1} at day 30. The highest EC was that of the vegetable-bacteria treatment (11.37 at day 5 and 13.32 at day 30) (Fig. 4).

Changes in nutrient content after 30 d of fermentation

Results of nutrient analysis showed that addition of *Bacillus* sp. increased the nutrient content in both vegetable and fruit waste, especially, organic matter. The nitrogen content was significantly higher in vegetable waste than that in fruit waste and there was no significant difference among control-vegetable, vegetable, and vegetable-bacteria samples. In contrast, the amount of phosphate was higher in the fruit waste substrate treatments where the highest content was in the fruit-bacteria treatment (225 mg L^{-1}). Interestingly, the amount of potassium did not depend on the initial material. There was no significant difference in potassium content among the vegetable (3076.33 mg L^{-1}), vegetable-bacteria (2950.33 mg L^{-1}), and fruit-bacteria (2929.33 mg L^{-1}) treatments, which were significantly higher than the potassium content in the other three treatments (Fig. 5). Finally, the fruit treatment had the highest organic matter content (7435.00 mg L^{-1}), followed by fruit-bacteria (6883.3 mg L^{-1}) (Fig. 5). Generally, *Bacillus* sp. showed an active role in fruit-bacteria fermentation which had the highest phosphate and organic matter content, while the potassium content was also high.

Biofertilizer and Liquid Organic Fertilizer Production

Nutrient content in raw carrier materials to produce dry and fresh inoculation

Nutrient content was variable among the three dry and two liquid raw materials. Total N (%) content in molasses (0.707%) was the highest, followed by rice straw (0.686%), which was significantly higher than that of the other raw materials. The rice-straw-based fertilizer had the highest total P (0.082%), whereas the molasses-based fertilizer had the highest amount of K (3.254%). There were no significant differences among the percentages of organic matter in molasses (88.7%), bagasse (83.9%) and corn husk (83.6%). The organic matter content of the distillery slop water was the lowest (Table 1).

Growth and survival of Klebsiella species in various substrates to produce biofertilizer

Corn husk, rice straw and bagasse, which was the control treatment and did not contain *Klebsiella* sp. at the time of inoculation at day 0, was not contaminated during the inoculation period. The number of *Klebsiella* sp. was highest at day 15 in all carriers and substrates but decreased at day 60 (Fig. 6). Rice straw substrate had the highest number of *Klebsiella* sp. bacteria (9.81 log CFU g^{-1}) at day 15 and it remained highest at days 30, 45 and 60 (Fig. 6). In distillery slop water and molasses, the number of *Klebsiella* sp. remained stable at around 7 log CFU g^{-1} until day 30, and then gradually decreased towards day 60. In the fermented fruit waste, the number of *Klebsiella* sp. was also highest at day 15 and decreased gradually to day 60.

In terms of the number of *Klebsiella* sp., rice straw was the best carrier in dry inoculation. The best substrate was fermented fruit waste, which was the best treatment in fresh inoculation. Compared with liquid organic fertilizer, *Klebsiella* sp. was the better biofertilizer, however, its number fell sharply during incubation time.

pH, EC and moisture changes during biofertilizer production

In general, except for rice straw, the pH of biofertilizers in the different substrates at the beginning was acidic and slowly increased from day 15 to day 60, while that of liquid organic fertilizers was alkaline at the beginning and rose towards day 45 and remained the same until day

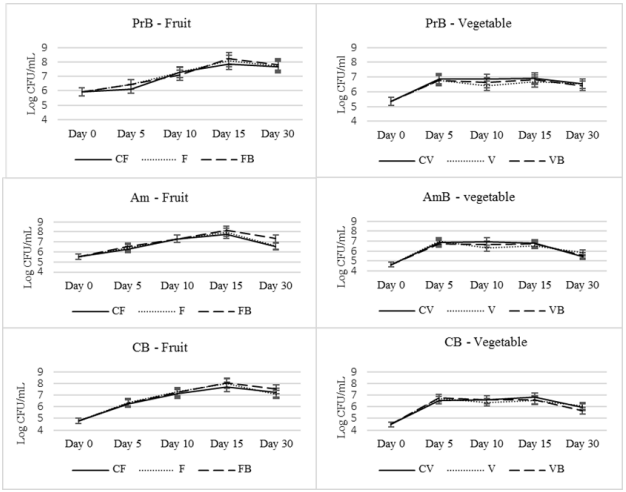

Fig. 3. Number of proteolytic (PrB), amylolytic (AmB) and cellulolytic (CB) bacteria in liquid fertilizer. (CF: Control Fruit, F: Fruit, FB: Fruit and bacteria, CV: Control vegetable, V: vegetable, VB: Vegetable and bacteria)

60. Among the dry carriers, the pH of the rice straw carrier was significantly higher than that of the other carriers (Fig. 7). The change in pH was remarkable in corn husk and fruit + bacteria fermented product with an increase in pH from about 5 on day 15 to about 7 on day 60.

In contrast to pH, EC decreased in all treatments. The lowest EC was recorded in liquid organic fertilizer with 2.1 μS cm^{-1} diluted distillery slop and 2.7 μS cm^{-1} in molasses at day 60 (Fig. 7). EC in dry inoculation ranged from 10.7 to 14.5 μS cm^{-1}.

The moisture content of all dry substrate cultures decreased gradually from day 15 to day 60 (Fig. 7). On day 15, the moisture content of the corn control husk (CC), corn husk (C), rice straw (R), and bagasse (S) treatments ranged from 62.8% to 65.8% and were significantly different from the moisture content of the control rice straw (CR) (60.7%) and the control bagasse (CS) (60.3%) treatments. There was no significant difference in moisture content on day 30. The lowest moisture content on day 45 was that of the rice straw culture (44.3%) and that of bagasse (31%) on day 60.

DISCUSSION

In this study, the total number of nitrogen-fixing, phosphate and potassium solubilization, proteolytic, amylolytic and cellulolytic bacteria were the highest in fruit plus *Bacillus* sp. Tanuwat and Penja (2009) showed that successful liquid fertilizer production was obtained from using fruit juice as the substrate of fermentation for 30 d, but with lower pH and EC. Fruit and vegetable wastes contain mainly organic acids, cellulose, soluble and insoluble starch (Stabnikova et al. 2005). Therefore, the indirect method to qualify the liquid product after fermentation was to determine the number of cellulolytic, proteolytic and amylolytic bacteria. In our study, the number of those enzymatically active bacteria in the fruit waste treatment was higher in vegetable waste and the lowest one was the number of cellulolytic bacteria, which play an important role in recycling cellulose in the biosphere (Li et al. 2009). Moreover, the higher number of proteolytic bacteria (10^7 CFU mL^{-1}) was detected in both vegetable and fruit waste treatments; peptide bonds in substrate were cleavaged by the protease subgroup of hydrolytic enzymes (Ghasemi et al. 2011).

In this study, both pH and EC in fruit waste treatments were lower than the pH and EC in the vegetable waste treatments. While pH must be stabilized within a certain range in liquid inoculum preparation (Pindi and Satyanarayana 2012), EC must be maintained low for nontoxic application in plant cultivation. The organic matter from fruit and vegetable waste is degraded by the microorganism and enzyme activities from bacteria, which was similar to the results of a study about soil organic matter decomposition by Xu et al. (2015). In addition, fruit waste plus *Bacillus* sp. had higher nutrient content and lower EC compared with other substrates and a suitable pH for survival of rhizobacteria. Therefore, the role of *Bacillus* sp. in the treatments is to enhance the degradation of substrates and to adjust pH and EC during the fermentation process which allows production of ideal liquid fertilizer to support plant growth.

The natural environment sometimes inhibits the growth of microorganisms. Therefore, it will be necessary to find a good carrier to maintain the number of beneficial bacteria in the soil for plant growth. In our study, rice straw, corn husk and bagasse were used as dry carriers, while distillery slop and molasses were used as fresh carriers. The initial inoculum of *Klebsiella* sp. in the carriers was 10^8, which increased until day 15. In general, growth of inoculated bacteria in a carrier is promoted until 15–30 d (Xu et al. 2015).

For the production of biofertilizer, a good carrier should have the following properties: low cost, nontoxicity to isolates, easy for sterilization, available in sufficient amount, good absorption for maintaining moisture, free of lump-forming and easy to process, suitable for seed germination and good pH buffering capacity (Somasegaran and Hoben 1994). In addition, the carriers should be evaluated based on the final results of microbial survival and multiplication during storage time using the plating method. In the study of Somasegaran and Hoben (1994), peat is the best carrier, although bagasse vegetable oils can be used as a substitute material

Table 1. Nutrient content in raw materials of dry and liquid substrates as carrier.

Type of Biofertilizer	Type of Carrier	Total N (%)	Total P (%)	Total K (%)	Organic Matter (%)
Biofertilizer	Bagasse	0.368^{c}	0.022^{d}	0.341^{e}	83.887^{ab}
	Corn husk	0.591^{b}	0.034^{c}	0.699^{d}	83.560^{ab}
	Rice straw	0.686^{a}	0.082^{a}	2.101^{b}	79.625^{b}
Liquid organic fertilizer	Distillery slop water	0.183^{d}	0.001^{e}	1.005^{c}	2.370^{d}
	Molasses	0.707^{a}	0.042^{b}	3.254^{a}	88.693^{a}
	Fruit waste	0.09^{e}	0.022^{d}	0.295^{f}	6.89^{c}
CV (%)		6.0	9.3	2.9	3.3

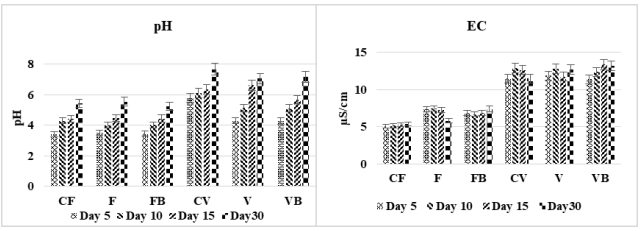

Fig. 4. pH and electrical conductivity (EC) of liquid organic fertilizer during fermentation time. CF: Control Fruit, F: Fruit, FB: Fruit and bacteria, CV: Control vegetable, V: vegetable, VB: Vegetable and bacteria.

over a wide range.

In our study, rice straw was the best carrier that retained a high number of bacteria in the range of 10^6–10^9 CFU g^{-1}, which fulfills the minimum standard (ranging from 5 x 10^7 to 1 x 10^9 Rhizobium cells/g of the product) in Thailand, Rwanda, the Netherlands and Australia (Lupwayi et al. 2000). A similar result reported that rice husk was the potential carrier for shelf life of bacteria as biofertilizer (Ogbo and Odo 2011). Ferreira and Castro (2005) also concluded that one way to fulfill characteristics of a good carrier is to support bacterial growth and survival at 10^9 bacteria/g carrier. All rhizobacteria strains require nutrients such as nitrogen and carbon sources for growth. In our study, rice straw and molasses were the two best carriers that provided nitrogen, phosphorus and potassium for *Klebsiella* sp. growth. Stephens and Rask (2000) demonstrated that

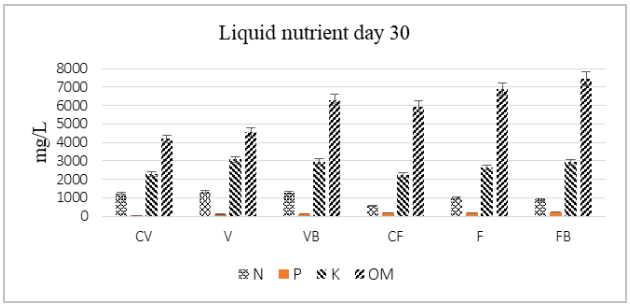

Fig. 5. Nutrient content in liquid organic fertilizer. (CF: Control Fruit, F: Fruit, FB: Fruit and bacteria, CV: Control vegetable, V: vegetable, VB: Vegetable and bacteria)

chemical and physical properties were indicators of a good substrate in inoculant technology. In our study, rice straw was the best carrier in keeping the moisture and suitable neutral pH for survival of *Klebsiella* sp. Lack of moisture and nutrient in carriers during incubation time caused a sharp decline in bacterial density.

When our results of nutrient compositions were compared with those of other studies (Khavazi et al. 2007), rice store nutrient was higher than that of rice husk and bagasse nutrient was lower than that of sugar cane. Khavazi et al. (2007) reported that the standard compost should have a pH of 5.5–8.5 and an EC ≤ 3500 μS cm^{-1}. In our results, pH and EC of both carriers and fresh inoculation were suitable for producing fertilizer. Concerning fresh inoculation, Rebah et al. (2002) concluded that the number of rhizobia remained successful until 80 d in wastewater sludge. However, in our study, fermented fruit waste was better than molasses and waste water for *Klebsiella* survival based on its higher nutrient content, lower EC and suitable pH. This result is strong evidence that fermented fruit wastes are the suitable substrate for producing liquid organic fertilizer. Although it has low nutrient initially, *Klebsiella* sp. survives well probably due to good pH and EC. Liquid fertilizer produced from fermented fruit wastes

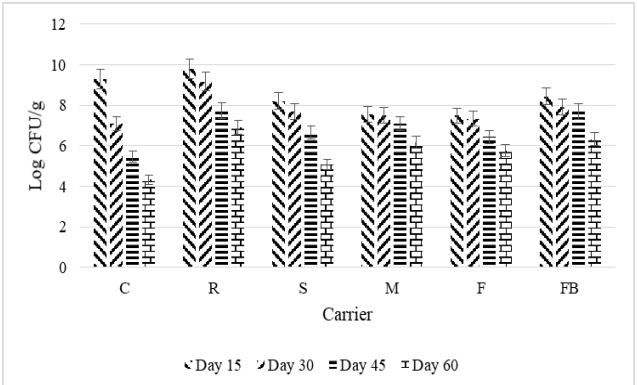

Fig. 6. Survival of *Klebsiella* sp. in various substrates during incubation time of 2 mo. (C: Corn husk, R: Rice straw, S: Baggase, F: diluted distillery slop water, M: molasses, FB: fermented fruit wastes in combination with *Bacillus* sp.)

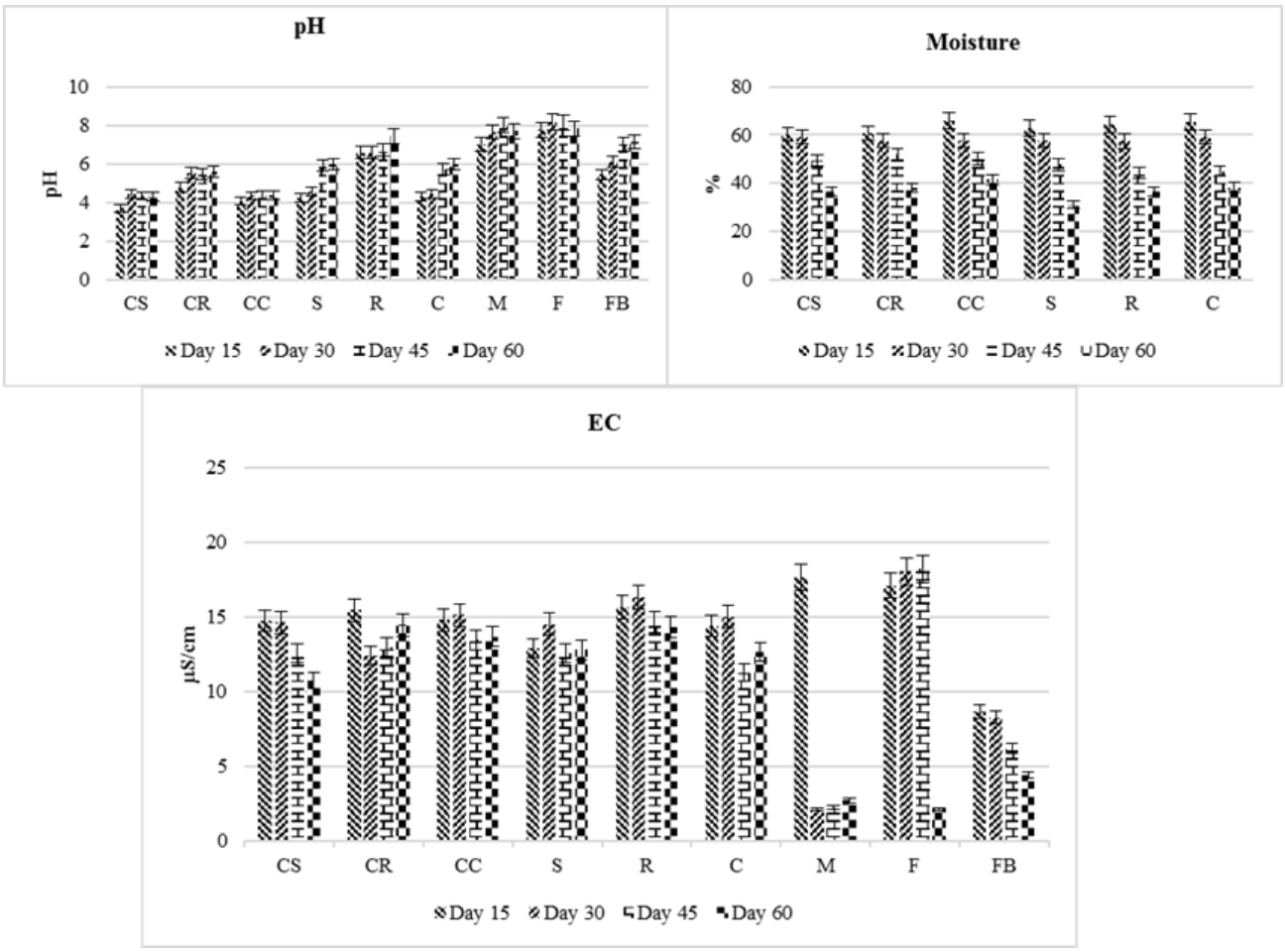

Fig. 7. pH, electrical conductivity (EC) and moisture of carrier during incubation time. (CS: control bagasse, CC: Control Corn husk, CR: Control Rice straw, S: bagasse, R: Rice straw, C: Corn husk, M: molasses, F: diluted distillery slop water, FB: fruit + bacteria fermented product)

can provide not only the microorganisms but also nutrients such as nitrogen, phosphate, potassium and organic matter for both bacteria and plant growth. Nutrient addition and plant growth will influence the structure of the bacterial community.

CONCLUSION

The results of our study suggest that waste substrates can be utilized to produce biofertilizer and liquid organic fertilizer, which are useful for plant crops. The fermentation process of fruit waste substrates was enhanced by adding *Bacillus* sp., resulting in high number of nitrogen-fixing, phosphate-solubilizing, potassium-solubilizing, proteolytic, amylolytic and cellulolytic bacteria. Additional inoculation of *Klebsiella* sp. as the plant-growth-promoting bacteria produced liquid fertilizer which provided not only the effective microorganisms but also nutrients for crop nitrogen, phosphate, potassium and organic matter. Rice straw substrate and molasses were the two best carriers for the survival of *Klebsiella* sp. up to 2 mo. The remaining moisture content, the suitable neutral pH, and the low EC were useful for the survival of *Klebsiella* sp. Lack of moisture and nutrient in carriers during incubation time caused the sharp decrease in *Klebsiella* sp. density.

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