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Potential of Legume and Maize Composts to Stimulate Population of Nitrogen- Fixing Bacteria, Phosphate-Solubilizing Bacteria and in Dole Acetic Acid Production

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ABSRACT

Organic matter in the form of compost contains nitrogen-fixing and phosphate-solubilizing bacteria that can produce indole acetic acid (IAA) to improve soil fertility. The objective of this study was to elucidate the potential of legume and maize to stimulate population of nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and indole acetic acid production. This study was conducted in three steps, i.e preparation of maize waste compost and legume waste compost, isolation of non symbiotic nitrogen-fixing bacteria and phosphate-solubilizing bacteria, and selection of bacteria with the highest ability on phosphate-solubilizing, nitrogen-fixing, and IAA production. Results of the study showed that seven isolates and thirteen isolates of phosphate-solubilizing bacteria were found in maize waste and legume waste, respectively. Three isolates of non symbiotic nitrogen-fixing bacteria were obtained from maize compost and two isolates were from legume compost. Isolate $K_2P_2$9.1 identified as *Bacillus subtilis* strain BS501α solubilized 5.24 ppm phosphate/ml, isolate K_2N_2 identified as *Pseudomonas putida* strain BN-St fixed 94.4 ppm nitrogen/hour, isolate $J_2P_1$9.2 identified as *Enterobacte*r sp EV-SA01 produced the highest IAA of 25.79 ppm/ml. The consortium of three bacteria grew well on 10 % molase media with amount of 1.01 x 10^9 after 24 hours incubation. Application of the 3 consortium bacteria to new maize and legume residues significantly increased N and P availability of the residues.
Key words: compost, legume, maize, nitrogen-fixing bacteria, phosphate-solubilizing bacteria, indole acetic acid.

INTRODUCTION

The increase of food need demands soil fertility improvement. Declining of soil organic matter content due to soil erosion and inorganic fertilization continuously damage soil and reduce plant production in Indonesia. Therefore, efforts of adding organic matter to soil have become main priority in order to increase soil fertility in Indonesia. Addition of organic materials can be made by applying farmyard manure, green manure or compost. Dahlin et al. [1] state that there possibilities for improving nitrogen use from organic materials in agricultural cropping. Compost is more effective than manures as it adds soil substance, contains high available nutrients, produces humus, generates growth hormone and suppresses pathogen organisms [2]. Compost can be made from various plant and animal residues having high microbial biodiversity, good compost may increase beneficial microbe and it is free from poisonous compound [3]. Nitrogen-fixing bacteria are widely found in symbiosis with plant. Such bacteria are able to use atmospheric nitrogen as nitrogen source for their growth that further provides nitrogen for plant and soil [4]. Phosphate-solubilizing bacteria generate metabolic compounds in their metabolism in the form of phosphates enzyme and organic acids such as citrate, occalate, and succinate that are able to solubilize unavailable P in the form of Ca-P,Al-P or Fe-P [5]. In addition, nitrogen-fixing and phosphate-solubilizing bacteria also have ability to generate Indole Acetic Acid (IAA) hormone with different ability [6].

The objective of this study was to elucidate the potential of legume and maize to stimulate population of nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and indole acetic acid production. In this study, nitrogen-fixing bacteria, phosphate-solubilizing bacteria were isolated and selected from compost made from maize and legume residues. It is very important to find the best isolate of nitrogen-fixing, phosphate solubilizing and IAA producing bacteria in order to give information the plant residues has ability to provide important bacteria that give benefit to soil fertility improvement and increase plant production.

MATERIALS AND METHODS

Sample collection and preparation of compost material

Compost materials used for this study were maize residue, bean (legume) residue and cow dung (Table 1). 500 kg of each plant residue (hybrid maize var. P-21 and bean), was collected from farm around experimental field of Faculty of Agriculture, University of Brawijaya at Jatikerto Malang, East Java than dried air for 1 week. The plant residues were chopped down to ± 2 cm. Each of the chopped residues was then mixed with cow dung with a ratio

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of 20:1 (5% cow dung). 250 kg of each residue-dung mixtures was diluted with 50 l of water. After dilution, the mixture was then piled up and covered up with thick plastic for 30 days. During the composting period, temperature was daily measured to maintain the temperature below 50°C. If the temperature reached > 50°C the mixtures was stirred and recovered. The composting process was stopped when the stable temperature of 28°C and the colour has turned to black. Compost observation was also done towards pH, C/N ratio, nitrogen fixing and phosphate solubilizing bacteria, available N and P and total microorganisms.

The isolation and selection of bacteria from compost

In order to get isolates bacteria from maize and bean compost, 100 g of each compost was soak with 90ml NaCl 0.85% for 30 minutes. After a moment, the clear solution was then diluted to seven (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7}). From each dilution, a 1 ml solution was poured into pretridish containing Picovskaya media that used Ca_3PO_4 as P source (Rao, 1994) for phosphate solubilizing bacteria and semi-solid N-fixing media (Dobereiner,1992) for isolating nitrogen fixing bacteria. Total microorganism was counted using Total Plate Count (TPC) method [7]. The degree of IAA of isolate bacteria that grew in the phosphate solubilizing media and N-fixing media was determined by Salkowsky method using a spectrophotometer with 530 nm wavelength [8]. In order to get high potential bacteria in providing soil P and N and ability to produce IAA, it was necessary to do scoring towards the result of parameter measurement to isolate found and choose 3 highest scores to test the growth and its activity and further identify with PCR 16S rRNA. The appropriate moment to harvest bacteria that would be used as the source of potential bacteria, hence it is necessary to test the growth ability of bacteria [7] and also growth with 10% Molase and Nutrient Broth as media.

Application of three consortium bacteria to compost

The three consortium bacteria selected from previous experiments were applied to new maize and bean compost to test their ability the increase P and N availability of the compost. The bacteria was growth on Molase, Nutrient Broth, and water media for 24 hours, and then applied to the mixture of maize and bean compost (50%;50% by weight) with a rate of 2 ml of bacterial culture / kg compost. The amount bacteria N and P as well as the amount of N and released from the compost were measured every week for four weeks.

RESULTS

Temperature measurement that was done daily indicated that the temperature of maize compost was higher than that of bean compost. A stable temperature of below 50°C was reached for both of compost after 13 days incubation (Figure 1). Isolate of phosphate solubilizing bacteria was dominated by coccus shape gram (-) bacteria, particularly for maize bean compost. The amount phosphate solubilized from maize compost ranged from 0 to 5.00 ppm/ml, whereas that solubilized from bean compost ranged from 0 to 12.413 ppm/ml (Table 2). Although the amount of P solubilized from bean compost was higher than that solubilized from maize compost, the amount of IAA produced by phosphate solubilizing bacteria isolated from maize compost was greater than that from bean compost (Table 2). Similarly for nitrogen-fixing bacteria, although the amount nitrogen fixed by nitrogen-fixing bacteria isolated from bean compost was higher than that from maize compost, the amount IAA produced by nitrogen-fixing bacteria isolated from maize compost was higher that that from bean compost (Table 3).

During composting, the change of temperature in maize compost was higher than in bean compost. This was due to the higher C content in the maize compost than that in the bean compost (Figure 1) that resulted in the higher bacterial growth in releasing CO_2 from the maize compost. Compost pH tended to decrease but was stable due to the production of organic acids and nutrient from decomposition of organic compounds in the compost (Figure 2). At the end of composting, the C/N ratio of maize compost was also higher than that of bean compost (Figure 3).

Table 1 Analysis of basic material of compost

Analysis	Method	Maize	Bean	Cow dung
pH (H2O)	(H2O)	7.2	8.0	7.2
pH (KCl)	(KCl)	7.0	7.9	7.1
C organic	Walkey Black	34.28%	22.83 %	33.67 %
N total	Kjeldahl	2.1%	1.83 %	1.98 %
C/N	Kjeldahl dan Walkey Black	16	12	17
P Total	HNO3+ HCO4	0.21 %	0.81 %	3449.75 mg/kg
K Total	HNO3+ HCO4	1.06 %	1.73 %	0.41 %
Na	HNO3+ HCO4	0.28 %	0.42%	0.34 %
Ca	HNO3+ HCO4	0.82 %	3.13 %	1.77 %
Mg	HNO3+ HCO4	0.34 %	0.51 %	0.68 %

Table 2 Isolation result and purifying of phosphate solubilizing bacteria and IAA production

Isolate origin	Number	Isolate code	Morphological characteristics			Clear zone index (cm2)	Phosphate solubilizing (ppm/ml)	IAA (ppm/ml)
			Shape	Gram	Colony			
Maize compost	1	J2P1 9.1	coccus	(-)	Yellow, small	1.219	2.891	4.684
	2	J2P1 9.2 ***)	bacil	(-)	Yellow, small	1.473	5.000	25.789
	3	J2P2	coccus	(-)	Red, medium	1.637	2.130	1.842
	4	J2P3 9.1 *)	coccus	(-)	Yellow, small	0.000	0.000	15.158
	5	J2P3 9.2 **)	coccus	(-)	Yellow, small	1.301	3.543	17.368
	6	J4P1 9.1	coccus	(-)	White, small	1.261	0.000	0.947
	7	J4P2	coccus	(-)	White, small	1.167	0.000	1.211
Bean compost	1	K2P1 9.1 *)	coccus	(-)	White, medium	1.444	3.674	5.421
	2	K2P1 9.2	bacil	(+)	White, medium	1.661	0.000	0.263
	3	K2P1 9.3	coccus	(-)	White, medium	0.000	0.000	2.842
	4	K2P2 9.1 ***)	bacil	(-)	White, small	1.774	5.261	3.789
	5	K2P2 9.2 **)	coccus	(-)	White, small	2.805	0.370	2.895
	6	K2P2 9.3	coccus	(-)	White, small	0.000	0.000	2.737
	7	K2P3 9.1	coccus	(-)	White, medium	1.592	1.783	1.579
	8	K2P3 9.2 *)	coccus	(-)	White, medium	3.908	10.783	2.895
	9	K2P39.3	bacil	(-)	White, medium	2.292	10.303	2.474
	10	K2P4 9.1	coccus	(-)	White, medium	3.412	8.391	0.211
	11	K2P5 8.1 ***)	coccus	(-)	Yellow, large	0.294	9.630	1.947
	12	K2P5 8.2 **)	coccus	(-)	Yellow, large	1.532	12.413	0.268
	13	K2P6 9.1	coccus	(-)	White, small	0.001	0.001	2.682

Table 3 Isolation and purifying of non-symbiotic N-fixing bacteria

Isolate origin	Number	Isolate Code	Morphological characteristics			Fixation ability (quantitative)	
			Shape	Gram	Colony	(ppm/h)	IAA (ppm/ml)
Maize compost	1	J_2N_1 **)	coccus	(-)	Small, white	90.4	7.158
	2	J_2N_2	coccus	(+)	Small, white	32.2	1.000
	3	J_2N_3	coccus	(-)	Small, white	69.7	4.789
Bean compost	4	K_2N_1	coccus	(-)	Small, white	52.4	5.526
	5	K_2N_2 ***)	coccus	(-)	Small, white	94.4	3.000

Table 4. Bacteria screening for biocompost production

Code	Origin	P solubilizing (ppm/ml)	Nitrogenase (ppm/h)	IAA (ppm/ml)	Antagonism	Score	Identification	Method
J_2P_1 9.2	Maize	5.000	nm	25.790	negative	***	*Enterobacter sp EV-SA01*	PCR 16S rRNA
J_2P_3 9.1	Maize	0.000	nm	15.160	negative	*	*Azotobacter sp*	UPGMA
J_2P_3 9.2	Maize	3.543	nm	17.380	negative	**	*Azospirilum sp*	UPGMA
K_2P_2 9.1	Bean	5.261	nm	3.789	negative	***	*Bacillus subtilis strain BS501α*	PCR 16S rRNA
K_2P_5 8.2	Bean	5.543	nm	0.263	negative	**	*Azospirilum sp*	UPGMA
CP_7 9.2	Cow dung	2.630	nm	1.632	negative	*	*Azotobacter sp*	UPGMA
K_2N_2	Bean	1.500	94.0	3.000	negative	***	*Pseudomonas putida strain BN-St*	PCR 16S rRNA
J_2N_1	Maize	0	90.4	7.158	negative	**	*Azotobacter sp*	UPGMA
C_2N	Cow dung	0	97.0	0.263	negative	*	*Bacillus subtilis*	UPGMA

Note: UPGMA = Unweighted Pair Group Method Using Arithmatic Average (clustering) ;nm= not measurable;
Score: *** = high, ** = medium, * = small

Table 5. Numbers of N and P bacteria molase, Nutrient Broth, and water media at 2 and 4 weeks

Media	N bacteria (x 10^9cfu/ml)		P bacteria (x10^9cfu/ml)	
	2 week	4 week	2 week	4 week
Water	8.00 a	0.07 a	0.13 a	1.33 a
Molase	8.13 a	0.71 b	1.77 a	11.33 ab
Nutrient Broth	30.67 b	4.47 b	2.07 a	14.67 b
L.S.D. 0.05	0.14	0.000*	0.098	0.043*

Table 6. Available P in compost with molase, Nutrient Broth, and water media at 1-4 weeks

Media	Available P (ppm)			
	1 week	2 weeks	3 weeks	4 weeks
Water	976.79 b	590.37 a	595.13 b	393.56 a
Molase	608.21 a	594.41 a	479.82 ab	408.67 a
Nutrient Broth	734.15 a	577.01 a	415.92 a	578.76 b
L.S.D.0.05	0.001*	0.316	0.024*	0.000*

*)significant

Table 7. Available N in compost with media air, molase, Nutrient Broth, and water media at 1-4 weeks

Media	Available N (ppm)			
	1 week	2 weeks	3 weeks	4 weeks
Water	122.0 a	977.78 a	888.89 a	913.33 a
Molase	857.78 b	842.22 a	955.56 a	1162.22b
Nutrient Broth	873.33 b	857.78 a	1000.00a	1188.89 b
L.S.D.0.05	0.043*	0.666	0.035*	0.008*

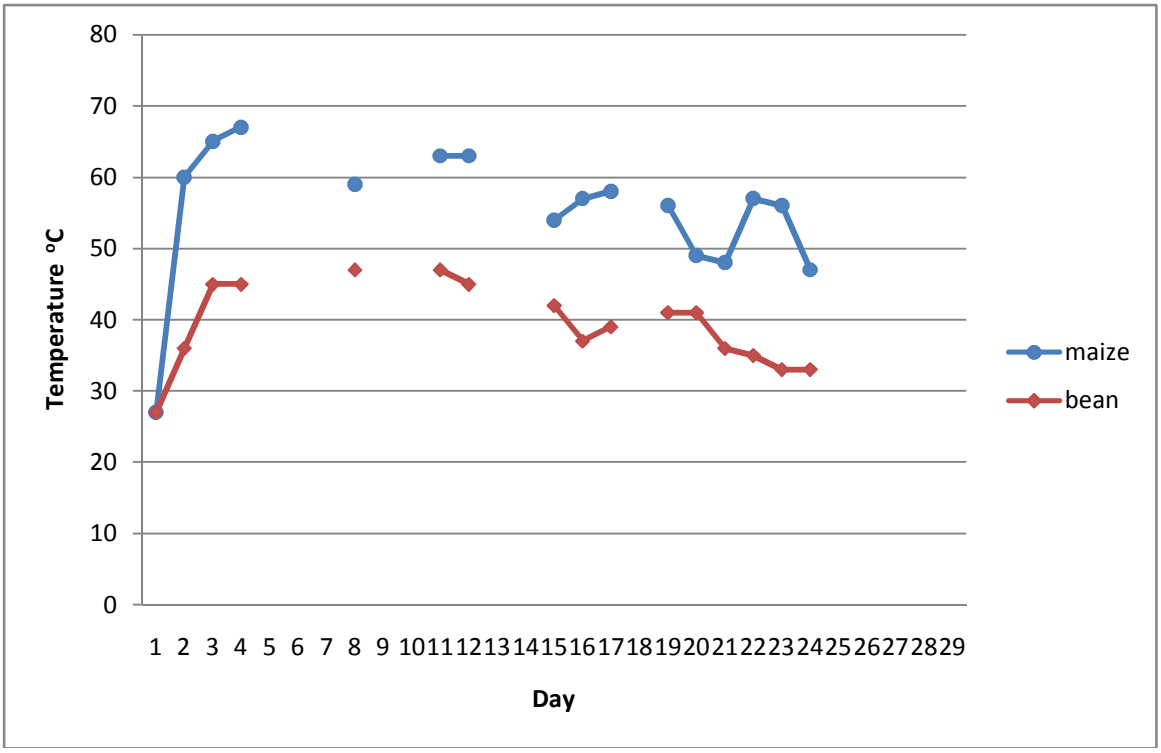

Figure 1. Compost temperature measurement

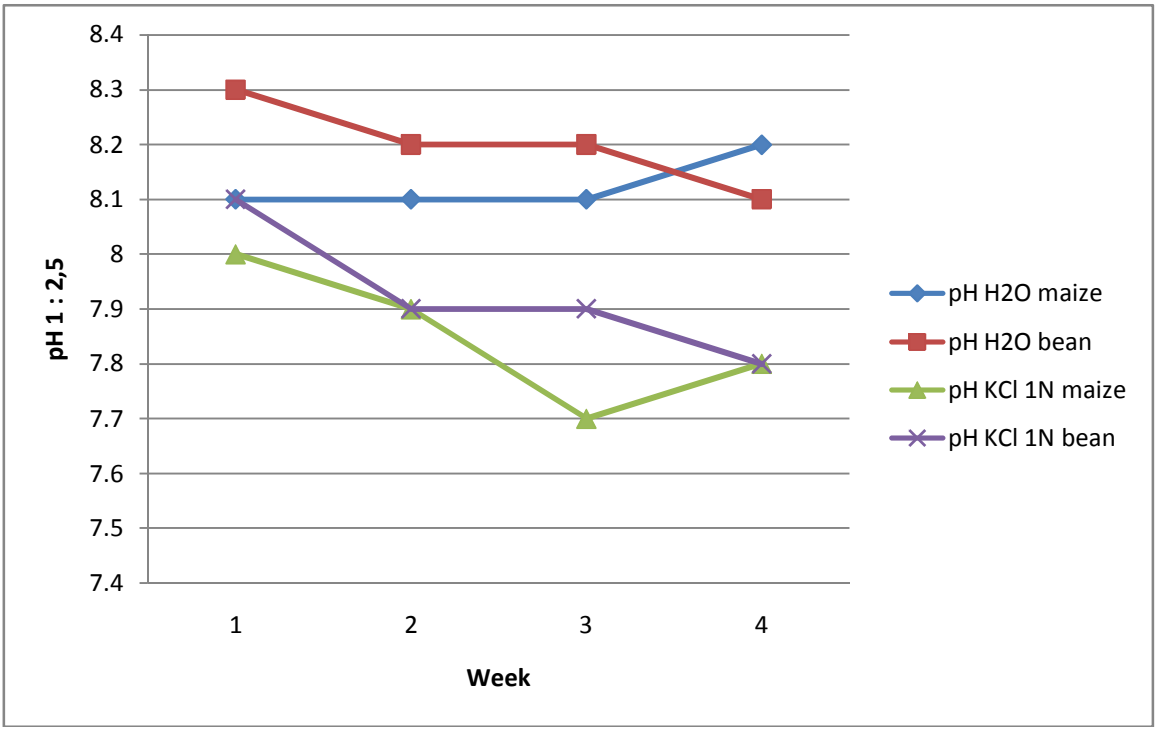

Figure 2. Compost pH measurement

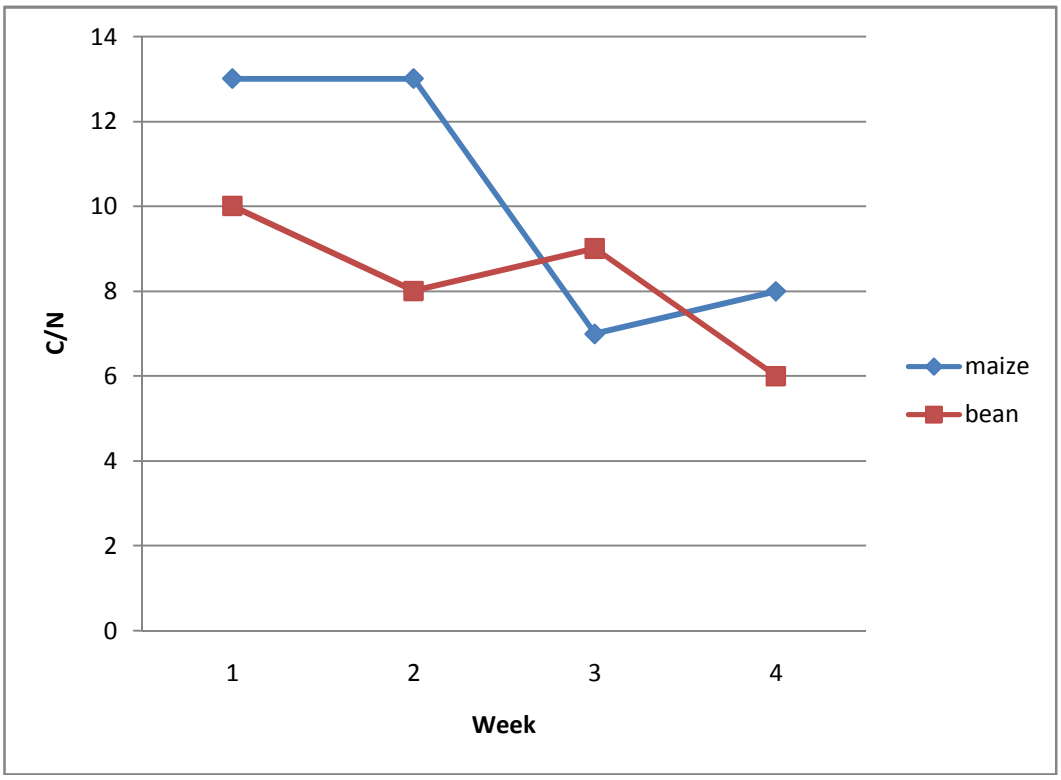

Figure 3. Compost C/N measurement

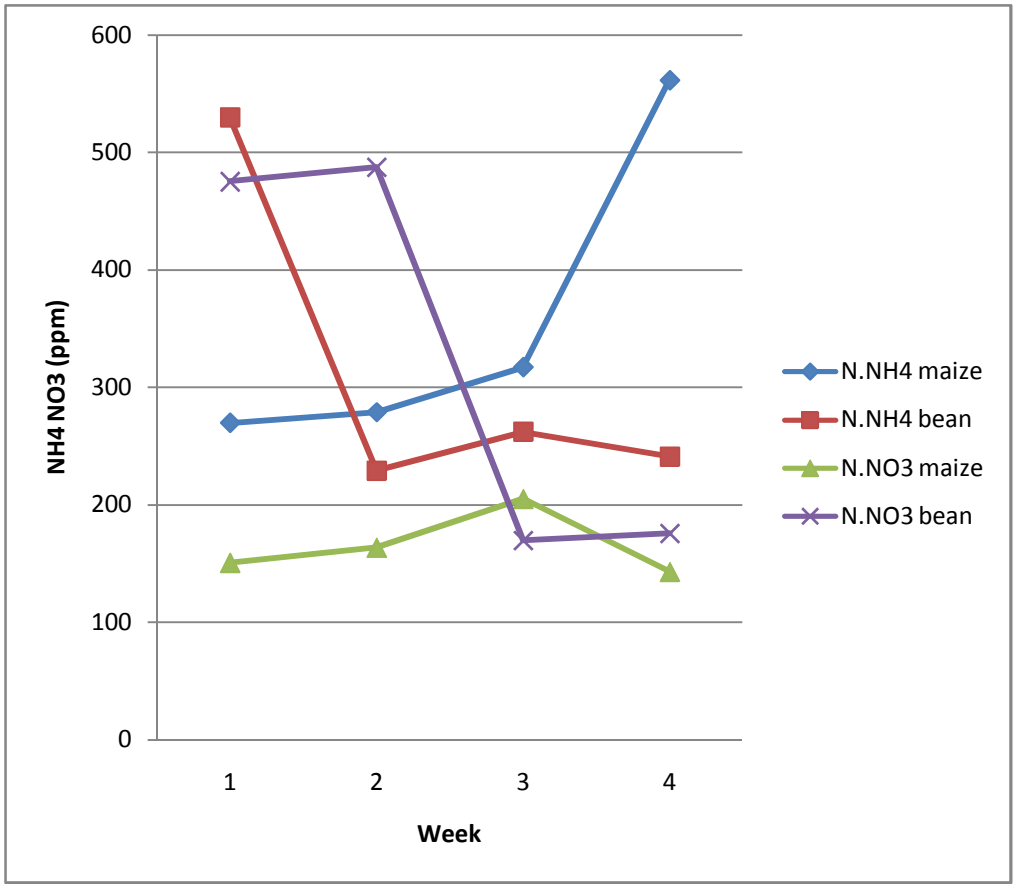

Figure 4. Compost available-N measurement

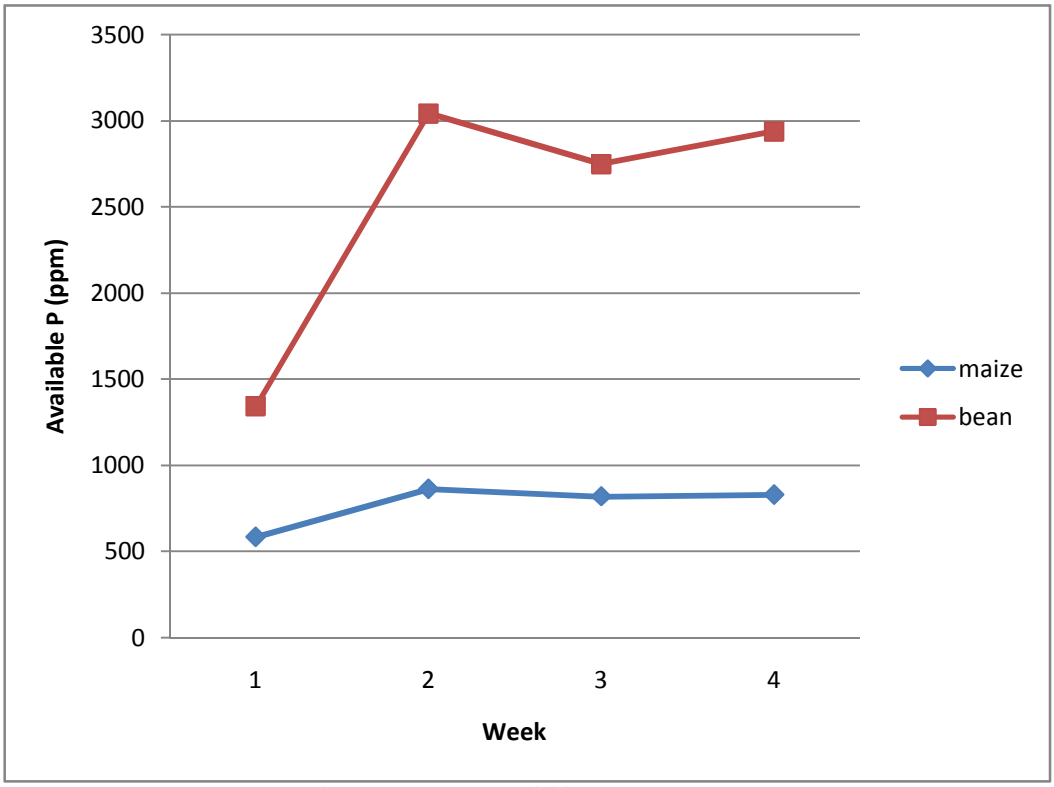

Figure 5. Compost available –P measurement

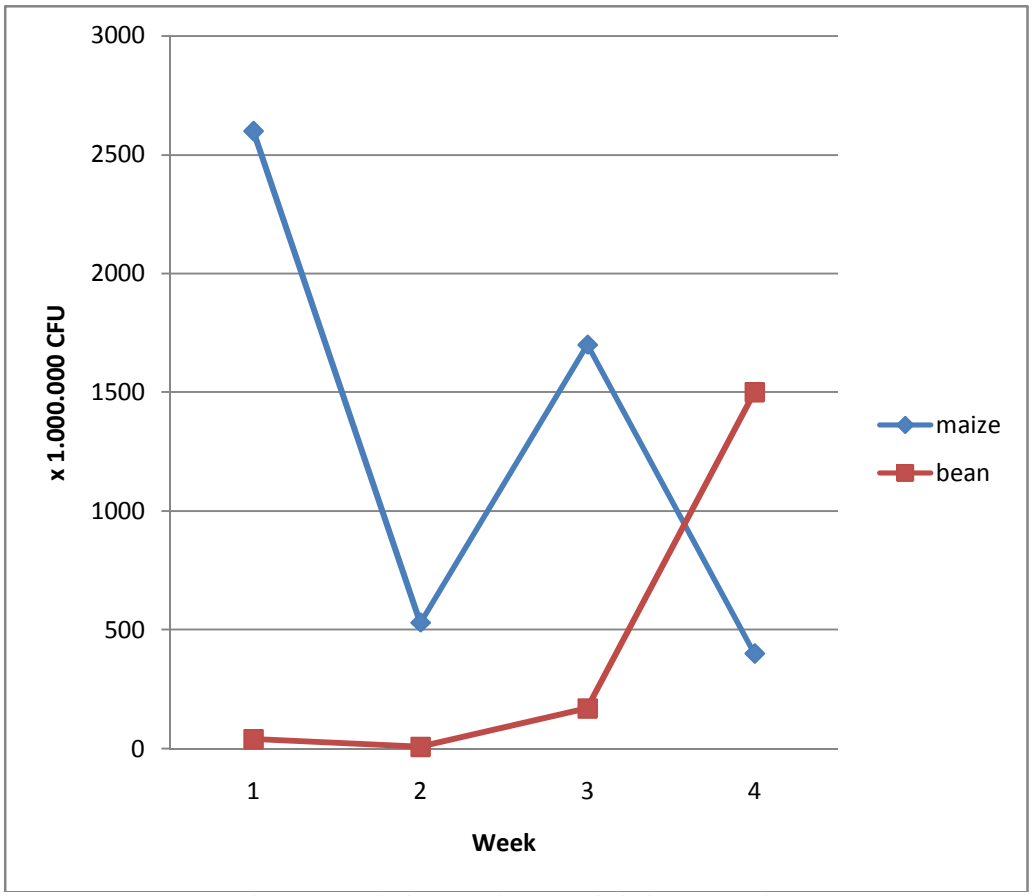

Figure 6. Total microorganism growth during composting

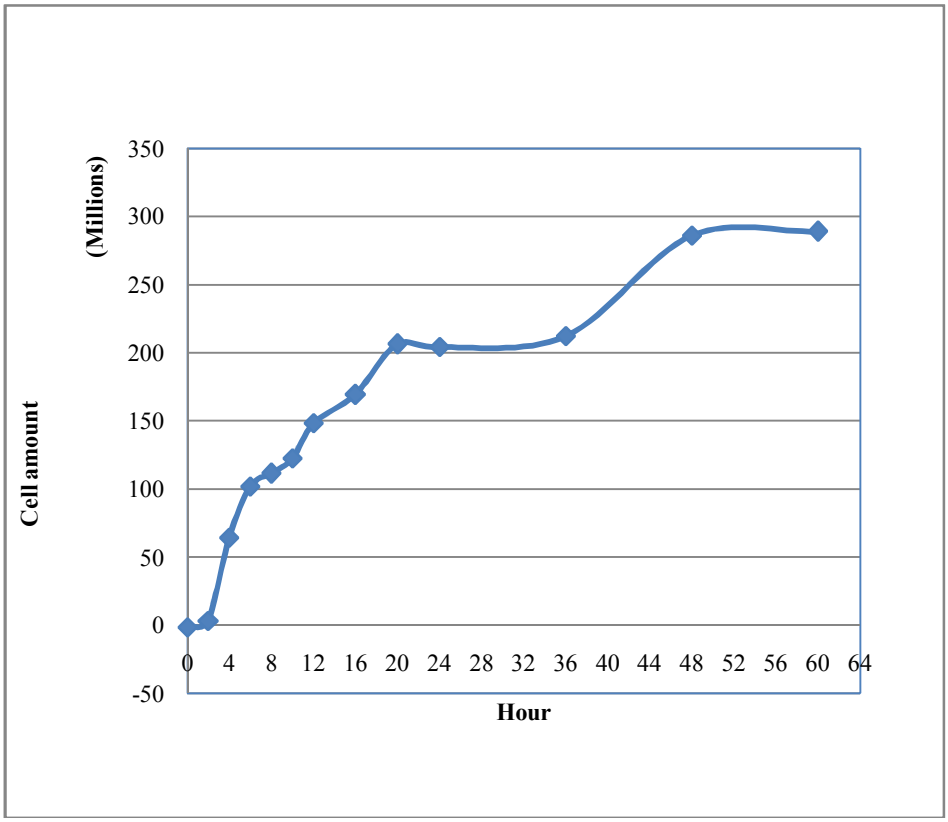

Figure 7. Growth Curve $J_2P_1$9.2

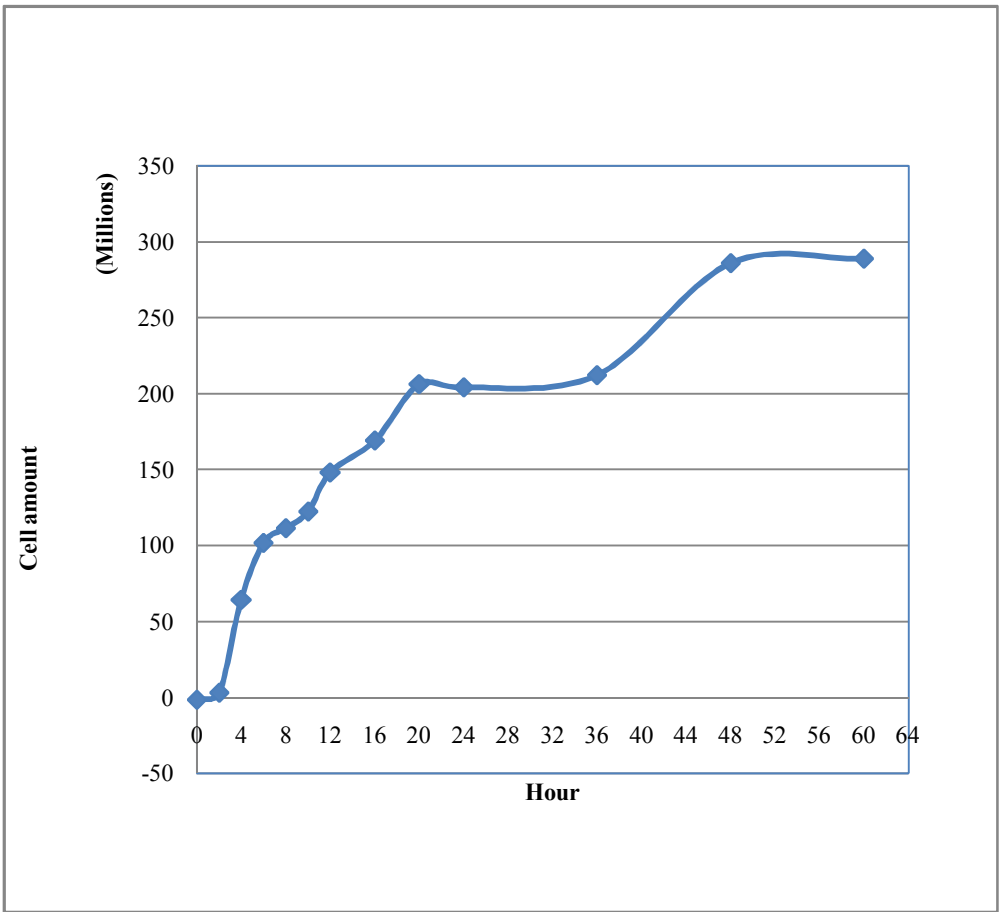

Figure 8. Growth Curve K_2P_2 9.1

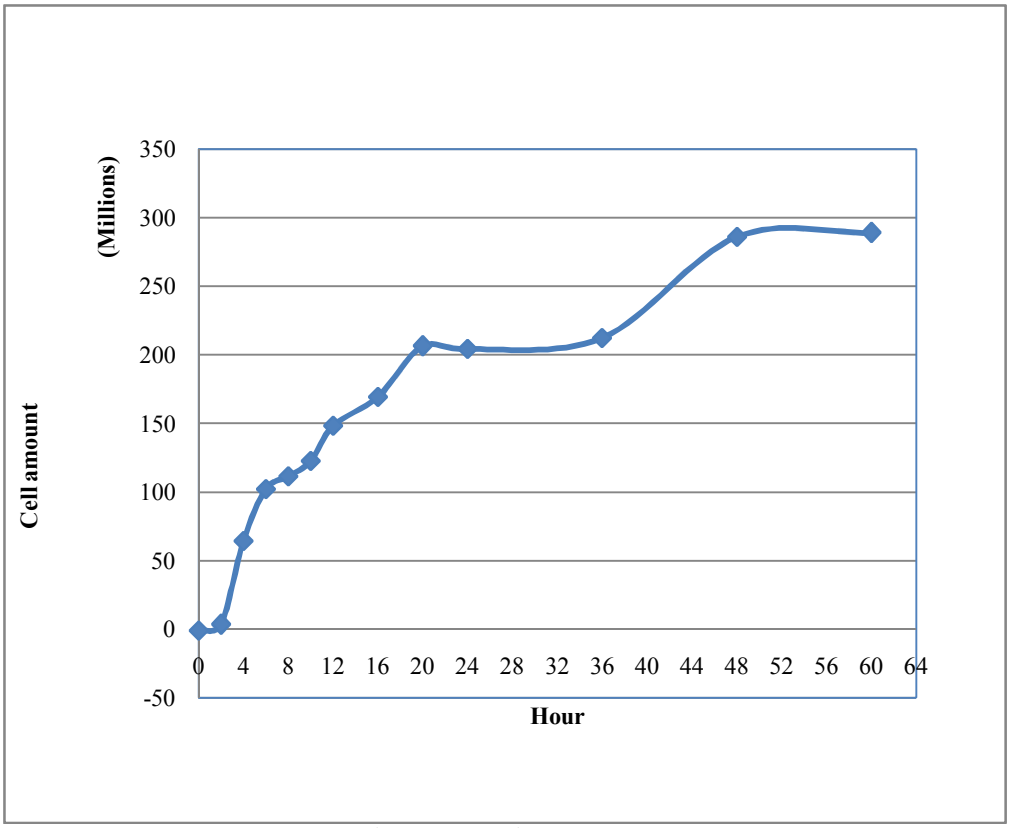

Figure 9. Growth Curve K_2N_2

DISCUSSION

As mentioned Broder and Wagner [9] that 68% of soybean loss weight on 32 days and maize only loss 47 % on 42 days that indicate soybean decomposed faster than maize. There were 7 phosphate solubilizing isolates and 3 N-fixing bacteria isolates gained from maize, whereas bean compost generated 13 phosphate solubilizing isolates and 2 N-fixing bacteria isolates (Table 4). These figures were related to the ability of bacteria body and substrates (Figures 4 and 5). The high P content in the compost caused the growth of phosphate solubilizing bacteria, and the N content in the compost induced amount of N-fixing bacteria to fix N_2 from the air. The production of IAA as growth hormone from each P and N isolates indicates differences. The production of IAA from maize compost was higher than that produced from bean compost. The highest value of IAA production was found in isolate $J_2P_19.2$ from maize compost that gaining 25.789 ppm IAA/ml. The highest ability of P bacteria to solubilize P was $K_2P_19.1$ isolate from bean compost that was capable to solubilize 5.24 ppm P /ml. This was related to the higher P content in bean compost than that in maize compost. The highest degree of N-fixed was observed for $K_2N_{2\ isolate}$ from bean compost that fixed 94.4 ppm N /hour. This was related to the association between bean and N-fixing bacteria.

The ability of N fixing bacteria and P solubilizing bacteria to produce IAA was related to the characteristic of basic material of the compost as substrate providers. Further identification using 16S rRNA indicated that the highest IAA production was observed for $J_2P19.2$ isolate which was identified as *Enterobacter sp* EVSA01 (100%similarity)(Tables 2 and 3). This is widely known species that are endophyte to maize (Patten and Glick, 2002). The K_2N_2 isolate that fixed the highest amount of N was identified as *Pseudomonas putida* strain BN-St(99%similarity), while the $K_2P29.1$ isolate that solubilized the highest amount of P was identified as *Bacillus subtilis* strain BS501α(100%similarity) . The growth curve of the $J_2P19.2$, K_2N_2 and $K_2P_29.1$ bacteria for 20-24 hours phase was presented in Figures 7, 8 and 9. Combination (consortium) of the 3 bacteria grown in 10% molase and Nutrient Broth Medias resulted in 1x 10^7cells. The growth of cell increased until 1x10^9cell within 24 hours. This indicates that molase can be used as carrier media.

Application of three consortium bacteria consisted of *Bacillus subtilis* strain BS501a, *Pseudomonas putida* strain BN-St and *Enterobacter* sp-SA01 significantly increased population of N and P bacteria 2 and 4 weeks after incubation, either in Molase media or Nutrient Broth media (Table 5). This indicates the growing capability of the bacteria in the composts to solubilize P and fix N (Tables 6 and 7) as also obtained by Abdelazis *et.al* [10] and Sarwar et al [11]

CONCLUSION

Compost from maize waste had higher potential for IAA production that compost from bean waste, but

compost from bean waste produced higher numbers of phosphate solubilizing and N-fixing bacteria than compost from maize bean. The characteristics and composition of compost materials affected bacterial diversity. Consortium of N fixing and P solubilizing bacteria can grow on career media having high C-organic content. Aplication consortium 3 bacteria (*Bacillus subtilis* strain BS501a, *Pseudomonas putida* strain BN-St and *Enterobacter* sp-SA01) with highest potential on solubilizing P, Nitrogen fixing and IAA production significantly increase P and N available on composting.

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