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Characterization of Phosphate Solubilizing and Potassium Decomposing Strains and Study on their Effects on Tomato Cultivation

Tin Mar Lynn[1], Hlaing Swe Win[1], Ei Phyu Kyaw[1], Zaw Ko Latt[1], and San San Yu[2]

[1]Department of Biotechnology,
Mandalay Technological University,
Mandalay, Myanmar

[2]Molecular Life Science Research Center,
Jacobs University,
Bremen, Germany

ABSTRACT: Seven strains were collected for phosphate solubilizing and potassium decomposing activities from Microbiology Laboratory, Department of Biotechnology, Shweziwa Biofertilizer Plant. When phosphate solubilizing activity of selected strains was qualitatively determined, all strains except from B1 strain, gave clear zone formation on NBRIP media. But when quantitatively determined by spectrophotometric method, all strains solubilized insoluble tricalcium phosphate. Among seven strains, Ps strain gave the highest soluble phosphate concentration (386 ppm). Potassium decomposing activity was also determined for qualitatively and quantitatively. For qualitative determination, potassium decomposing activity was screened for clear zone formation on potassium decomposing media. Among seven strains, B1 and Y strains cannot give clear zone around their colonies. But when determined by AAS method, all strains can decompose potassium mica by giving soluble potassium concentration. Y strain gave the highest soluble potassium concentration (8.45 ppm). Phosphate solubilizing and potassium decomposing strains were combined differently for four treatments to study their effects on tomato cultivation. Chemical fertilizer was also applied to compare with selected strains. Among all treatments, T-4 showed better result on total yield although yields were not significantly different.

KEYWORDS: Phosphate solublizing, Potassium decomposing, NBRIP, AAS method, Tomato.

1 INTRODUCTION

Myanmar is the agricultural country and so business is mainly dependent an agriculture. Therefore, it is important to increase agricultural production and the improvement of soil is one of the most common strategies. The currently used agricultural inputs are mostly chemical. The poor farm management technique and improper use of agrochemical has a result in both soil quality and environmental degradation. In order to avoid these problems, application of biofertilizer is considered today to limit the use of mineral fertilizer and supports an effective tool for desert development under less polluted environment, decreasing agricultural costs, maximizing crop yield due to providing them with an available nutritive elements and growth promoting substances [1].

Biofertilizers are living microbial inoculants that are added to the soil to improve the plant growth and can be used as an alternative source of chemical fertilizer [2]. Use of soil microorganisms which can either fix atmosphere nitrogen, solubilizing phosphate, synthesis of growth promoting substances, will be environmentally begin approach for nutrient management and ecosystem function [3].

Nitrogen (N), Phosphorous (P) and Potassium (K) are major essential macronutrients for plant growth and development. Phosphorus is one major plant nutrients, second only to nitrogen in requirement. However, a greater part of soil phosphorus, approximately 95–99% is present in the form of insoluble phosphates and hence cannot be utilized by the plants. To

Corresponding Author: Tin Mar Lynn (light.lynn@gmail.com)

circumvent the P deficiency in soils, P fertilizers are applied. However, after application, a considerable amount of P is rapidly transformed into less available forms by forming a complex with Al or Fe in acid soils or Ca in calcareous soils [4] before plant roots had a chance to absorb it [5]. It has been reported that many soil fungi and bacteria can solubilize inorganic phosphates. The solubilization effect is generally due to the production of organic acid [6]. Last but not least, phosphate not only increase seed germination and early growth, it also can stimulates blooming, hastens maturity, enhance bud seed formation and aids in seed formation [7].

Potassium is one of the most important macronutrient for the growth and reproduction of the plants [8]. Potassium ions serve to activate certain enzymes especially those involved in photosynthesis, respiration and in starch and protein synthesis [9]. Moreover, opening and closure of stomatal guard cells or daily changes in the orientation of leaves are affected by potassium concentration [10]. Potassium is available in four forms in the soil which are K ions (K+) in the soil solution, as an exchangeable cation, tightly held on the surfaces of clay minerals and organic matter, tightly held or fixed by weathered micaceous minerals, and present in the lattice of certain K-containing primary minerals [11]. Inoculation with bacteria, which can improve P and K availability in soils by producing organic acids and other chemicals, stimulated growth and mineral uptake of plants ([12], [13]).

The aim of this research work was to increase the production of crop yields in order to improve farmers' profit by using biofertilizer in place of chemical fertilizer.

2 MATERIAL AND METHODS

2.1 STRAIN COLLECTION

Phosphate solubilizing and potassium decomposing strains were collected from Microbiology Laboratory, Department of Biotechnology, Shweziwa Biofertilizer Plant, Kyaukse District, Mandalay Region, Myanmar.

2.2 IDENTIFICATION OF SELECTED STRAINS

Selected strains were cultured on their respective media (Pikovaskaia's and potassium decomposing media) to study colonial morphology. Microscopic morphology of selected strains was examined by Gram's staining method. Biochemical characteristics of selected strains were also studied.

2.3 QUALITATIVE DETERMINATION OF PHOSPHATE SOLUBILIZING ACTIVITY

Selected strains were checked for phosphate solubilizing activity. All selected strains were inoculated in National Botanical Research Institute Phosphate (NBRIP) broth media, and incubated in water batch shaker at 37°C for three days. After incubation, the culture broth was centrifuged at 6000 rpm for 15 minutes and 50µl of supernatant was added to well that was punched on NBRIP media. The culture plates were incubated at 37°C. After incubation, clear zone formation around the well was recorded for every day.

2.4 QUANTITATIVE DETERMINATION OF PHOSPHATE SOLUBILIZING ACTIVITY

Selected strains were further evaluated for their phosphate solubilizing ability. Phosphate solubilization in Pikovaskaia's broth media was quantified in a flask (10 ml) and incubated in water batch shaker at 37°C for five days. Uninoculated medium served as control. After incubation, the culture broth was passed through the cation exchange resin and $(PO_4)^{3-}$ solution was reacted with color forming reagent (Sodium Molybdate and Hydrazium Sulphate). After blue color development, phosphate solubilizing activity was measured by UV-vis spectrophotometric method at 830 nm.

2.5 QUALITATIVE DETERMINATION OF POTASSIUM DECOMPOSING ACTIVITY

Potassium decomposing activities of selected strains was qualitatively screened on potassium decomposing media. Selected strains were firstly inoculated in potassium decomposing broth media and incubated in water batch shaker at 37°C until optimum growth. After getting optimum growth, the bacterial broth was centrifuged at 6000 rpm for 15 minutes and 50 µl of supernatant was added to well that was punched on media. After incubation, clear zone formation around well was recorded for potassium decomposing activity.

2.6 Qualitative Determination of Potassium Decomposing Activity

Selected strains were cultured as described in qualitative determination. After centrifugation, amount of soluble potassium in supernatant was measured by Atomic Absorption Spectrometry method (AAS).

2.7 Study on Co-existence Growth of Phosphate Solubilizing and Potassium Decomposing Strains

Co-existence growth of phosphate solubilizing and potassium decomposing strains was studied on Pikovaskaia's and Potassium Decomposing media by cross culturing of strains with each other. After incubation at 37°C, co-existence growth of strains was studied.

2.8 Preparation of Pellet Form Biofertilizer Using Selected Strains

For preparation of pellet form biofertilizer, P-solubilizing and K-decomposing strains were cultured in Pikovaskaia's and potassium decomposing broth media and incubated until optimum growth. When the bacterial broth culture was ready in use, the compost was crushed to obtain the finely powdered compost. The compost was mixed with zeolite and the bacterial broth culture was added to these mixtures. After mixing well, the mixture was placed into the pelletizing machine. While pelletizing, gypsum was used to coat the mixture of compost and zeolite. After pelletizing, the pellet form biofertilizer was dried in room temperature.

2.9 Study on Effect of Pellet Form Biofertilizer on Tomato Cultivation

Phosphate solubilizing and potassium decomposing strains were applied in tomato cultivation in pellet form biofertilizer to know their effects. Experiments were designed by randomized completely block design (RCBD). After three months of cultivation period, data analysis was taken and compared among treatments.

3 Results and Discussion

3.1 Strains Collection

Seven strains, two phosphate solubilizing, four potassium decomposing bacteria and one potassium decomposing yeast strain were collected.

3.2 Identification of Selected Strains

Four potassium decomposing and one phosphate solubilizing bacteria were gram-negative cocci and the other phosphate solubilizing bacteria was gram-positive in rod-shaped. Their biochemical characteristics were shown in Table 1. One collected yeast strain was Gram positive and its sugar assimilation and fermentation patterns were shown in Table 2. Antibiotic sensitivity patterns were shown in Table 3. According to colonial and microscopic morphology and biochemical characteristics, four potassium decomposing bacteria may be *Pseudomonas* spp. and the other phosphate solubilizing bacteria may be *Bacillus megaterium*.

Table 1. Microscopic Morphology and Biochemical Characteristics of Selected Strains

Biochemical Tests	Ps	B-1	Y	K-3	K-5	KA-2	KA-35
Gram's Reaction	- (cocci)	+ (rod)	+ (cocci)	- (cocci)	+ (cocci)	- (cocci)	- (cocci)
Motility	+	+	ND	+	+	+	+
Methyl Red	-	+	ND	+	+	+	+
Voges Prosakur	+	+	ND	-	+	+	+
Citrate Utilization	+	+	+	+	+	+	+
Starch Hydrolysis	-	-	+	-	-	+	-
Gelatin Agar	-	-	ND	-	-	-	-
Urease	-	-	ND	+	-	-	-
Indole	-	-	ND	-	-	-	-
Catalase	+	+	ND	+	+	+	+

Antibiotic sensitivity patterns were shown in Table 3. In antibiotic sensitivity pattern, Ps, K3 and A35 strains resistant to only Ampicillin, they were sensitive to other four antibiotics. But, all other strains were sensitive to all tested antibiotics.

Table 2. Sugar Assimilation and Fermentation Patterns of Yeast

Sugars	Assimilation Patterns	Fermentation Patterns
Glucose	+	+
D-xylose	+	+
Sucrose	+	+
Maltose	+	+
Lactose	-	-
Raffinose	+	+
Arabinose	-	-
Myo-Inositol	-	-

Table 3. Antibiotic Sensitivity Patterns of Selected Strains

Antibiotics	Ps	B 1	Y	K3	K5	KA2	A35
Ampicillin	R	S	S	R	S	S	R
Gentamycin	S	S	S	S	S	S	S
Kanamycin	S	S	S	S	S	S	S
Chloramphenicol	S	S	S	S	S	S	S
Tetracycline	S	S	S	S	S	S	S

R = Resistance S = Sensitive

3.3 Qualitative and Quantitative Determination of Phosphate Solubilizing Activity

According to plate screening for clear zone formation, all selected strains, except from B1, gave clear zone formation around the well. Index for clear zone formation of these strains was shown in Table 4. As they gave clear zone, it can be assumed that these strains have phosphate solubilizing activity. In clear zone formation, K3 strain gave the largest zone formation. During five days incubation, clear zone formation of all these strains was larger and larger.

After plate screening, Phosphate solubilizing activity of all selected strains was quantitatively determined by UV-vis spectrophotometric method at 830 nm using KH_2PO_4 as standard. Amount of solubilized phosphate of all selected strains were shown in Figure 1. Although K3 strain gave the largest clear zone in plate screening method after 5 day incubation (1.375 in Table-4), it showed the lowest amount of solubilized P in quantitative analysis (Fig-1). According to quantitative analysis, Ps strain was the best phosphate solubilizer by giving 386 ppm but the clear zone diameter of Ps was not the largest among all strains. Although B1 strain gave no clear zone formation on plate screening, soluble phosphate concentration of B1 could be quantitatively determined (306 ppm). So, it was known that clear zone diameter formation was not directly proportional to the amount of solubilized phosphate concentration. All these findings revealed that one should not rely only on qualitative method while isolating and screening the P solubilizing microorganisms. It is wise to supplement qualitative method with quantitative measurement of P solubilizing for getting more reliable inferences [14].

Similar results have been reported in ([15], [16], [17]). It has also been reported that many isolates which did not show any clear zone in qualitative method i.e. NBRIP medium- agar plate assay) solubilized insoluble inorganic phosphates in quantitative method ([18], [19], [16]). Thus, the plate screening method fails where the clear zone is inconspicuous or absent. This may be because of the varying diffusion rates of different organic acids secreted by an organism [20]. Contrary to qualitative method (indirect measurement) of phosphate solubilization by plate screening method, the quantitative analysis by UV-spectrophotometer in broth culture method resulted in reliable results [16].

Identification of individual isolates by colonial, microscopical and biochemical characteristics showed that these bacteria were different. But, these variations were not well depicted with phosphate solubilizing trait. Phosphate solubilizing activity

is determined by microbial biochemical ability to produce and release organic acids, which their carboxylic groups chelate the cations (mainly Ca) bound to phosphate converting them into soluble forms [21].

Table 4. P-solubilizing Activity of Selected Strains on NBRIP Media (in terms of Solubility Index)

Strains	Day 1	Day 2	Day 3	Day 4	Day 5
Ps	0.25	0.5	0.625	0.75	0.875
B 1	-	-	-	-	-
Y	0.125	0.25	0.375	0.5	0.625
K 3	0.875	1	1.125	1.125	1.375
K 5	0.25	0.375	0.5	0.625	0.75
KA 2	0.625	0.875	1.125	1.125	1.125
KA 35	0.25	0.5	0.75	0.75	1

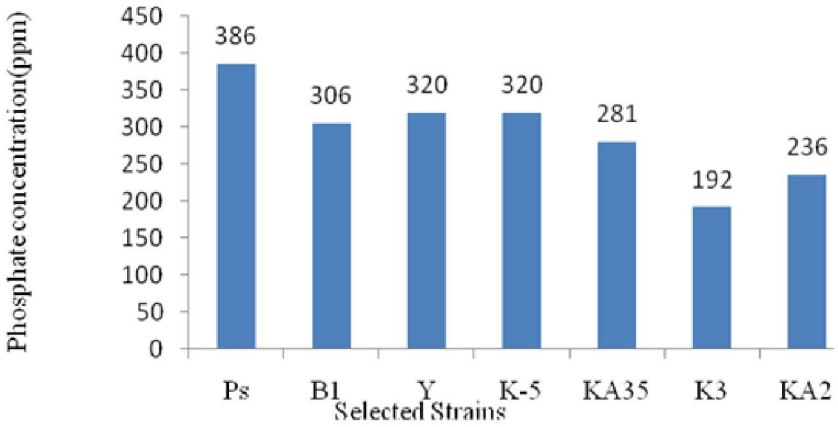

Fig. 1. P-concentration Solubilized by Selected Strains in Pikovaskia Broth after 5 Days Incubation by Spectrophotometric Method

Potassium decomposing activities of selected strains were firstly screened for clear zone formation on potassium decomposing media. The potassium solubility index of all selected strains was shown in Table 5. Like in P-solubilization by plate screening, clear zone formation of all strains, except from B1 and Y strains, was larger and larger until 5 days incubation. In qualitative determination, solubility index for all strains was almost the same. Quantitative measurement of potassium decomposing activity by AAS method was shown in Figure 2. Although B1 and Y strains cannot give clear zone formation when screened on media, they can solubilize potassium mica by giving 6.63 ppm and 8.45 ppm when measured by AAS method. In potassium decomposing activity, Y strain gave the highest amount of soluble potassium concentration and B1 strain was the second highest. Although Ps was the best strain in phosphate solubilizing activity, potassium decomposing activity was the lowest among all these strains. The ability of bacteria to release K largely depends on the nature of the mineral compounds [22]. The variability among the bacteria indicates the importance of exploration of different mineral potassium solubilizing bacteria and their solubilizing mechanisms.

Table 5. K-decomposing Activity of Selected Strains on K-decomposing Yeast Media (in terms of Solubility Index)

Strains	Day 1	Day 2	Day 3	Day 4	Day 5
Ps	0.75	0.875	0.875	1	1.125
B 1	-	-	-	-	-
Y	-	-	-	-	-
K 3	1	1.125	1.25	1.25	1.25
K 5	0.75	0.875	1	1	1.125
KA 2	0.625	0.75	0.875	1	1.125
KA 35	0.625	0.875	1.125	1.125	1.25

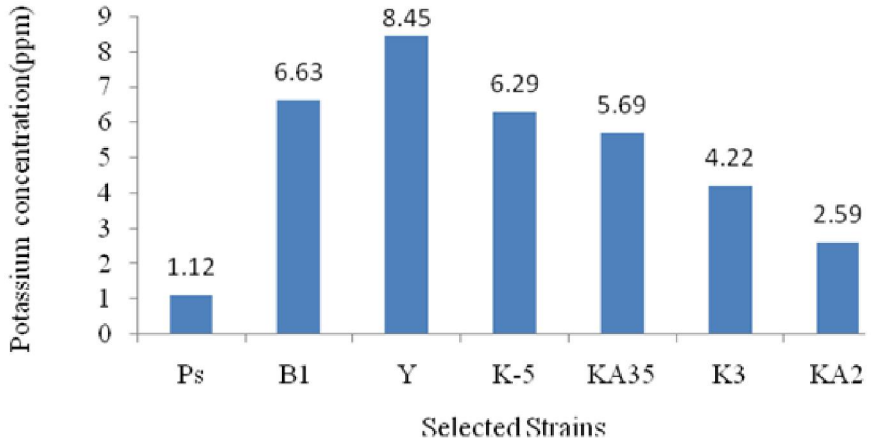

Fig. 2. K-decomposing Activity of Selected Strains by Atomic Absorption Spectrometry (AAS) Method

3.4 Study on Shelf-life of Pellet Form Biofertilizer

For convenience application, a carrier material is used as a vehicle for the microorganisms to be used as biofertilizer. Moreover, such materials may have a role in maintaining the viability (shelf-life) of the microorganisms prior to its release into the field as well as they also provide a suitable microenvironment for rapid growth of the organisms upon their release [23]. After formulating phosphate solubilizing and potassium decomposing strains as pellet form biofertilizer, microbial populations were checked for every month. It was shown in Table 6. Microbial populations were counted for 16 weeks. Among four different combinations, microbial population of all combinations was around 10^4 after 16 weeks. So, it was seen that there was no effect of combination on microbial population.

Table 6. Microbial Population in Formulated Biofertilizer

Treatment	0 week	2 week	4 week	6 week	8 week	10 week	12 week	14 week	16 week
Ps	$2.8x\ 10^7$	$1.08x10^8$	$2.4x10^7$	$4.4x10^6$	$1.2x10^6$	$6.8x10^5$	$7.3x10^4$	$5.2x10^4$	$3.6x10^4$
B 1	$4.8x10^8$	$1.0x10^8$	$2.0x10^7$	$5.2x10^6$	$7.6x10^5$	$6.0x10^5$	$6.0x10^4$	$4.8x10^4$	$2.4x10^4$
Y	$6.8x10^7$	$7.6x10^7$	$3.2x10^7$	$4.4x10^6$	$1.2x10^6$	$7.6x10^5$	$4.6x10^4$	$1.2x10^4$	$1.2x10^4$
K 3	$1.68x10^7$	$8.4x10^7$	$8.2x10^6$	$8.0x10^6$	$3.2x10^6$	$1.2x10^6$	$6.0x10^4$	$2.4x10^4$	$2.4x10^4$

3.5 Study on Effect of Pellet Form biofertilizer on Tomato Cultivation

With four different combinations of phosphate solubilizing and potassium decomposing strains, their effects were studied on tomato cultivation. Water was used as negative control and combination of compost, gypsum and zeolite served as positive control. And only chemical fertilizer was used as Treatment 1. So, there were five treatments.

According to data analysis, all of these treatments had no differently resulted on height of tomato plants, number of branches, and fruits per plant (data not shown). But, T4 gave the better results on total yields of tomato plants at 126 days after sowing. Yields of tomatoes from all treatments were shown in Table 7 and Fig. 3. All treatments gave the better results when compared with negative control, but when compared with positive control, total yield of T1 and T5 were lower than positive control. Although there was no significance difference on total yield and yield per plant among all treatments, T4 was suitable for biofertilizer formulation according to this study. The results are in agreement with [3] who recorded increase in yield significantly in maize crop and also improve soil properties such as organic content due to co-inoculation of KSB and PSB. The results are also comparable with [24], whose studied the increase rice grain yield in a field experiment due to effect of silicate solubilizing bacteria recorded 5218 kg ha grain yield than control 4419 kg per ha.

Table 7. Treatment System for Tomato Cultivation and Yield of Tomatoes after 120 DAS

Treatments	Formulation	Total Yield (Kg)	Yield Per Plant (Kg)
Negative Control	Water	36.602	1.22±0.28
Positive Control (PC)	Compost, Gypsum, Zeolite	34.66	1.16±0.18
Treatment 1	Chemical Fertilizer	35.47	1.18±0.28
Treatment 2	PC+Ps+B1+Y+K5	37.89	1.19±0.19
Treatment 3	PC+Ps+B1+Y+K5+KA35	37.62	1.25±0.3
Treatment 4	PC+ Ps+B1+Y+K5+KA35+K3	48.95	1.63±0.14
Treatment 5	PC+ Ps+B1+Y+K5+KA35+K3+KA2	35.82	1.19±0.1

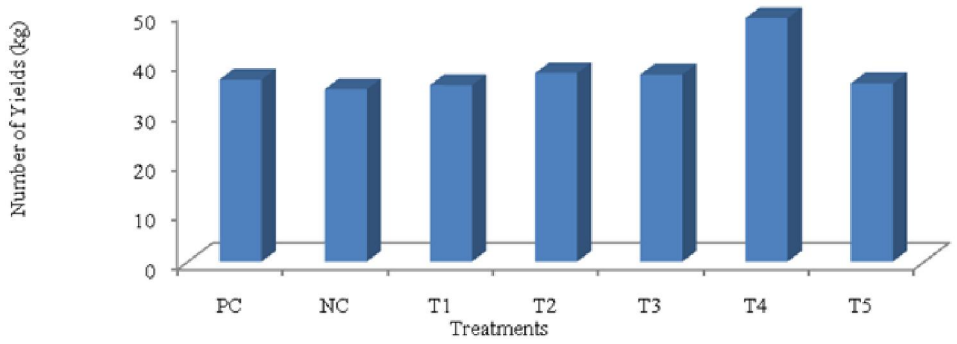

Fig. 3. Total yields of tomatoes at 126 days after sowing

Among seven strains, K-3 showed largest clear zone formation but Ps gave the highest solubilized phosphate amount when quantitatively determined. For potassium decomposing activity, A35 and K3 were the best for clear zone formation. But, yeast strain was the best K-decomposer with the highest concentration of soluble potassium (8.45 ppm). These seven strains were formulated as pellet form biofertilizer with different combinations for field trial observation. Among five treatments, T-4 was the best for yields of tomatoes when compared with other treatments. Use of these bacteria and yeast strain as bioinoculants will increase the available phosphate and potassium in soil and promote plant growth.

4 CONCLUSION

Among seven strains, K-3 showed largest clear zone formation but Ps gave the highest solubilized phosphate amount when quantitatively determined. For potassium decomposing activity, A35 and K3 were the best for clear zone formation. But yeast strain was the best K-decomposer with the highest concentration of soluble potassium (8.45 ppm). These seven strains were formulated as pellet form biofertilizer with different combinations for field trial observation. Among five treatments, T-4 was the best for yields of tomatoes when compared with other treatments. Use of these bacteria and yeast strain as bio-inoculants will increase the available P and K in soil, helps to minimize the chemical fertilizer application, reduces environmental pollution and promotes sustainable agriculture.

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