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USE OF THE EFFECTIVE MICRORGANISMS AS BIOFERTILIZER FOR RICE GROWTH AND PRODUCTION

[1]Ei Mon Myo (Author), [2]Myo Myint, (Author), [3]Zaw Khaing Oo (Author),
Biotechnology Research Department
Ministry of Education
Kyaukse, Myanmar
eimonmyo@gmail.com

***Abstract*— Soil sample was collected from the vicinity of the rice and chick pea plants. Forty four bacterial strains and seven strains of actinomycetes had been isolated. Among forty four strains, one strain was Serratia and four strains were Azotobacter species due to their distinct colonial morphology, microscopic morphology and biochemical characterization. From the study on each fertilizing activity, many effective microbial isolates, four strains as nitrogen fixer, six strains as P-solubilizer, ten K-decomposing strains, were occurred. In P-solubilizing activity, Serratia was the most efficient one, having 35 mm in zone diameter on plate screening and 750.88 ppm by UV spectrophotometric measurement. In quantitative analysis by AAS, the k-decomposing strain defined as K23 could solubilize 10.46 ppm and strain K11 could solubilize 9.92 ppm respectively. In the study on N2 fixing activity of Azotobacter species by ammonium test kit, A1 and A4 could fix nitrogen from 0.2 to 0.5%. The selected strains had co-existence on one another and so these strains were studied in field trial application on rice cultivation. The panicle length, seed number per panicle, 1,000 seed weight and estimated yield of the rice plot which use biofertilizer was greater than the others which were splashed with organic fertilizer and urea. The estimated rice yields from the use of biofertilizer, organic fertilizer and urea were 100, 70 and 80 baskets/acre respectively**.

***Index Terms*— Nitrogen fixer, P-solubilizer, K-decomposing, , co-existence, bioertilizer, rice yield.**

I. INTRODUCTION

Myanmar is an agricultural country and so the business is mainly dependent on agriculture. The extensive use of chemical fertilizer and pesticide causes soil erosion, a decrease in microbial diversity and even illness and death to the farmers. The improper use of agrochemicals has also resulted in both soil quality and environmental degradation [6].

In order to avoid these problems, new techniques that can provide an increasing and abundant supply of nutrition and safe foods should be developed. Bio-fertilizers 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 [4].

Recent development in soil microbiological research makes it possible to produce various kinds of biofertilizer. It showed that ample beneficial traits of soil microbes in enhancing plant growth and the possibility of a microbial strain to have more than one of functional traits [1, 2, 3].

The microbe-plant interaction in the rhizosphere can be beneficial, neutral, variable or deleterious for plant growth. Rhizobacteria that exert the beneficial effects on plant development are termed as plant growth promoting rhizobacteria (PGPR) [7].

Nitrogen, phosphorus and potassium are essential nutrients required by both plants and microorganisms and their major physiological roles are the accumulation and and release of energy during cellular metabolism [5].

Phosphorus is generally deficient in most natural soils, because it is fixed as water-insoluble iron and aluminum phosphates in acidic soils or calcium phosphate in alkaline soil. Therefore, the inoculation of soil with phosphate solubilising microorganisms may alleviate this problem [8]. P-solubilizing bacteria were successfully used for plant N and P nutrition and growth yield.

It is known that at least sixty enzymes involve in activating the plant growth. And, this may be its most important function in the plant. Potassium is also known as the quality nutrient because of its important effects on quality factors such as size, shape, color, taste, shelf life, fiber quality and other quality measurements. Potassium is an important plant nutrient and is taken up in large quantities by many species. It has a wide range of functions in plants and can be taken up in excess when surplus potassium is present in the soil [9].

Nitrogen (N) is an extremely important element in plant nutrition. In both nature and in agricultural situations, plant growth is often limited by a lack of nitrogen, and addressing shortfalls results in visible and significant plant growth.

There are many benefits of biofertilizer upon chemical fertilizer. Biofertilizer is very cost effective and increases crop yield by 10-30%. It is a supplement to chemical fertilizer and replaces chemical fertilizer up to 25%. Biofertilizer also stimulates plant growth and biologically activates the soil. It restores natural soil fertility and also provides protection against some soil borne diseases.

II. MATERIALS AND METHODS

A. Sample Collection

All the bacterial samples were isolated from the rhizosphere of the rice and chick pea plants located in Patheingyi Agricultural Site, Myanmar.

B. Preparation and Inoculation of the Sample

One g of soil sample was mixed with 10 ml of sterile 1% normal saline in the test tube. Afterwards, the tube was shaken to mix the sample and normal saline thoroughly. Then, it was stood for 10 min and the supernatant was streaked on the nutrient agar for the bacterial growth. The colonies which have different cultural characteristics were sub-cultured to obtain the pure isolates.

C. Screening of P-solubilizing Activity

P-solubilizing activity of the bacterial strains was screened by using Pikovaskia's medium (PVK) and National Botanical Research Institute Phosphate medium (NBRIP) using tricalcium phosphate and rock phosphate as substrate. Each bacterial isolate was inoculated on the above media containing bromothymol blue indicator and also without it for one week, measuring the yellow zone and clear zone diameters. The strains which showed the highest P-solubilizing activity on the plate were selected for further study.

D. Quantitative Measurement of P-solubilizing Activity

Firstly, the selected P solubilizing strains and two reference strains were cultured in PVK broth for one week and then the samples were centrifuged with 6500 g for 10 min. Afterwards, 0.5 ml of sodium molybdate solution and 0.2 ml of hydrazine sulfate solution were added into 2.5 ml of each sample and they were heated in boiling water for 10 min. Formation of the color blue of the samples was observed after 10 minutes, showing the P-content in each sample. They were stood for a while to be cool enough and then the color intensity was measured by UV spectrometer at 830 nm wavelength.

E. Screening of Nitrogen Fixing Activity of the Microbes

All the strains were cultured on nitrogen free medium for the screening of N2 fixation for 1 week. Only the bacterial isolates which can grow on this medium were further inoculated on GNF medium with bromothymol blue indicator for 1 week. And then, color change from the original green to blue could be supposed to have N2 fixing activity. Four Azotobacter species which show the strong color development were selected for further study, quantitative measurement by ammonium test kit.

F. Quantitative Analysis of N2 Fixing Activity

The bacterial isolates were cultured in GNF broth for one week. After one week incubation, the samples were centrifuged at 6500 rpm for 10 min and then 1 ml of each supernatant was added in each test tube. Then, half spoon of solution 1 and one drop of solution 2 were added to the respective test tubes. After 10 minutes at room temperature, one drop of solution 3 was added into the test tubes. The color development of each sample was observed after 15 min. This can be assumed as the percentage of ammonium which was converted from nitrate to ammonium by microbes.

G. Screening of the Bacterial Isolates to Decompose Potassium

Firstly, the K-decomposing activity of the bacterial strains was screened by K-decomposing medium with 0.12% K-mica. After two days, the clear zone around some colonies was observed due to degradation of potassium in culture. These strains were selected for quantitative analysis.

H. Quantitative Determination of K-decomposing Activi

To investigate the K-decomposing activity, a single colony of each bacterial isolate was cultured in K-decomposing medium supplemented with 0.12 % mica. Bacterial cultures were inoculated at 37˚C for 1 week. According to the literature, this incubation time is enough to solubilize potassium by microbes.

The samples were centrifuged at 6500 rpm for 10 min to get the cell free supernatant. Then, 100 ml of each supernatant was sent to Metallugical Research and Development Centre, Ela to analyse the potassium content in cell free supernatant by Atomic Absorption Spectrometer.

I. Study on Co-existence of the Effective Strains

The co-exist condition of the selected strains was investigated on nutrient medium. In this experiment, cross-line inoculation method was used to observe their inhibition or co-exist condition on one another. The cultural pattern was studied for 5 day inoculation..

J. Study on Field Trial Application on Rice Cultivation in Rainy Season

The growth stimulating effect of the selected and most efficient strains on plant was studied on field trial on rice cultivation in rainy season. Firstly, a single colony of each isolated bacterium was inoculated in their respective medium (broth) for 5 days. After this incubation period, 2 L of each culture broth was mixed with the compost from Shwe Zi Wa (before adding the microbes into it). Then, this biofertilizer was used in field trial application on rice and organic fertilizer and urea were also used in comparison. Each fertilizer was splashed on respective rice plot for two times in total during the cultivation period. The dosages used were 250 kg plus 5 kg urea per acre for bio and organic fertilizers treatments. For urea, 50 kg per acre was applied as a positive control. After three month cultivation period, data analysis from each rice plot was taken and compared with each other.

III. RESULTS AND DISCUSSIONS

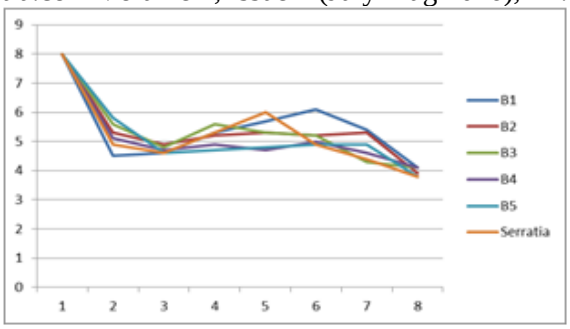
A. Microbial Isolation from the Rhizosphere of the Rice Plants

Fifty one bacterial strains could be isolated from rhizosphere in Patheingyi by using plate count agar base, GNF and actinomycetes selective media. Among them, eight strains were Azotobacter sp., seven species were actinomycetes, one strain was Serratia sp. and others may be Bacilli and Pseudomonas species according to their distinct colony morphology, microscopic morphology and biochemical tests.

B. Determination of the P-solubilizing Activity of the Bacterial Isolates

Firstly, P-solubilizing activity of the bacterial isolates was screened by Pikovaskia's medium and NBRIP medium with a trace amount of BTB indicator and also without it. Six strains (B1, B2, B3, B4, B5and Serratia sp.) were selected as the most efficient strains by plate screening and zone diameter was recorded in millimeter. Measurement of the pH of each bacterial isolate in PVK broth was also studied in daily for one week and it was found that the pH of each bacterial culture declined gradually during the incubation period.

Almost all of the strains solubilized tricalcium phosphate in maximum at five day incubation period. At sixth day, the activity decreased a little. However, the P-solubilizing activity of the bacterial isolates increased again at seven and eight day incubation period. Therefore, it can be assumed that the P-solubilizing activity of the bacterial isolates should be compared at five day incubation period. According to the literature, Serratia was so great in P-solubilizing activity. In this study, it was also found that it was the most effective strain in P-solubilizing activity, 750.88 ppm. Other effective strains were B1, and B3 having 684.42 ppm and 673.52 ppm respectively. Other strains possessed the P-solubilizing activity above 500 ppm only.



X= Incubation time, Y= pH
Fig. 1. Measurement of the pH during the incubation time

TABLE I. P-SOLUBILIZING ACTIVITY OF THE BACTERIAL STRAINS BY UV SPECTROPHOTOMETER

Strain No.	Conc.: (mg/l)			
	5th day	6th day	7th day	8th day
B1	684.42	556.21	602.11	580.12
B2	526.89	438.77	501.89	473.10
B3	673.52	472.72	563.50	533.25
B4	539.78	421.80	456.57	430.72
B5	556.33	470.65	385.79	405.56
Serratia	750.88	596.49	572.48	550.78

C. Study on N2 fixing Activity of the Microbial Strains

N2 fixation of the species was screened by GNF medium including the BTB indicator. After one week, color change from the original green to blue was observed due to the conversion of nitrate to ammonium. Selected strains were further studied by ammonium test kit for quantitative analysis. The content of ammonium in each sample was mentioned as the unit of percentage.

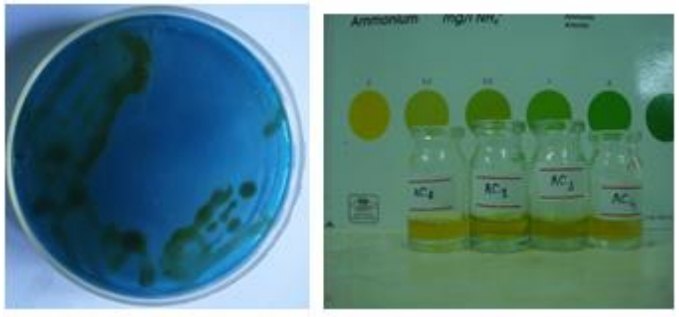


Fig.2. Investigation of nitrogen fixing activity of the effective strains by Ammonium Test Kit

TABLE II. N2 FIXING ACTIVITY OF THE SELECTED STRAINS BY AMMONIUM TEST KIT

Strain No.	Ammonium content (%)
A_1	0.2 – 0.5
A_2	0 - 0.2
A_3	0 - 0.2
A_4	0.2 – 0.5

D. Determination of the Bacterial Activity to Decompose Potassium

Bacterial activity to decompose potassium was screened by K-decomposing medium with 0.12% K-mica. The K-solubilizing activity of the selected strains was quantitatively measured by Atomic Absorption Spectrometer.

In this study, although no. 13 showed the k- decomposing activity on the plate screening method, it was K decomposing activity negative in AAS measurement. So, it can be said that the acid production of the bacteria is not completely related with their K-decomposing activity. It was seen that K-decomposing activity of strain K23 was greater than other bacterial isolates, having 10.46 ppm. Serratia, and strain K8 had the low K-decomposing activity in AAS measurement.

TABLE III. MEASUREMENT OF THE K-DECOMPOSING ACTIVITY OF THE BACTERIAL STRAINS BY ATOMIC ABSORPTION SPECTROPHOTOMETER (AAS)

Strain No.	Cal. Conc.: (ppm)
K8	3.35
K11	9.92
K12	9.46
K13	_
K23	10.46
Serratia	6.92

E. Investigation of Co-existence of the Selected Strains

In biofertilizer formulation, some products are liquid and others are solid as bio-organic fertilizer. And so, it is an important factor for the microbes to survive together in liquid or solid for a long shelf-live. The inoculated strains should not be parasitism on one another. Therefore, it is important for microbes to have the co-existence for carrier consideration. In this study, their co-exist condition was studied on nutrient medium for one week and it was found that the isolated effective strains have the co-existence. In addition, these bacterial isolates had been isolated from the same habitat and so they can easily survive in soil again. So, it is clear that these selected strains can be applied for biofertilizer formulation for plant growth promoting activities..

F. Field Trial Application on Rice in Rainy Season

The most effective bacterial strains were used in field trial on rice cultivation in rainy season. After harvesting, data analysis such as average panicle length, seed number of a panicle on average, thousand seed weight and estimated yield was taken and recorded.

In field trial application, the use of biofertilizer containing the selected strains for each fertilizing activity had the plant growth stimulating effects on rice significantly. Other rice plots which used organic fertilizer and urea were not as good as biofertilizer. From this study, it was known that the average panicle length of each fertilizer was not greatly different. The average seed number of a panicle was 90, 74 and 75 for biofertilizer, organic fertilizer and urea respectively. So, it can be seen that biofertilizer was more effective than the other fertilizers for the rice productivity. The thousand seed weight of the rice from the plot which uses biofertilizer was also greater than organic fertilizer and urea, 22.52, 21.01 and 21.1 g respectively. So, it is seen that the rice from the application of biofertilizer was fatter than the other fertilizers. The estimated yield of rice from the use of biofertilizer, organic fertilizer and urea was 100, 70 and 80 baskets per acre respectively.

Fig.3.Rice ultivation by using biofertilizer urea organic fertilizer biofertilizer.

TABLE IV. FIELD TRIAL APPLICATION OF RICE CULTIVATION IN RAINY SEASON

Data analysis (mean value)	Biofertilizer	Organic Fertilizer	Urea
Panicle Length (mm)	19.5	19	18
Seed No. per Panicle	90	74	75
1,000 Seed Weight (g)	22.52	21.01	21.1
$6' \times 6'$ Yield (baskets)	0.19	0.14	0.16
Estimated Yield (baskets/acre)	100	70	80

IV. CONCLUSION

In conclusion, from the study on phosphorus solubilizing, nitrogen fixation, k-deposing and field trial application on rice cultivation, it was seen that biofertilizer containing the isolated plant growth promoting bacteria can improve the rice productivity and the cost is also less than the application of chemical fertilizer. So, it is undoubtedly certain that it can replace the use of chemical fertilizer for sustainable development on soil fertility.

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