

Isolation of *Rhizobium* from Vermicomposts and Testing with Effects on Germination of Rice

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Abstract

Rhizobiums were isolated from vermicomposts. Vermicomposts were collected from FAME Pharmaceuticals, Organic Farming Project at Kywal Nar Htauk Village, Pyin Oo Lwin Township, Mandalay Division, Myanmar. In the screening program, bacteria were identified by serial dilution method and biochemical tests. According to the results, these strains were identified as genus *Rhizobium*. In the fermentation studies, 48hrs ages and the maximum activity reached at 7 days fermentation. Seed culture of the best of 48hrs fermented solution was observed. The best results of fermented solution (FS) and the comparison of H₂O (control) of paddies were carried out to measure the plant height at 14 days. In this observed, the different time of fermentation, 10hrs the best of plant heights (cm) were higher than controls. *Rhizobium* effects as fertilizer application on germination of *Oryza sativa* L. were tested. *Rhizobium* is a very important fertilizer for agriculture and are an invaluable fertilizer for various plants.

Keywords : isolation, *Rhizobium* and biochemical test

INTRODUCTION

Today, so many chemical fertilizers are being used in the crops and vegetables fields that soil conditions become acidic and unfertile. Chemical residues remain in crops and vegetables. That cause uncomfortable health and toxic side effects to men and animals. The number and types of organisms present in vermicomposts strongly depend on the availability of nutrients and water, pH and the environment. There are plenty of microenvironments in vermicomposts supporting different organisms.

Several microorganisms influence nutrient uptake in plants. Those that directly benefit the plant's metabolisms have considerable biofertilizer potential. Important groups in this category are symbiotic nitrogen fixers and mycorrhizal fungi. Agricultural important microorganisms that fix atmospheric nitrogen and help maintain or restore vermicomposts fertility are the vermicomposts *Rhizobium*, *Azotobacter*, *Azospirillum*, *Acetobacter*, several cyanobacteria and mycorrhiza (A. Cruger, 1998).

A biofertilizer (also bio-fertilizer) is a substance which contains living microorganisms, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.

Biofertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in biofertilizers restore the soil's natural nutrient cycle and build vermicomposts organic matter. Through the use of biofertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the vermicomposts. Since they play several roles a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria"(PGPR). Therefore, they are extremely advantageous in enriching vermicomposts fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through

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microorganism and their byproducts. Hence, biofertilizers do not contain any chemicals which are harmful to the living vermicomposts (Vessey, J.K. 2003).

Biofertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers. Biofertilizers such as *Rhizobium*, *Azotobacter*, *Azospirillum* and blue green algae (BGA) have been in use a long time. *Rhizobium* inoculant is used for paddy crops. The aim of the present work is to use the isolate strain effects as biofertilizer.

MATERIALS AND METHODS

Collection of Soil Sample

In the present work, the samples of vermicomposts which were used as sources of screening of useful microorganisms were collected from FAME Pharmaceuticals, Organic Pharming Project at Kywal Nar Htauk Village, Pyin Oo Lwin Township, Mandalay Division, Myanmar.

Isolation of Microorganisms

Serial dilution method (Collins 1965, Pelczar and Chan, 1972)

Serial dilution techniques of Collins 1965, Pelczar and Chan (1972) with slight modification were applied in the screening and isolation of microbes from the vermicomposts.

Preparation of Media

Bacteria strains of culture media YPD agar, YEM agar and PDA media were used during the research.

Staining Reactions of Isolated Strain

The morphology of the isolated strain was recorded and it was subjected to Gram staining. The staining reaction were carried out according to the methods of Santra et.al, (1993) and Rajan (2001).

Biochemical Tests

- (1) Catalase activity
- (2) Test for starch hydrolysis
- (3) Urea hydrolysis
- (4) Nitrate reduction

Isolation of Pure Culture by Pour Plate Methods

15 ml of YPD Agar media (Yeast extract - 1g, Peptone-2g, Dextrose-2g, Agar-1.5 g) was separately distributed into test tubes. The test tubes were plugged with non-absorbent cotton wool and sterilized by autoclaving them at 15 pounds pressure per square inch for 15 minutes at 121°C. The sterilized mediums contained in each of the test tubes were cooled down to about 45°C and separately poured into the petridishes containing the vermicomposts solution.

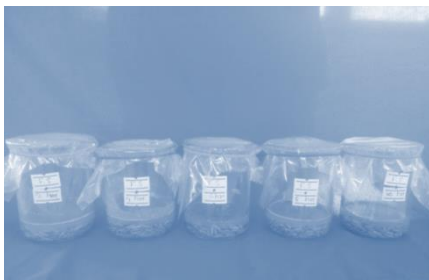
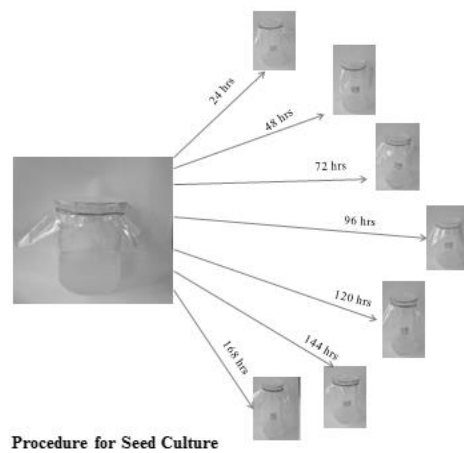
When the agar was solidified, the plates were inverted and incubated at 30°C for 48 hours. The individual colonies developed on the YPD Agar media were separately streak over the sterile YPD Agar media contained in another set of separate sterile petridishes by inoculating needle loop. The second set of inoculated plates was again incubated at 30°C for 48 hours.

On each of these plates were then separately transferred to YPD Agar media slants in order to obtain a pure culture of bacterium. For further study, the slants were separately sub-cultured by streaking method to obtain pure cultures.

Then the pure cultures of isolated microbial strains were kept in specific slants at 4° C in the refrigerator for the study of morphological and biochemical characters.

Seed Culture

Isolated strain were incubated for 7 days old culture of isolated strains were inoculated into the YPD media (Peptone-2g, Dextrose-2g, Yeast extract-1g, Agar-1.5g, D.W-100ml). The sample culture (1ml) was checked in 12 hrs intervals for the growth. According to seed culture method ages of inoculum 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 144hrs, 168hrs were utilized for fermentation.



Seeds in FS (Fermented solution)



Seeds in H₂O (Control)

Experiment: Effects of plant height at 14 days

Effects of isolated bacterial fermented solution from vermicomposts samples on germination and plant height of paddy. This study was carried out at Botany Department of Hinthada University. Bacterial fermented solutions were obtained from the isolation of microbes in vermicomposts sample in organic farming Pyin Oo Lwin, Township. About 40 seeds of paddy were soaked in each bacterial fermented solution at 2 hrs, 4hrs, 6hrs, 8hrs, and 10hrs respectively. The soaking seeds in each solution and control (water soaking) were sown in uniform size of mud filled trays at room temperature. The data were collected at 14 days after sowing to compare seed germination percentages and plant height in different solution.



Experimental work

RESULTS

Microorganisms affect our daily life in many ways. The balance of nature depends upon microbes. Without essential microbial activities, life on earth could not have been continued. Microbes also have great economic importance and commodities such as cheese, wine, enzymes, organic acids and antibiotics are produced by microorganisms. Microbial growth, on the other hand, also causes animal and plant diseases as well as biodeterioration of various commodities.

Vermicomposts Isolation

The isolated strains were detailed procedure of serial dilution method.
(Collins 1965 and Pelczar and Chan, 1972)

Morphological Characters of *Rhizobium*

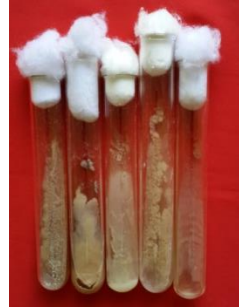
The *Rhizobium* is gram negative and rod shaped bacteria. Member of this genus characteristically able to invade root hairs of paddy plants and incite production of root nodules where in the bacteria occur as intracellular symbionts. The ability to cause nodule formation is more stable than the ability to fix nitrogen in symbiosis. The bacteria were isolated from vermicomposts. The morphological character of isolated bacterium was observed rod shaped, gram-negative, aerobic, white and mucoid. The bacterium are white colour on PDA medium. They showed positive result in oxidation test, positive result in catalase test, positive result in nitrate reduction test, negative result in starch hydrolysis and negative result in urea hydrolysis.



Isolated strain of PDA medium



Isolated strain of YPD medium
(3 days old culture)



Isolated strain of YPD medium
(7 days old culture)

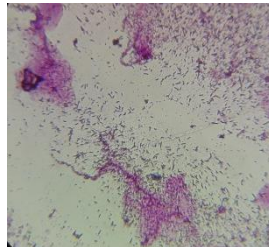
Figure 1. Isolated strain of culture medium



Colony of YPD medium



Colony of PDA medium



Micrograph of *Rhizobium* 100X

Figure 2. Colony and cell morphology of *Rhizobium*

Staining Reactions of Isolated Strain

In the identification of bacterium division, gram staining tests was utilize as the first step. It can also be distinguished to the group of bacteria, namely Gram positive and Gram negative group. After testing with Gram staining, colour of bacterium change to dark purple is Gram positive, while change to red colour is gram negative. Isolated strain was Gram negative bacterium.

Biochemical Test for Characterization of Bacteria

1. Catalase activity

If some bubble appears within 20 seconds, the organism showed positive catalase activity. Absence of bubble shows negative catalase activity. Isolated strain was showed catalase activity.

2. Starch Hydrolysing activity test

The appearance of clear zone around the line of growth of each isolated strain indicated starch hydrolysis, shows positive reaction. *Rhizobium* was not this activity.

3. Urea Hydrolysis

Positive test shows all cultures are discarded to purple red colour. *Rhizobium* was showed positive activity.

4. Nitrate Reduction

Nitrate reduction tested for nitrates with sulfanilic acid and α -naphthylamine reagent. Bacteria that reduce nitrate to nitrite turn the reagents red or pink. *Rhizobium* was showed positive reaction.



Test for Nitrate hydrolysis



Test for starch hydrolysis



Test for urea hydrolysis

Figure 3. Biochemical test

Seed Culture

Seed culture of fermented solution for 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 144hrs and 168hrs intervals respectively, at the 7 days culture. The results of fermented solution of 48hrs were most suitable condition.

Table 1. Effects of Fermentation Medium

Culture Time(Ages of culture, hrs)	Growth of inoculum (mm)
24	1.67
48	3.15
72	0.05
96	0.15
120	0.24
144	0.26
168	0.83

Experiment .Effects of plant height at 14 day

Plant heights of paddy at 48hrs fermented solutions and different soaking times (2hr,4hr,6hr,8hr,10hr) were shown in figure and table. Plant height of paddy treated with 48 hrs fermented solution was tallest at 14 days than control.

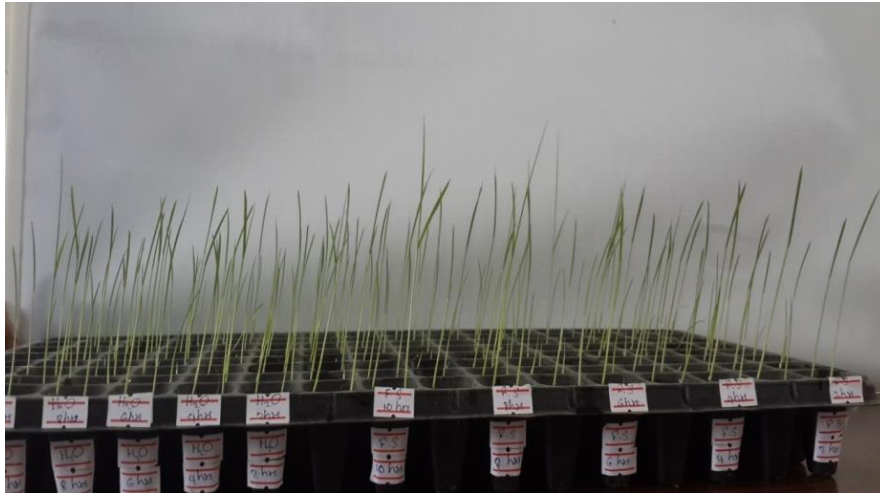


Figure 4. Seeds germination test in seedling tray

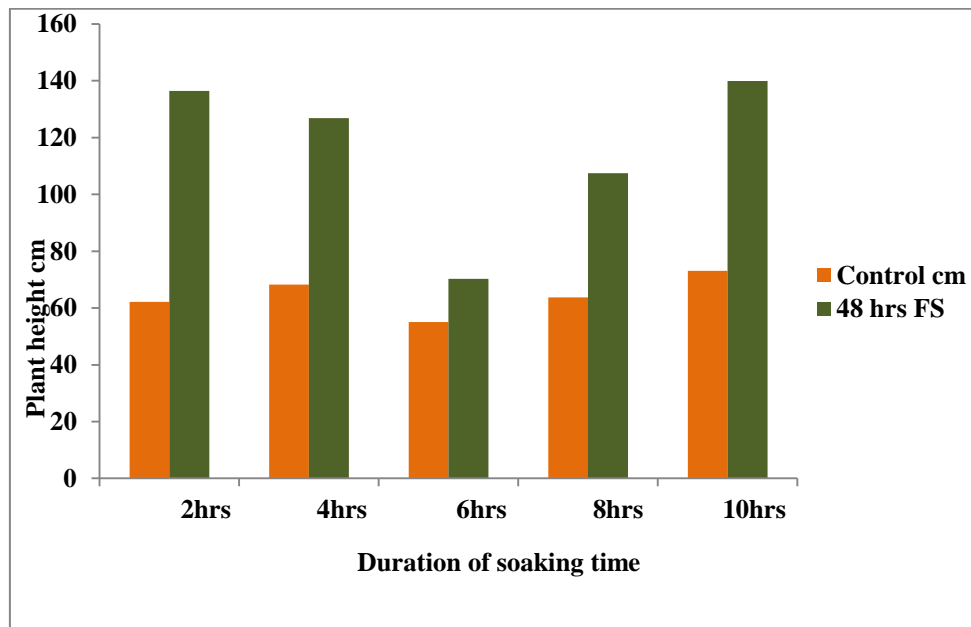


Figure 5. Effects of plant height 14 days

Table 2. Effects of plant height at 14 days

Duration of soaking time	Control (H ₂ O)	48hrs FS
2hrs	62.18 cm	136.41 cm
4hrs	68.19 cm	126.84 cm
6hrs	55.00 cm	70.22 cm
8hrs	63.75 cm	107.44 cm
10hrs	73.12 cm	139.94 cm

DISCUSSION AND CONCLUSION

Microorganisms were isolated from vermicomposts samples. Highly active microorganisms were selected by using agricultural plants. Microorganisms were isolated from vermicomposts in FAME Pharmaceuticals, Organic Pharming Project at Kywal Nar Htauk Village, Pyin Oo Lwin Township, Mandalay Division, Myanmar.

The study was carried out to investigate the effects of isolated bacterial fermented solution in vermicomposts samples. Genus *Rhizobium* was first selected from field of Pyin Oo Lwin Township because of Dichotomous key leading to the Identification of Genus *Rhizobium* by using the method of Harley and Prescott 2002.

The morphological character of isolated bacterium was observed that rod shaped, gram-negative, aerobic, white and mucoid. The bacteria were found as white colour on PDA medium. They showed positive result in oxidation test, positive result in catalase test, positive result in nitrate reduction test, negative result in starch hydrolysis and negative result in urea hydrolysis.

In the study, *Rhizobium* bacteria can be inoculated onto paddy seeds to fix nitrogen in the soil, germination percentage, plant height of paddy was showed as the highest in FS compared to control in each experiment. The higher number of flowers causes the higher number of fruits lead to greater production of yield of paddy. A. Cruger (1998) agreed that bacteria included in FS such as soil bacteria being agricultural important microorganisms that fix atmospheric nitrogen and help maintain or restore soil fertility.

Fermented solution (FS) for 24hrs, 48hrs, 72hrs, 96hrs, 120hrs, 144hrs and 168hrs intervals respectively at the 7 days culture. The results of fermented solution of 48hrs (3.15mm) were most suitable condition.

In this observed that the different time of soaking solution 10hrs the best of plant heights (139.94cm) were higher than controls and 2hrs the second best of plant heights (136.41cm) and 4hrs the third best of plant heights (126.84cm) at 14 days.

Tin Soe, (2004) revealed that soaking the seeds for carefully determine the length of time before sowing would save valuable time of growth period and dry seeds spend a great deal of time just absorbing water from the vermicomposts.

In the present work, the application of different hours soaked in 48hrs bacterial fermented solution (FS) was the most suitable and it can be improved for production of high yields compared to seeds without soaking solution for cultivation of the paddy cultivars.

Therefore, as stated as the introduction, since the chemical fertilizers are more expensive and not safe for health due to chemical residue, it was assumed that this biofertilizer may be used instead of chemical fertilizers. However, this study should be necessary to proceed that of applications and also modified it for the further studies of growing paddy and other crops in Myanmar.

It was concluded that biofertilizer may be usual in production of agricultural crops in stead of chemical fertilizers. According to present study the *Rhizobium* is sure to be the best fertilizer for growth rates of not only rice but also other crops during the season.

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