Enhancement of rice yield, N_2 uptake and fertilizer use efficiency using biofetilizer of diazotrophs

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Introduction

Rice, *Oryza sativa*, is the staple food for more than 2 billion people in Asia and Latin America. By the year 2020, rice production must increase from 520 million mt to 760 million to feed the increasing demand of the global population. However, the yield of rice has been declining over the years and this is mainly due to the decreased in nitrogen uptake and declining use of fertilizer due to the rising costs of fertilizers. To maintain the current yield, higher inputs of fertilizer, such as urea-based fertilizer must be supplied. However, increased use of nitrogenous fertilizers will exacerbate the problems of groundwater and environmental pollution due to the leaching, denitrification, ammonia volatilization of these chemicals into the environment. It has been estimated that as much as 60% of the N applied to the rice is loss. Furthermore rice crop is considered inefficient in nutrient uptake. As an alternative, the use of diazotrophs (nitrogen-fixing bacteria) is proposed. Several studies have shown the benefits of using microbial biofertilizers that associate intimately with the roots of wetland rice crop. During this association, the bacteria could supply nitrogenous compounds to the growing rice plants thus reducing the dependency on chemical fertilizers. These bacteria also produced plant growth hormones which can enhanced the growth and uptake of nutrients. In this study several isolates of bacteria from wetland rice located over various sites of paddy field of Peninsular Malaysia was tested as a potential biofertilizer for rice crop and its efficiency as biofertilizer for rice. The study was also conducted to determine the possibility of complementing or replacing chemical fertilizers with microbial biofertilizers derived from diazotrophs isolated locally.

Materials and Methods

Soil and rice crops were sampled from various paddy fields in Kelantan, Kedah, Selangor and Johor. At each location about twenty samples of soils and rice plants were collected. In the laboratory, bacteria were isolated from soils, rhizosphere and roots by growing in various selective growth media. The number of bacteria was narrowed down to twenty isolates which were then subjected to various characterization tests based on colony and cell morphology (microscope study), patterns of intrinsic antibiotic resistance, ability to fix nitrogen using acytelene reduction assay (ARA) to determine the presence of nitrogenase which can fixed gaseous nitrogen into ammonium, production of plant growth hormones such as IAA (indole-3-acetic acid) and growth promotion of several rice cultivars. A glasshouse experiment was also conducted to determine the efficiency of six selected diazotrophs in nutrient uptake and promotion of growth by inoculating them to rice plants growing in different levels of chemical fertilizers (i.e. 0 N, ¼ N, 1/3 N, ½ N, ¾ N and full N.). Plant and root biomass was recorded during growth and nutrient analysis of plant tissue was carried out at harvest.

Results and Discussion

Twenty-one isolates were identified based on colony characteristics such as morphology, colour and surface texture. All cultures were found to be Gram-positive rods with various isolates showing short and thick rods, whilst others showed extended rods. Fifteen isolates were microaerophilic i.e. requires low oxygen tension for growth whereas others are aerobic. All isolates were resistant to Nystatin but showed a differential response to penicillin and only two isolates were resistant to streptomycin. Only 25% of the isolates were able to fix nitrogen and about twelve isolates were shown to produce IAA. In the glasshouse experiment, the results showed that different isolates could increase the nutrient uptake of different elements. Rice plants inoculated with rhizobacteria E44 showed the tendency to increase nitrogen content, rhizobacteria E38 tends to increase plant phosphorus content, and rhizobacteria E40 inoculation tends to increase dry weight of rice plants. Meanwhile, inoculation of rhizobacteria E40 showed the tendency to increase shoot magnesium content while rhizobacteria F23 tends to increase root magnesium content. The results of this study showed the diversity of bacteria found on rice roots and rhizosphere based on basic cell and colony morphology. Each isolate

also showed a different characteristic indicating that rice rhizosphere can support a diverse range of bacteria species. Some of the isolates showed the potential as biofertilizer by having the ability to fix nitrogen and hence can supply rice plant with nitrogenous compounds essential for growth and grain filling. Yet, other showed the ability to produce plant growth hormones which enhance root biomass thus increasing the efficiency of nutrient uptake as confirmed by the results in the glasshouse experiments. Each species tend to increase the nutrient uptake of different element and thus having different species of bacteria within the rhizosphere can complement the use of chemical fertilizers for rice production. Several of the isolates can be recommended as potential biofertilizer of rice crops.

Conclusions

Bacteria isolated from local rice soils and rhizosphere has the potential as biofertilizers because they are diazotrophic and fixed nitrogen and also produce IAA. Different isolates can increase the nutrient uptake of different essential elements for rice growth thus increasing the efficiency of nutrient uptake. It is expected that a biofertilizer based on a mixed culture of several bacteria can be used as biofertilizer for wetland rice production in this country.

Benefits from the study

Several isolates have been identified to be able to fix nitrogen and produced plant growth hormones. In addition they also increased the efficiency of plant nutrient uptake. Selected isolates can be further improved and developed as biofertilizers of locally grown rice crop.

Patent(s), if applicable:

Nil

Stage of Commercialization, if applicable:

Nil

Project Publications in Refereed Journals:

Nil

Project Publications in Conference Proceedings

1. Tan G.H., Halimi M.S., Zulkifli H. S. and Khanif, M.Y. (2000). Determination of Indole-3-acetic Acid Produced by Bacteria from Rice Using Modified Colorimetric Methods. Proceedings 23rd Symposium of the Malaysian Society for Microbiology, P. Langkawi, Kedah, Malaysia. p 238-240

Graduate Research

Name of Graduate	Research Topic	Field of Expertise	Degree Awarded	Graduation Year
Tan Geok Hun	Isolation and identification of rhizobacteria from wetland rice (*Oryza sativa* L.) as potential biofertilizer	Soil Microbiology of Rice	MSc	2002
Neo Sye Peng	Nutrient uptake by rice plant inoculated with microaerophilic rhizobacteria isolated from selected rice soils	Soil Microbiology of Rice	MSc	2002

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