



UNIVERSITI PUTRA MALAYSIA

**EFFECT OF HYDROLYTIC ENZYMES PRODUCTION BY
DIAZOTROPHS
AND UREA FERTILIZATION ON ROOT COLONIZATION AND
GROWTH OF RICE (*Oryza sativa* L.)**

ASILAH ABDUL MUTALIB

ITA 2010 2



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**MASTER OF SCIENCE
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By

ASILAH ABDUL MUTALIB

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

October 2010



Dedicated

to

My parents

Kasmah Shafie
Abdul Mutalib Ahmad

My aunts

Jemilah Mohamed
Hadijah Mohamed

My brothers

Khair Yazid Abdul Mutalib
Muhammad Khairul Abdul Mutalib



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**EFFECT OF HYDROLYTIC ENZYMES PRODUCTION BY DIAZOTROPHS
AND UREA FERTILIZATION ON ROOT COLONIZATION AND
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ASILAH ABDUL MUTALIB

October 2010

Chairman : Associate Professor Radziah Othman, PhD

Institute : Institute of Tropical Agriculture

Production of hydrolytic enzyme by diazotrophs and their association with rice plant may increase the N₂-fixing activity and subsequently plant growth. The ability to produce hydrolytic enzymes is an important mechanism of diazotrophs to enter the plant roots. Hydrolytic activities are important during colonization of the surface and interior of root by associative and endophytic diazotrophs. A series of experiments were conducted in laboratory and glasshouse conditions with the following objectives; (i) to screen for hydrolytic enzyme production in diazotrophs isolated from rice rhizosphere, (ii) to determine the effect of diazotrophs on hydrolytic enzyme activity, root colonization, root development and N₂-fixing activity of rice, (iii) to determine the effect of urea-N on hydrolytic enzyme production, N uptake and growth of rice inoculated with diazotrophs. Twelve bacterial isolates were screened for the endoglucanase (EG) and endopolymethylgalacturonase (EPMG). The production of the



hydrolytic enzymes, root colonization and root development of MR219 rice inoculated with selected diazotrophs (Sb34, Sb41 and Sb42) was determined *in vitro*. The effect of urea-N on production of these hydrolytic enzymes in rice inoculated with diazotrophs was evaluated under glasshouse condition. The inoculated (Sb42 strain) and non-inoculated plants were treated with three rates of urea-N (0, 30 and 60 kg/ha). Plants were harvested at 45 days after transplanting and analyzed for soluble protein content, specific enzymes activity, plant growth, concentration and uptake of N and photosynthetic activity. Results showed that nine out of 12 bacterial strains were positive with carboxymethyl cellulose (CMC) and pectin reactions as indicated by appearance of halo zone around the colonies. There was significant ($P \leq 0.05$) effect of different diazotrophic inoculation on the rice rhizosphere and endosphere populations. Populations of inoculated diazotrophs were found higher in the rhizosphere than the endosphere of rice. Inoculated plants showed significantly ($P \leq 0.05$) higher specific enzymes activity and soluble protein content compared to non-inoculated control. Inoculation significantly ($P \leq 0.05$) increased root growth of rice with substantial increases in root length, volume and surface area. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) observations revealed the ability of diazotrophs to colonize the surface and interior of roots. Glasshouse study showed that growth of rice was significantly ($P \leq 0.05$) affected by diazotrophs and urea-N application. Low availability of nitrogen in soil stimulated the bacterial activity. Application of urea at 30 and 60 kg N negatively affected the interaction between diazotroph and rice plants. Inoculated plants without urea-N showed significantly ($P \leq 0.05$) higher specific hydrolytic enzymes activity, soluble protein content, plant

biomass, photosynthesis activity and N uptake compared to other treatments. In general, diazotrophs produced substantial amount of hydrolytic enzyme which can improve nitrogen fixing activity in inoculated plants. Colonization of diazotrophs on the surface and interior of rice root is important for nitrogen fixation and plant growth enhancement. The association of these diazotrophs with the plants significantly ($P \leq 0.05$) increased the production of hydrolytic enzymes in the root system which is an important mechanism for endophytic colonization and subsequently nitrogen fixation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**KESAN PENGHASILAN ENZIM HIDROLITIK OLEH DIAZOTROF
DAN PEMBAJAAN UREA KE ATAS PENGKOLONIAN AKAR DAN
PERTUMBUHAN PADI (*Oryza sativa* L.)**

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Penghasilan enzim hidrolitik oleh diazotrof dan gabungannya dengan pokok padi boleh meningkatkan aktiviti pengikatan nitrogen dan seterusnya pertumbuhan pokok. Kebolehan untuk menghasilkan enzim hidrolitik adalah satu mekanisme penting oleh diazotrof untuk masuk ke dalam akar pokok. Aktiviti hidrolitik adalah penting semasa pengkolonian pada permukaan dan di dalam akar oleh diazotrof asosiatif dan endofitik. Beberapa siri kajian telah dijalankan di dalam makmal dan di rumah kaca dengan objektif-objektif berikut; (i) untuk menyaring penghasilan enzim hidrolitik dari diazotrof yang diasingkan daripada rizosfera padi, (ii) untuk menentukan kesan diazotrof ke atas aktiviti enzim hidrolitik, pengkolonian akar, pengembangan akar dan aktiviti mengikat nitrogen pada padi, (iii) untuk menentukan kesan urea-N ke atas penghasilan enzim hidrolitik, pengambilan N dan pertumbuhan padi yang diinokulasi dengan diazotrof. Dua belas jenis bakteria telah disaring untuk endoglukanase (EG) dan

endopolimetilgalakturonase (EPMG). Penghasilan enzim hidrolitik, pengkolonian akar dan pengembangan akar padi MR219 yang diinokulasi dengan diazotrof terpilih (Sb34, Sb41 dan Sb42) telah ditentukan secara *in vitro*. Kesan urea-N ke atas penghasilan enzim hidrolitik ini dalam padi yang diinokulasi dengan diazotrof telah ditentukan di bawah kajian di rumah kaca. Pokok yang diinokulasi (strain Sb42) dan yang tidak diinokulasi telah dirawat dengan tiga kadar urea-N (0, 30 dan 60 kg/ha). Pokok telah dituai pada hari ke-45 selepas dipindahkan dan dianalisis untuk kandungan protein boleh larut, aktiviti spesifik enzim, pertumbuhan pokok, kepekatan dan pengambilan N dan aktiviti fotosintetik. Keputusan menunjukkan bahawa sembilan daripada 12 strain bakteria adalah positif dengan reaksi carboxymethyl cellulose (CMC) dan pektin yang ditunjukkan dengan kehadiran zon halo di sekeliling koloni. Terdapat kesan yang signifikan ($P \leq 0.05$) terhadap inokulasi diazotrof yang berlainan ke atas populasi rizosfera dan endosfera padi. Populasi diazotrof yang diinokulasi telah dijumpai lebih tinggi dalam rizosfera berbanding dengan endosfera padi. Pokok yang telah diinokulasi menunjukkan aktiviti spesifik enzim dan kandungan protein boleh larut yang lebih tinggi secara signifikan ($P \leq 0.05$) berbanding dengan kawalan yang tidak diinokulasi. Inokulasi telah meningkatkan pertumbuhan akar padi secara signifikan ($P \leq 0.05$) dengan pertumbuhan yang besar dalam panjang, isipadu dan kawasan permukaan akar. Pemerhatian dari Mikroskop Pengimbas Elektron (SEM) dan Mikroskop Pemancar Elektron (TEM) mendedahkan kebolehan diazotrof untuk menkolonisasi permukaan dan dalam akar. Kajian di rumah kaca menunjukkan pertumbuhan padi telah memberikan kesan yang signifikan ($P \leq 0.05$) oleh diazotrof dan aplikasi urea-N. Ketersediaan nitrogen yang rendah dalam tanah telah merangsang aktiviti bakteria.

Aplikasi urea pada 30 dan 60 kg N telah memberikan kesan yang negatif pada interaksi antara diazotrof dan pokok padi. Pokok yang diinokulasi tanpa urea-N menunjukkan spesifik aktiviti enzim hidrolitik, kandungan protein boleh larut, biomas pokok, aktiviti fotosintesis dan pengambilan N yang lebih tinggi secara signifikan ($P \leq 0.05$) berbanding dengan rawatan-rawatan yang lain. Secara amnya, diazotrof dapat menghasilkan jumlah enzim hidrolitik yang banyak di mana ia dapat memperbaiki aktiviti pengikatan nitrogen dalam pokok yang diinokulasi. Pengkolonian diazotrof di atas permukaan dan dalam akar padi adalah penting untuk pengikatan nitrogen dan peningkatan pertumbuhan pokok. Gabungan diazotrof ini dengan pokok telah meningkatkan penghasilan enzim hidrolitik secara signifikan ($P \leq 0.05$) dalam sistem akar yang menjadi mekanisme penting untuk pengkolonian endofitik dan seterusnya pengikatan nitrogen.

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I certify that a Thesis Examination Committee has met on 6 October 2010 to conduct the final examination of Asilah binti Abdul Mutalib on her thesis entitled “Effect of Hydrolytic Enzymes Production by Diazotrophs and Urea Fertilization on Root Colonization and Growth of Rice (*Oryza sativa* L.)” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

ASILAH BINTI ABDUL MUTALIB

Date: 6 October 2010



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