UNIVERSITI PUTRA MALAYSIA

INFLUENCE OF SUGAR CANE MOLASSES ON GROWTH OF NITROGEN FIXING BACTERIA (Stenotrophomonas maltophilia) AND RICE YIELD

NOOR HIDAYAH HASHIM

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By

NOOR HIDAYAH HASHIM

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfillment of the Requirement for the Degree of Master of Science

May 2016

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edicated To,

My lovely mother, Mama…
My late father, Ayoh…
My sweet sisters…
My handsome brothers…
Thank you so much…i love you all..

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

INFLUENCE OF SUGAR CANE MOLASSES ON GROWTH OF NITROGEN FIXING BACTERIA (*Stenotrophomonas maltophilia*) AND RICE YIELD

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May 2016

Chairman : Associate Professor Radziah Othman, PhD
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Sugar cane molasses is an important carbon source for the growth of nitrogen fixing bacteria. Nitrogen fixing bacteria utilize root exudate carbon compound and form association with plant and fix nitrogen. Sugar cane molasses as an external carbon sources for nitrogen fixing bacteria is low-cost efficient growth factor. Thus, addition of sugar cane molasses to the rice plant may be able to provide carbon substrate to nitrogen fixing bacteria and also improved rice yield parameter. A series of experiments were conducted in the laboratory and glasshouse conditions with the following objectives: 1) To determine the effect of different concentrations of molasses on growth of *Stenotrophomonas maltophilia* (Sb16) in culture media incubation and soil amended with molasses and urea N, 2) To determine the effect of sugar cane molasses on growth of N_2-fixing bacteria and effect of *S. maltophilia* (Sb16) and molasses and urea-N on total starch, total sugar and rice yield and to elucidate their distribution of N partitioning in the plant parts. Laboratory and glasshouse experiments were arranged in a factorial experiments in complete randomized design (CRD) with 3 replication and factorial completely randomized design (RCBD) with 4 replications using SAS statistical program version 9.3. In the glasshouse study, each block consist of 2 bacterial treatments: control (0) and *Stenotrophomonas maltophilia* (Sb16), 3 amendments treatment: control (0), urea application (60 kg N ha^{-1}) and molasses application (2 tan ha^{-1}). Mean separation was carried out using Tukey's Studentized Range (HSD) at the 5% level of probability. An experiment was conducted to test the growth of *Stenotrophomonas maltophilia* (Sb16) on different media in laboratory and consecutively test the growth of *S. maltophilia* (Sb16) on soil. The different media for *S. maltophilia* growth was made by adding molasses as carbon (C) sources, compared to commonly used Nutrient broth and Trypic Soy broth. In the soil incubation study molasses and urea were added to soil inocculated with *S. maltophilia* (Sb16). A glasshouse study was conducted to determine the effect of N_2 fixing bacteria, *S. maltophilia* (Sb16) and sugar cane molasses on total starch and total sugar and growth of rice. Two rates of sugar cane molasses (0 and 2 tan ha^{-1}), urea (0 and 60 kg N ha^{-1}) and *S. maltophilia* (Sb16) were applied to MR 219 rice variety. Plant growth and yield, total starch and sugar, N uptake and biomass of plants were determined. The highest

growth of *S. maltophilia* strain Sb16 (14.84 mg mL^{-1}) was observed at sugarcane molasses concentration of 20% w/v at 24 hour incubation. Total reducing sugar increased with increasing molasses concentrations. High total N (505 mg L^{-1}) was produced in 7.5% w/v molasses concentration. Application of urea significantly influenced total population and total reducing sugar of *S. maltophilia* (Sb16). Highest of total bacterial population (7.97 log10 cfu dry weight $soil^{-1}$) and total reducing sugar (0.098 mg g^{-1} glucose) were observed in urea treatment at day 7. While, application of molasses significantly increased total N (0.31%) in the soil applied with *S. maltophilia* (Sb16). Application of molasses to uninoculated MR 219 rice significantly increased tiller $plant^{-1}$, aboveground dry matter, grain yield, 100-grain weight, straw yield, harvest index (HI) and biological yield. Application of molasses and urea to rice plant inoculated with *S. maltophilia* (Sb16) significatly increased grain yield, with application of molasses also showed significantly increased grain weight and aboveground dry matter compared to inoculated plants. Highest tiller number $plant^{-1}$, aboveground dry matter, grain yield, HI and biological yield were observed in application molasses with uninoculated MR 219 rice plant. High N content and N uptake in leaf blade were observed with uninoculated plant applied with molasses. Application of molasses to inoculated plants showed high total sugar of leaf blade and leaf sheath of rice plant. While, application of molasses to uninoculated plants resulted in high total sugar of stem, panicle and grain. Application of molasses to uninoculated plants showed high total starch in leaf blade, leaf sheath, panicle and grain, while, application molasses to inoculated plants was observed to produce high total starch in stem. The results suggested that sugar cane molasses can provide carbon source and increase *S. maltophilia* (Sb16) growth and yeild parameters of rice.

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

PENGARUH GULA MERAH TEBU PADA PERTUMBUHAN BAKTERIA PENGIKAT NITROGEN (*Stenotrophomonas maltophilia*) DAN HASIL PADI

Oleh

NOOR HIDAYAH HASHIM

Mei 2016

Pengerusi : Professor Madya Radziah Othman, PhD
Fakulti : Pertanian

Gula merah tebu adalah sumber karbon yang penting untuk pertumbuhan bakteria pengikat nitrogen. Bakteria pengikat nitrogen menggunakan komponen karbon dalam eksudat akar dan membentuk hubungan dengan pokok serta mengikat nitrogen. Gula merah tebu adalah sumber luar karbon untuk bakteria pengikat nitrogen sebagai faktor pertumbuhan berkos rendah yg efisien. Oleh itu, penggunaan gula merah tebu pada tanaman padi dapat menyediakan karbon sebagai substrat kepada bakteria pengikat nitrogen dan juga menambah baik parameter hasil padi. Satu siri eksperimen telah dijalankan di makmal dan rumah kaca dengan objektif seperti berikut; untuk menentukan kesan kepekatan berbeza gula merah tebu ke atas pertumbuhan *Stenotrophomonas maltophilia* (Sb16) dalam inkubasi media pertumbuhan dan tanah yang ditambah dengan gula merah tebu dan urea, dan untuk menentukan kesan gula merah tebu pada pertumbuhan bakteria pengikat nitrogen serta kesan *S. maltophilia* (Sb16), gula merah tebu dan urea ke atas jumlah kanji, jumlah gula dan hasil padi dan menjelaskan sebaran pembahagian N pada bahagian-bahagian tumbuhan. Ujian makmal dan rumah kaca telah disusun dalam ujikaji faktoran dalam reka bentuk rawak lengkap (CRD) dengan 3 replikasi dan faktoran reka bentuk rawak lengkap dalam blok (RCBD) dengan 4 replikasi menggunakan SAS statistik program versi 9.3. Dalam kajian rumah kaca, setiap blok terdiri 2 rawatan bakteria: kawalan (0) dan *S. maltophilia* (Sb16), 3 rawatan bahan tambah untuk tanah: kawalan (0), aplikasi urea (60 kg N ha^{-1}) dan gula merah tebu (2 tan ha^{-1}). Semua data dianalisis menggunakan Tukey Standard Studentized (HSD) pada keberangkalian 5%. Satu eksperimen telah dijalankan untuk menguji pertumbuhan *S. maltophilia* (Sb16) pada media yang berbeza dalam makmal dan juga inkubasi dalam tanah. Rawatan media yang berbeza telah dibuat untuk *S. maltophila* (Sb16) dengan menambah gula merah tebu sebagai sumber karbon. Dalam inkubasi tanah gula merah tebu dan urea telah ditambah untuk memantau tanah diinkubasi dengan *S. maltophilia* (Sb16). Kajian rumah kaca telah dijalankan untuk menentukan kesan bakteria pengikat N_2, *S. maltophilia* (Sb16) dan gula merah tebu ke atas jumlah kanji dan jumlah gula dan pertumbuhan pokok padi. Dua kadar gula merah tebu (0 dan 2 tan ha^{-1}), urea (0 dan 60 kg N ha^{-1}) dan *S. maltophilia* (Sb16) telah digunakan untuk varieti padi MR 219. Pertumbuhan tumbuhan

dan hasil, jumlah kanji dan gula, pengambilan N dan berat kering pokok padi telah ditentukan. Pertumbuhan tertinggi *S. maltophilia* (Sb16) (14.84 mg mL^{-1}) diperhatikan dengan rawatan gula merah tebu dengan kepekatan 20% w / v pada 24 jam inkubasi. Jumlah gula meningkat dengan peningkatan kepekatan gula merah tebu. Jumlah N tertinggi (505 ng L^{-1}) adalah kepekatan gula merah tebu pada 7.5% w/v. Penambahan urea mempengaruhi jumlah populasi dan jumlah gula *S. maltophilia* (Sb16). Jumlah ppopulasi bakteria yang tinggi (7.97 log10 cfu berat kering $tanah^{-1}$) dan jumlah gula (0.098 mg g^{-1} glukosa) diperhatikan dalam rawatan urea pada hari ke-7. Walau bagaimanapun, penggunaan gula merah tebu ketara meningkat dengan jumlah N (0.31%) dalam tanah dengan *S. maltophilia* (Sb16). Penambahan gula merah tebu pada varieti padi MR 219 meningkatkan dengan ketara bilangan pokok padi, jumlah berat kering pokok padi, hasil padi, berat 100-biji padi, hasil jerami, HI dan hasil biologi. Penggunaan gula merah tebu dan urea pada pokok padi yang ditambah dengan *S. maltophilia* (Sb16) ketara meningkatkan hasil padi, dengan penggunaan gula merah tebu juga menunjukkan peningkatan ketara berat padi dan jumlah berat kering pokok padi. Bilangan tertinggi bilangan pokok, jumlah berat kering pokok padi, hasil padi, HI dan hasil biologi diperhatikan dalam penggunaan gula merah tebu pokok padi varieti MR219. Kandungan N yang tinggi dan pengambilan N oleh daun pokok padi dengan rawatan pokok dengan gula merah tebu tanpa inokulasi oleh *S.maltophilia* (Sb16). Penggunaan gula merah tebu pada pokok yang diinokulasi oleh *S. maltophilia* (Sb16) tinggi dalam jumlah gula pada daun padi dan sarung daun pokok padi. Walau bagaimanapun, penggunaan gula merah tebu meningkatkan pertumbuhan tumbuhan pokok yang tidak diinokulasi dengan *S. maltophilia* (Sb16) jumlah gula pada batang, tangkai dan biji padi. Penambahan gula merah tebu pada pokok padi yang tidak diinokulasi oleh *S. maltophilia* (Sb16) menunjukkan jumlah kanji yang tinggi di dalam daun, sarung daun, tangkai dan biji padi, manakala, penggunaan gula merah tebu pada pokok padi yang diinokulasi *S. maltophilia* (Sb16) diperhatikan menunjukkan jumlah kanji yang tinggi di dalam batang. Hasilnya menunjukkan bahawa gula merah tebu boleh memberikan sumber karbon dan meningkatkan pertumbuhan *S. maltophilia* (Sb16) dan parameter hasil padi.

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I certify that a Thesis Examination Committee has met on 6 May 2016 to conduct the final examination of Noor Hidayah Hashim on her thesis entitled "Influence of Sugar Cane Molasses on Growth of Nitrogen Fixing Bacteria (*Stenotrophomonas maltophilia*) and Rice Yield" 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.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Member of
Supervisory
Committee:----------

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cfu	Colony forming unit
SAS	Statistical Analysis System
NB	Nutrient Broth
NA	Nutrient Agar
TSB	Trypic Soy Broth
PSB	Phosphate Saline Buffer
BNF	Biological Nitrogan Fixation
ATP	Adenosine triphosphate
DCM	Dry Cell Mass
DAT	Day after transplanting
PHA	Polyhydroxyalkanoates
ARA	Acetylene Reduction Assay

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food for more than half of the global population. There is a need to increase rice production to ensure security in rice production of about 70% of national requirement (Arshad et al., 2011). In order to increase rice production due high rice demand, high chemical fertilizer input is required. Nitrogen (N) is one of the most yield-limiting nutrients in lowland rice production (Fageria and Barbosa Filho, 2001). Nitrogen fertilizers are being applied to increase plant growth and yield production. Application of high N fertilizer can lead to environmental pollution. Chemical production of nitrogen fertilizers during combustion of fossil fuel may release greenhouse gases such as CO_2 and N_2O. Up to 50% of the applied fertilizer is lost by leaching of NO_3^- resulted highly contaminated ground water with nitrate, eutrophication and blue-baby syndrome (By and Professionals, 2002). Nitrification of ammonium-N from nitrogen may cause soil acidification which can lead to decreases in plant nutrient availability.

One of the strategies to reduce N fertilizer application and enhanced nitrogen use efficiently is utilization of the biological nitrogen fixation (BNF). The BNF process has been known to be important in sustainable rice cultivation (Saikia and Jain, 2012; Houlton et al., 2008). The use of N-fixing bacteria also known as diazotrophs can fix atmospheric N_2 and supply nitrogen for the plant and, thus, makes the crop less dependent on N fertilizer (Prakamhang et al., 2009). Some species of these N_2 fixing bacteria can colonize the plant's rhizosphere and at the same time grow endophytically. Most plants benefits by harbouring these endophytic microbes (Compant et al., 2005). Several diazotrophs have been isolated from rice and able to colonize the surface and interior of roots and improved root surface area, root volume and N uptake of rice seedlings (Biswas et al., 2000; Naher et al., 2009a). Nitrogen fixation by associative diazotrophs is an immediate process in condition where soil N is limited and the adequate carbon sources are available (Kennedy et al., 2004). The nitrogen fixing bacteria utilized carbon from soil plant system as their energy sources and fix atmospheric N_2. About 0.11 moles of glucose must be burned to drive fixation of one mole of ammonia equal to 1.44g of glucose is needed for one gram of N fixed. In biological N_2 fixation, glucose supplies energy (carried as ATP) and also the material reductant, hydrogen (H_2). It is proven that when an energy source such as starch was added to stable compost, the expression to fix nitrogen is enhanced (Mulder, 1975). Addition of glucose stimulated N_2-fixation in compost (Keeling et al., 1998). It is proven that the development of N_2-fixing bacteria is favored by the presence of considerable amount of available carbon compounds (Stella and Suhaimi, 2010). Availability of cheap carbon sources for these nitrogen fixing bacteria to fix N_2 is important for sustainable rice production. Sugar cane molasses also alters the C/N ratio in soil and affects the soil microbiota which in turn affects the available nutrients for plant (Schenck, 2001). Addition of a range of carbon sources (lactate and sucrose) has been shown to regulate N_2-fixation in the rhizosphere of plant such as *Zostera noltii* (Welsh et al., 1997). Sugar cane molasses is a cheap carbon and energy source that is suitable medium to grow N_2-fixing bacteria. In addition to glucose, sugar cane

molasses also contain sucrose and fructose that can be used by other microorganisms (Olbrich, 2006). Application molasses to paddy soil is as an additional carbon sources to N_2-fixing bacteria in the soil to fix N and available to rice plant, thus also can improved yield of rice. Sugar cane molasses can be functionally used as organic fertilizer that may replace urea as main N source commonly used in paddy fields. Therefore, the objectives of the study were:

1. To determine the effect of different concentrations of molasses on growth of *Stenotrophomonas maltophilia* (Sb16) in culture media and in soil amended with molasses and urea.
2. To determine the effect of *Stenotrophomonas maltophilia* (Sb16), molasses and urea on rice yield and to elucidate the distribution of N in the plant parts.

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