
**UNIVERSITI PUTRA MALAYSIA**

***BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF FLUORESCENT PSEUDOMONAS FOR POTENTIAL OF PYRICULARIA ORYZAE***

**MANSOUREH SADAT SHARIFI NOORI**

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# BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF FLUORESCENT PSEUDOMONAS FOR POTENTIAL OF PYRICULARIA ORYZAE

**By**

**MANSOUREH SADAT SHARIFI NOORI**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Doctor of Philosophy**

**February 2013**

**This thesis dedicated to:** All I love specially

To my father's soul, who encouraged me all the way long. I wish he was here to see his dream come true,

To my mother for her consistent love, help and support throughout all levels of my life and education,

To my beloved husband, Sasan, who made his own sacrifices to allow me the time needed to finish my study, I could not have done this work without him,

and last but not least, to my children,

Mohammad Reza,

Melika,

and Fatima

who have always been showing great patience during these years and had to spend most of the time without me...

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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**Chairman : Associate Professor Halimi Mohd Saud, PhD**

**Faculty : Agriculture**

The present study emphasizes on the isolation and characterization of strains of fluorescent *Pseudomonas* collected from rice plants obtained from different localities in Peninsular Malaysia. Genetic diversity of *Pseudomonas* isolates were determined using molecular markers, including 16S rRNA gene and Rep-PCR and the 16S ribosomal RNA gene sequences of selected plant growth-promoting rhizobacteria (TS3C4 isolate), and were analyzed for possible predictive gene/protein using molecular bioinformatic. Twenty strains employed in this study were initially isolated from rhizosphere of healthy rice plants from three states of Peninsular Malaysia (Penang, Malacca and Selangor).

The isolates were purified and subjected to identification based on morphological and biochemical characters. Through biochemical and morphological characterization, these isolates were tentatively identified up to generic level. Following API 20NE biochemical identification kit of the 20 isolates, 16 strains were identified as *Pseudomonas fluorescens*, two isolates belong to the species of the *P. luteola*, one isolate to the *P. putida* and a single isolate (TS3C4) showed a doubtful identification. It was observed that different strains of bacteria produced various concentration of indole acetic acid. Overall, out of twenty isolates nineteen of them were able to form zone of solubilization on the NBRIP medium. Based on the dual culture assay study, results clearly indicated the potential of nineteen isolates to inhibit the *Pyricularia oryzae*, the causal agent of rice blast disease, while isolate TS3A1 did not show inhibitory activity against this fungus. The results indicated the ability of all the isolates to produce both HCN and siderophores. In addition isolates such as DL26, DL17, TS11, and TS3A2 showed significantly higher production of siderophores compared to other isolates. All twenty isolates were tested for production of protease enzyme but only six isolates i.e. TS3C8, TS3B5, TS3C4, TS25, TS11, and reference strain NCIM 2099 showed protease activity. Results from the greenhouse study for biocontrol activity of selected plant growth promoting rhizobacteria indicated that out of four strains tested (TS3B5, TS3C4, DL22, and NCIM 2099 as reference strain) for their bio-control potential against *Pyricularia oryzae* in greenhouse, TS3B5, TS3C4 and NCIM 2099 exhibited 13.50% reduction in disease index whereas, isolate DL22 showed disease index reduction in the range of 16.88% relative to control. Out of the four promising strains, TS3C4 emerged as the best organism in plant growth promotion and was selected for sequence analysis of 16s

rRNA gene for prediction of useful gene/protein which may involve in plant growth promotion and biocontrol traits. Most of the isolates identified as *Pseuodomonas fluorescens* based on API 20NE kit, were clustered together (cluster B), while cluster A was divided into two sub-clusters which was identified as *Pseudomonas aeroginosa.* Cluster C contained two isolates characterized as *Pseudomonas luteola.* Surprisingly, the isolate TS3C4 showed doubtful identification by API 20NE kit, was placed in cluster D which was very distinct from the others. This spatial separation of isolate TS3C4 suggests that this strain might be another strain of rhizobacteria. The results of clustering of phylogeny tree with 16s rRNA gene sequencing showed that all the Malaysia isolates were classified in b1 subgroup, while similar strains from NCBI were placed in different group and sub-group. This research clearly demonstrated that *Pseudomonas* isolates originating from different geographic region in Peninsular Malaysia were phylogenetically similar to each other compared to similar strains from NCBI which were isolated from different country. In silico 16S rRNA gene sequence analysis of selected isolate TS3C4 was able to predict potential useful gene/protein from small open reading frames such as kinase, outer membrane ferric siderophore receptor, Chain X, 1.8a Crystal Structure of the Pa2412, protease, 3-oxoacyl-ACP synthase III and carboxyl-terminal protease proteins which involved in biological control activity when composed with protein BLAST data bank. Although some of these secondary metabolites were confirmed through biochemical characterization, these approaches must be accompanied with in vivo evidences for validation of its beneficial mechanisms and characteristics.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN BIOKIMIA DAN MOLEKUL *PSEUDOMONAS* BERPENDARFLOUR UNTUK POTENSI *PYRICULARIA ORYZAE***

Oleh

**MANSOUREH SADAT SHARIFI NOORI**

**Februari 2013**

**Pengerusi: Profesor Madya Halimi Mohd Saud, PhD**

**Fakulti: Pertanian**

Kajian ini memberi tumpuan kepada pengasingan dan pencirian *Pseudomonas* berpendafluor yang diasingkan dari tanaman padi dari beberapa lokasi di Semenanjung Malaysia. Kepelbagaian genetik asingan *Pseudomonas* ditentukan melalui penanda molekul, termasuklah gen 16S RNA dan 'rep-PCR' dan jujukan gen ribosom 16s rRNA dan asingan terpilih strain T53C4 telah dianalisis untuk meramal kehadiran gen/protein melalui kaedah bioinformatik molekul. Dua puluh (20) strain telah dipencil dari tanaman padi yang sihat dari tiga (3) negeri di Semenanjung Malaysia (Pulau Pinang, Melaka dan

Selangor). Pencilan ini ditulenkan dan pencirian dibuat berdasarkan ciri morfologi dan biokimia. Pencilan ini dikenalpasti ke paras generik berdasarkan morfologi dan ciri biokimia. Daripada 20 pencilan, 16 strain dikenalpasti sebagai *Pseudomonas* fluorescens, dua (2) sebagai *P. luteola*, satu (1) sebagai *P. putida* dan satu (1) pencilan (TS3C4) yang menunjukkan identiti yang meragukan dengan mengguna kit biokimia API 2ONE. Pemerhatian menunjukkan strain yang berlainan menghasilkan kepekatan IAA yang berlainan. Sembilan belas (19) dari dua puluh (20) pencilan mampu membentuk zon pelarutan dalam media NBRIP. Keputusan asai kultur dua (2) menunjukkan potensi sembilan belas (19) pencilan untuk merencat Pyricularia oryzae, agen penyebab penyakit karah padi manakala pencilan TS3A1 tidak menunjukkan sebarang aktiviti perencatan terhadap kulat ini. Keputusan ini menunjukkan keupayaan pencilan untuk menghasilkan HCN dan siderofor. Tambahan lagi pencilan seperti DL26, DL17, TS11 dan TS3A2 menunjukkan hasil siderofor yang lebih signifikan dan banyak berbanding pencilan yang lain. Kesemua dua puluh (20) pencilan diuji untuk penghasilan enzim protease tetapi hanya enam (6) pencilan – TS3C8, TS3B5, TS3C4, TS25, TS11 dan strain rujukan NCIM2099 menunjukkan aktiviti protease. Tiga (3) dari empat (4) pencilan yang diuji iaitu TS3B5, TS3C4 dan NCIM 2009 mengurangkan indeks penyakit sehingga 13.50% manakala pencilan DL22 mengurangkan indeks penyakit pada julat 16.88% berbanding kawalan. Daripada empat (4) pencilan tersebut, strain TS3C4 adalah yang terbaik dalam penggalakkan tumbesaran. Seterusnya TS3C4 dipilih untuk analisis jujukan gen rRNA 16s untuk meramal gen/protein berfaedah yang mungkin terlibat dalam penggalakkan tumbesaran dan ciri biokawalan. Sebahagian besar pencilan dikenalpasti dan dikelompokkan sebagai *Pseudomonas fluorescens* berasaskan

kit API 2ONE. Kelompok A dibahagikan kepada dua (2) sub-kelompok dikenalpasti sebagai *Pseudomonas aeuriginosa*. Kelompok C terdiri dari dua (2) pencilan Pseudomonas luteola. Manakala pencilan TS3C4 mempunyai identiti yang diragui, diletakkan dalam kelompok D yang terasing dari lain-lain kelompok. Penyisihan spatial TS3C4 memberi gambaran bahawa ia adalah strain shizobakteria yang berbeza. Keputusan pengkelompokan dari rajah filogeni jujukan gen rRNA 16s menunjukkan pencilan dari Malaysia ini adalah berbeza berbanding strain yang hampir sama dalam NCBI dan diletakkan dalam sub-kumpulan b1. Kajian ini dengan jelas menunjukkan bahawa pencilan *Pseudomonas* dari kawasan geografi yang berlainan di Semenanjung Malaysia adalah sama secara filogenetik berbanding strain yang hampir sama dari negara lain dalam pangkalan data NCBI. Analisis 'in-silico' jujukan gen rRNA 16s strain terpilih TS3C4 mampu meramal gen/protein yang berfaedah berasaskan ORF (Open Reading Frame) enzim kinase, penerima membran luar siderofor ferik, rantaian X, struktur hablur Pa2412, protease, 3-oksoasil-ACP sintase III dan protein karboksil-terminal yang terlibat dalam aktiviti biokawalan menggunakan kaedah perbandingan data bank dan perisian BLAST. Walaupun beberapa metabolit sekunder disahkan melalui ciri biokimia tetapi pendekatan ini perlu diikuti dengan pendekatan dan bukti 'in-vitro' untuk tujuan pengesahsahihan.

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**IN THE NAME OF ALLAH**

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I certify that a Thesis Examination Committee has met on September 2012 to conduct the final examination of Mansoureh Sadat Sharifi Noori on her thesis entitled "Biochemical and Molecular Charachterization of Fluorescent Pseudomonas Isolates to Control Rice Blast In Malaysia" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1988. The Committee recommends that the student be awarded the Doctor of Philosophy.

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This thesis was submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfillment for the degree of Doctor of Philosophy. The members of the Supervisory committee were as follows:

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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 or not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

**MANSOUREH SADAT SHARIFI NOORI**

Date:

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