UNIVERSITI PUTRA MALAYSIA

CHARACTERIZATION OF Streptomyces SP FROM OIL PALM RHIZOSPHERE FOR BIOLOGICAL CONTROL OF Ganoderma boninense

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

CHARACTERIZATION OF *Streptomyces* SP FROM OIL PALM RHIZOSPHERE FOR BIOLOGICAL CONTROL OF *Ganoderma boninense*

By
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The objective of this study was to isolate, screen and identify actinomycetes from the rhizosphere of oil palm for biological control of Basal Stem Rot (*Ganoderma boninense*), a very destructive disease of oil palm in Malaysia. Rhizosphere samples from top soils of healthy palms amongst the infected palm on laterite, coastal, inland and peat were used in this study. A total of 1050 of actinomycetes was isolated with 355 isolates (33.8 %) from laterite, 235 (23.38%) isolates from peat, 206 (19.62 %) isolates from coastal and 254 isolates (24.19 %) isolated from inland soil with the highest occurrence of actinomycetes was observed in peat area. About 600 isolates of *actinomycetes* with different morphological appearance and matched the spore-producing and fast growing criteria were tested for their inhibitory effects on *G. boninense* mycelia growth on dual culture plates.

From 600 isolates screened, 13.5% of the isolates showed potential antagonistic activity with more than 80% of Percent Inhibition of Radial Growth (PIRG). Out of this number, 21 isolates showed highest PIRG with observable abnormal growth of *G. boninense*. The isolates were further tested for their culture filtrates in the form of liquid and powder (freeze-dried) for effect towards G. *boninense* growth. Culture filtrate from six isolates, in both forms showed inhibition towards *G. boninense* with intermediate PIRG value (50% to 80%) with similar inhibition rate observed for the standard antifungal agent (Nystatin) at 0.1 mg/ml and 1 mg/ml. Screening of the culture filtrate on *Ganoderma* plate showed halo zone with diameter 12.5 – 23.5 mm compared to nystatin at 30 mg/ml and 50 mg/ml from four isolates. Lower inhibition activity obtained might be affected by the concentration of bioactive compound available in the supernatant. Four isolates; AGA 043, AGA048, AGA347 and AGA 506 were highlighted for their ability to inhibit and exhibit potential metabolites against *G. boninense*. Based on their morphological and cultural characteristic, the potential actinomycetes were identified as a member of *Streptomyces* genus. Analysis of a partial 16S rRNA gene sequence (512 bp) of AGA043, AGA048, AGA347 and AGA506 exhibited

a very high level of 16S rDNA sequence similarity (>98%) with sequence related to different *Streptomyces* species deposited to the database. However the 16S ribosomal DNA amplification of AGA 043 and AGA048 were only able to be identified up to genus level. The 16S ribosomal DNA amplification of AGA347 and AGA506 revealed that the isolates are closely related to *Streptomyces hygroscopicus* subsp. *hygroscopicus* and *Streptomyces ahygroscopicus* with 99% of similarity percentage

All of the *Streptomyces* isolates were formulated in vermiculite to evaluate their ability to reduce disease incidence in oil palm seedlings. Pre-inoculating the seedlings with AGA347 (T3) showed the most effective in suppressing BSR based on 73.11 % of disease reduction compared to standard antifungal producer, *Streptomyces nourseii* (T5), AGA043 (T1), AGA048 (T2) and AGA506 (T4) with 47.41 %, 30.11 %, 54.83 % and 44.08 % respectively.

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

PENCIRIAN *Streptomyces* sp. DARI RIZOSFERA SAWIT SEBAGAI KAWALAN BIOLOGI *Ganoderma boninense*

Oleh
SHARIFFAH MUZAIMAH BT SYED ARIPIN @ ISA
JULAI 2013

Pengerusi : Prof. Dzolkhifli Omar, PhD
Fakulti : Pertanian

Objektif bagi kajian ini adalah untuk memencil, menguji serta mengenalpasti aktinomiset daripada rizosfera sawit bagi tujuan kawalan biologi penyakit reput pangkal batang (*Ganoderma boninense*), suatu penyakit yang merosakkan sawit di Malaysia. Sampel rizosfera dari pokok sihat yang dikelilingi pokok yang telah dijangkiti dari kawasan laterit, *coastal, inland* serta gambut telah digunakan dalam kajian ini. Sejumlah 1050 aktinomiset telah dipencilkan dengan 355 pencilan (33.8%) dari kawasan laterit, 235 (23.38%) daripada kawasan gambut, 206 (19,62%) dari kawasan 'coastal' dan 254 (24,19%) dari kawasan 'inland' dengan kehadiran aktinomiset yang lebih tinggi direkodkan di kawasan tanah gambut. Sejumlah 600 pencilan aktinomiset yang menunjukan morfologi berbeza serta berupaya untuk cepat tumbuh dan menghasilkan spora matang telah disaring untuk menguji kesan perencatan kepada pertumbuhan miselia *G. boninense* di atas piring *dual culture.*

13.5 % daripada 600 pencilan yang disaring telah menunjukan potensi dalam aktiviti antagonistic dengan peratusan perencatan pertumbuhan (PIRG) melebihi 80%. 21 pencilan menunjukan PIRG tertinggi dengan pertumbuhan *G. boninense* yang tidak normal. Pencilan-pencilan ini seterusnya diuji bagi mengukur keupayaan perencatan terhadap pertumbuhan *G. boninense* menggunakan kultur tapisan didalam bentuk cecair dan serbuk daripada kultur tapisan yang telah dibeku-kering (freeze-dried). Kultur tapisan oleh enam pencilan, menunjukkan perencatan terhadap *G. boninense* dengan peratusan perencatan diantara 50% hingga 80% didalam kedua-dua bentuk. Nilai perencatan yang lebih rendah yang diperolehi ini, adalah berkemungkinan disebabkan oleh kepekatan bahan bioaktif yang terdapat didalam kultur tapisan. Bagaimanapun, nilai ini adalah setara dengan nilai yang diperolehi dari serbuk antikulat, Nystatin yang diuji terhadap *G. boninense* pada 0.1 mg/ml dan 1 mg/ml. Saringan keatas piring yang mengandungi kultur *Ganoderma* menunjukan zon '*halo*' dengan diameter antara 12.5 hingga 23.5 mm dengan nilai setara diperolehi dari penggunaan nystatin pada kepekatan 30 mg/ml dan 50 mg/ml.

Empat pencilan AGA 043, AGA048, AGA347 dan AGA 506 telah di pilih berdasarkan kebolehan untuk merencat serta menghasilkan bahan bioaktif berpotensi terhadap *G. boninense*. Berdasarkan ciri-ciri morfologi, kesemua pencilan ini dapat

dikelaskan kedalam kumpulan dengan genus *Streptomyces*. Analisis jujukan 16S rRNA (512 bp) menunjukan AGA043, AGA048, AGA347 and AGA506 memberikan nilai kesamaan yang tinggi (>98%) dengan jujukan 16S rDNA yang terdapat dalam pangkalan data. Bagaimanapun, amplifikasi DNA ribosom 16S bagi AGA 043, AGA048 hanya dapat mencirikan kedua pencilan ini sehingga ke peringat genus. Manakala, amplifikasi amplifikasi DNA ribosom 16S bagi AGA347 dan AGA 506 telah *Streptomyces hygroscopicus* subsp. *Hygroscopicus* and *Streptomyces ahygroscopicus* (dengan nilai kesamaan ~99 %).

Berdasarkan potensi aktiviti antagonistik terhadap *Ganoderma* secara *in vitro*, empat pencilan *Streptomyces* telah dipilih bagi menguji keberkesanan setiap satunya dalam mengawal reput pangkal batang (BSR) ke atas anak benih kelapa sawit di tapak semaian. Semua pencilan dirumuskan dalam vermikulit. Pra-inokulasi benih dengan AGA347 (T3) menunjukkan kebolehan dalam mengurangkan kadar insiden penyakit BSR berdasarkan 73. 11% nilai pengurangan penyakit berbanding *Streptomyces nourseii* (T5), AGA043 (T1), AGA048 (T2) dan AGA506 (T4) dengan 47. 41%, 30. 11%, 54. 83% dan 44. 08% setiap satu.

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I certify that.....

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

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