De Novo SEQUENCING OF A BACTERIUM CAPABLE OF INHIBITING THE GROWTH OF *Ganoderma boninense*

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ABSTRACT

De Novo SEQUENCING OF A BACTERIUM CAPABLE OF INHIBITING THE GROWTH OF *Ganoderma boninense*

Eleven rhizobacteria and fifteen root endophytic bacteria were isolated from oil palm plantation site located at the school of sustainable agriculture, UMS Sandakan Campus, Sabah. Dual culture test was carried out on PDA media to select bacteria isolate with antagonistic character against *Ganoderma* sp. BRIUMSa. The bacteria isolates were characterized based on morphological features and identified through 16s rRNA gene sequencing and Gen-III Biolog MicroStation™ automated identification system. Results revealed degrees of inhibition toward mycelial growth of *Ganoderma* sp. BRIUMSa vary accordingly to the bacterial isolates. Amongst the eleven rhizospheric bacterial isolates, only seven bacterial isolates have showed positive inhibitory effect against *Ganoderma* sp. BRIUMSa *in vitro.* However, only three bacterial isolates from the genera *Pseudomonas* and *Bacillus* have showed percentage inhibitions of radial growth (PIRG) ranging from 30% to nearly 60% in the dual culture test. They were *Pseudomonas aeruginosa* (*Pseudomonas* sp. SPLUMS-1) (PIRG=59.0%), *Pseudomonas tolaasii* (PIRG=48.7%) and *Bacillus subtilis* (PIRG=33.4%). Nevertheless, isolate *B. subtilis* which initially shown to inhibit mycelial growth of *Ganoderma* sp. BRIUMSa was eventually overlay by the fungus after 10 days of post inoculation suggesting the inhibitory effect of *Bacillus* sp. is only temporally. On the other hand, only one root endophytic bacterial isolate *Burkholderia cepacia* (*Burkholderia* sp. SPLUMS-2) has showed potential inhibitory effect against *Ganoderma* sp. BRIUMSa with its PIRG recorded was close to 50% (48.1%). Bacterial isolates *Pseudomonas* sp. SPLUMS-1, *Burkholderia* sp. SPLUMS-2 and *Pseudomonas tolaasii* were further evaluated using agar amendment test and culture filtrate test. These were done by aseptically incorporating overnight bacterial culture or sterile-filtered culture filtrate into the PDA media. The percentage of inhibition (PI) value of *Pseudomonas* sp. SPLUMS-1 against *Ganoderma* sp. BRIUMSa was nearly 100% for both tests. Meanwhile, there was significant different ($P<0.05$) of PI value between agar amendment (20.14%) and culture filtrate (89.0%) tests for *Burkholderia* sp. SPLUMS-2; and 95.6% and 52.3%, respectively for *P. tolaasii.* Disease severity index for seedlings pre-treatment with *P. aeruginosa*, *B. cepacia* and *P. tolaasii* were 15%, 20% and 25% respectively, while seedlings post-treatment with the same bacteria were 20%, 25% and 25%, respectively in comparison to *Ganoderma* sp. inoculated seedlings (50%).

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ABSTRAK

Sebelas rhizobakteria dan lima belas bakteria endofitik akar telah dipencilkan daripada tapak ladang kelapa sawit yang terletak di sekolah pertanian lestari, UMS Kampus Sandakan, Sabah. Ujian dwi kultur telah dijalankan di atas media PDA untuk memilih isolat bakteria dengan watak antagonistik terhadap *Ganoderma* sp. BRIUMSa. Isolat bakteria telah disifatkan berasaskan kepada ciri-ciri morfologi dan dikenal pasti melalui penjujukan 16s rRNA gen dan sistem pengenalan automatik Gen-III Biolog MicroStation™. Hasil kajian menunjukkan darjah perencatan terhadap pertumbuhan miselia *Ganoderma* sp. BRIUMSa berbeza mengikut isolat bakteria. Di antara sebelas isolat rhizobacteria, hanya tujuh pencilan bakteria telah menunjukkan kesan perencatan yang positif terhadap *Ganoderma* sp. BRIUMSa *in vitro*. Walau bagaimanapun, hanya tiga pencilan bakteria dari genus *Pseudomonas* dan *Bacillus* telah menunjukkan peratusan perencatan radius pertumbuhan (PPRP) yang melingkungi 30% hingga 60% dalam ujian dwi kultur. Mereka ialah *Pseudomonas aeruginosa* (*Pseudomonas* sp. SPLUMS-1) (PPRP=59.0%), *Pseudomonas tolaasii* (PPRP=48.7%) dan *Bacillus subtilis* (PPRP=33.4%). Walau bagaimanapun, isolat *B. subtilis* yang pada awalnya menunjukkan perencatan terhadap pertumbuhan miselia *Ganoderma* sp. BRIUMSa akhirnya telah ditindih oleh kulat tersebut selepas dieramkan selama 10 hari mencadangkan kesan perencat *Bacillus* sp. adalah seketika sahaja. Sebaliknya, hanya satu isolat bakteria endofitik akar *Burkholderia cepacia* (*Burkholderia* sp. SPLUMS-2) telah menunjukkan kesan perencat yang potensi terhadap *Ganoderma* sp. BRIUMSa dengan catatan PPRP menghampiri 50% (48.1%). Ujian selanjutnya ke atas isolat bakteria *Pseudomonas* sp. SPLUMS-1, *Burkholderia* sp. SPLUMS-2 dan *Pseudomonas tolaasii* telah dinilai melalui ujian pindaan agar dan ujian turasan kultur. Ini dilakukan dengan mencampurkan kultur bakteria yang telah dieramkan semalaman atau turasan kultur yang steril-ditapis ke dalam media PDA secara aseptik. Nilai peratusan perencatan (PP) *Pseudomonas* sp. SPLUMS-1 terhadap *Ganoderma* sp. BRIUMSa adalah menghampiri 100% untuk kedua-dua ujian. Sementara itu, terdapat perbezaan yang bererti ($P < 0.05$) daripada nilai PP di antara ujian pindaan agar (20.14%) dan ujian turasan kultur (89.0%) bagi *Burkholderia* sp. SPLUMS-2; dan 95.6% dan 52.3% masing-masing bagi *P. tolaasii*. Indeks keparahan penyakit untuk anak benih pra-rawatan dengan *P. aeruginosa*, *B. cepacia* dan *P. tolaasii* adalah 15%, 20% dan 25% masing-masing, manakala anak benih pasca-rawatan dengan bakteria yang sama adalah 20%, 25% dan 25% masing-masing berbanding dengan anak benih yang diinokulasi dengan *Ganoderma* sp.(50%).

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