UNIVERSITI TEKNOLOGI MARA

MOLECULAR RELATIONSHIP AND BIOSYNTHETIC POTENTIAL OF ENDOPHYTIC BACTERIA

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ABSTRACT

Bacilli are well known for producing antimicrobial products. From 1000 endophytic *Bacillus* spp. that were isolated from tissues of plants collected in every part of the Malaysian Peninsula by collaborators from AIMST University, Malaysia, 83 of them identified as *Bacillus altudinis, B. amyloliquefaciens* or *B. thuringiensis* were selected for this study. The study was divided into two parts, i.e. a computational part (bioinformatics) and a wet laboratory part. The aim of the bioinformatic part was to analyse any possible evolutionary relationships of the selected bacilli by building a phylogenetic tree for the readily available 16S rRNA sequences. The objectives of the wet laboratory part were to find suitable media to enhance secondary metabolite production in bacilli, to isolate any promising compound, and to screen for antimicrobial properties of the extracts and metabolites. The 16S rRNA sequences of the selected bacilli were aligned in the MEGA5.0 software and a phylogenetic tree was built by Neighbour-Joining method. From the tree, there was no detectable genetic difference between the isolates of the same species, although they were isolated from different geographical regions as well from different host plants. The selected bacilli were screened for metabolites production in the presence of stress factors, including $FeSO_4$, $ZnSO_4$, and $CuSO_4$, glycerol, DMSO, glucose, arabinose and sucrose. The nutrient medium at different strengths was also used as one of the stress factors. After being incubated, the cultures were extracted with ethyl acetate and the resulting extracts profiled by RP-HPLC. The combination of half-strength medium and addition of DMSO significantly enhanced the metabolite production for three *Bacillus* spp. The extracts showing most promising metabolite profiles were selected to undergo semi–preparative HPLC. From the collected fractions, one compound from the *B. thuringiensis* extract was in sufficient amount for full NMR and MS-TOF analyses. It was identified as 3-methyl-*N*-(2-phenylethyl)–butyramide. This compound is known as an anti-quorum sensing agent and thus prevents bacteria from forming biofilms. However, it did not reduce significantly the viability of the test microbes. An antimicrobial susceptibility test was carried out for the collected fractions on ATCC strains, including *E. coli*, *S. aureus*, and *C. albicans*. Only one fraction, F5 from *B. altitudinis* shows inhibition towards test microbes. However, its low amount did not allow identification of the active compound.

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