PROJECT REPORT

Project title: Isolation of actinomycetes from Sabah and the screening for inhibitor against eukaryotic signal transduction

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Synopsis

Actinomycetes strains isolated from 504 soil samples collected from Sabah terrestrial ecosystem were studied and screened for novel bioactive compounds inhibitory against eukaryotic signal transduction. All the soil samples were collected under trees identified to species or genus level. Isolation of *Streptomyces* and non-*Streptomyces* actinomycetes on HV (humic acid + B-vitamins) medium yielded 569 strains. Morphology characterisation of the isolated actinomycetes was carried out including aerial mycelium, substrate colour, diffusible pigment and spore morphology on oatmeal medium while chemotaxonomic identification based on Diaminopimelic Acid. All actinomycetes strains were grown under aerobic condition in liquid culture and extracted with acetone. In this research, yeast MAPK kinase and MAP kinase phosphatase are the molecular level targeted proteins. The screening system was developed for searching MAPK kinase and MAP kinase phosphatase inhibitors. $MKK1^{P386}$ and $MKK1^{P386}$-MSG5 mutant yeast were used to screen for inhibitors, as these yeast kinase and phosphatase have homologous proteins in the MAP kinase signal transduction pathway in human. Strain H7553 and H7597 showed potential MAPK kinase inhibitors. The *in vivo* Ras/Raf interaction with the yeast two hybrid screening system was used to screen against Ras/Raf protein interaction inhibitor. Strain H7520 showed potential inhibitor for yeast Type 1 protein serine/threonine phosphatase (GLC7) screening system. Extract H7944 showed inhibition effect in the ERK signal transduction (the chain-reaction of phosphorylation from MEK1/2 to ERK1/2) when inhibited phosphorylation of MEK to ERK. Thus, strain H7944 (MBA94-2) was able to prevent activation of MEK. Strain H7944 was a potential MEK 1/2 inhibitor since β-galactosidase assay confirmed that H7944 do not inhibited the Ras/Raf pathway.

Aktinomiset dipencilkan daripada 504 sampel tanah dikutip dari ekosistem Sabah dikaji dan penyaringan compaun bioaktif terhadap perencatan transduksi isyarat eukariot. Semua sampel tanah dikutip di bawah pokok yang dikenalpasti sehingga peringkat spesies atau genus. Pemencilan aktimomiset *Streptomyces* dan bukan *Streptomyces* menggunakan media asid humik-vitamin (HV) agar berjaya memencilkan sebanyak 569 strain aknomiset. Pencirian morfologi strain aktinomiset yang dipencilkan dilakukan melalui pencirian warna aerial miselium, warna substrak dan penyebaran warna pigment ke atas media agar oatmeal manakala pencirian kimiataksonomi dilakukan melalui teknik isomer asid diaminopimelik. Semua strain aktinomiset dikulturkan secara aerobik dalam media cecair dan diekstrak dengan menggunakan aseton. Dalam kajian ini, MAPK kinase dan MAP kinase fosfotase adalah sasaran protein molekular dalam yis. Sistem penyaringan dibangunkan untuk mencari perencat untuk MAPK kinase dan MAP kinase fosfotase. Yis mutant, $MKK1^{P386}$ dan $MKK1^{P386}$-MSG5 digunakan untuk tujuan penyaringan disebabkan kinase dan fosfotase yis mempunyai persamaan dengan protein dalam transduksi isyarat MAP kinase dalam manusia. Strain H7553 dan H7597 menunjukkan potensi perencat untuk MAPK kinase. Sistem penyaringan *in vivo* interakasi Ras/Raf dalam yis dual-hybrid digunakan untuk menyaring perencat protein interakasi Ras/Raf. Ekstrak daripada aktinomiset H7520 menunjukkan potensi perencat untuk protein serine/threonine fosfotase (GLC7) dalam yis dalam penyaringan yang dijalankan. Ekstrak H7944 menunjukkan kesan perencatan dalam isyarat transduksi apabila ia merencat fosforilasi terhadap MEK daripada ERK. Ini bermakna H7944 berupaya mencegah pengaktifan MEK. Strain H7944 mempunyai potensi menjadi perencat MEK 1/2 kerana kajian β-galaktosidase yang dijalankan menunjukkan ia tidak merencat kitaran Ras/Raf