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May happiness be a crown your wear for all days.

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ABSTRACT

Actinobacteria are well known for their production of an extensive array of chemically diverse and medically important secondary metabolites. Fifty two strains of actinobacteria used in this study were divided into 20 colour groups based on their aerial and substrate mycelium colour as well as diffusible pigment production on Yeast Extract Malt Extract (ISP) medium. Antifungal activity was determined against *Candida albicans*, *Candida parapsilosis*, *Fusarium oxysporum*, *Ganoderma boninense*, *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* by the agar plug-diffusion method. Ten of the fifty two actinobacterial strains showed strong antifungal activity. Two strains (MA04119 and RHRS) were active against *S. pombe*, four strains (9, A21, OPSa and RC3) against *S. cerevisiae*, two strains (OPSa and A21) against *C. albicans*, one strain (MA04020) against *C. parapsilosis*, one strain (H23) against *F. oxysporum* and two strains (9 and PDA4) against *G. boninense*. The identification of actinobacterial strains were done by using molecular approaches including enterobacterial repetitive intergenic consensus (ERIC)-PCR and 16S rRNA gene sequencing. By using the (ERIC)-PCR fingerprinting technique, all the tested strains have been de-replicated into three groups. The ten actinobacterial strains which showed antifungal activity were also screened for the presence of polyene-specific CYP genes and only four of them (strains H23, RC3, MA04020, and RHRS) showed the presence of the expected 350bp DNA fragment of the polyene CYP internal region. This suggests that these four strains might produce the cryptic polyene biosynthetic compound. 16S rRNA gene sequence analysis showed that nine of the strains, (H23, RHRS, MA04119, MA04020, OPSa, RC3, PDA4, A12 and OPSa) belong to the genus *Streptomyces*, while strain 9 was identified to be a *Brevibacterium* sp.

ABSTRAK

Aktinobakteria berkeupayaan untuk menghasilkan metabolit sekunder yang pelbagai dan mempunyai keupayaan dari segi perubatan. Lima puluh dua strain aktinobakteria yang dikaji dalam eksperimen ini telah dikategorikan kepada 20 kumpulan, berdasarkan warna miselium dan penghasilan pigmen pada media Yeast Extract Malt Extract (ISP). Aktiviti antikulat telah dikenalpasti ke atas *Candida albicans*, *Candida parapsilosis*, *Fusarium oxysporum*, *Ganoderma boninense*, *Schizosaccharomyces pombe* and S*accharomyces cerevisiae* dengan menggunakan kaedah penyerapan agar. Daripada lima puluh dua strain yang dikaji ini, sepuluh daripadanya telah menunjukkan aktiviti antikulat yang kuat. Dua strain (MA04119 dan RHRS) adalah aktif ke atas *S. pombe*, empat strain (9, A21, OPSa dan RC3) ke atas *S. cerevisiae*, dua strain (OPSa dan RHRS) ke atas *C. albicans*, satu strain (MA04020) ke atas *C. parapsilosis*, satu strain (H23) ke atas *F. oxysporum* dan dua strain (9 dan PDA4) ke atas *G. boninense*. Pengenalpastian strain aktinobakteria telah dilakukan dengan menggunakan teknik molekul termasuk *enterobacterial repetitive intergenic consensus* (ERIC)-PCR dan amplifikasi PCR ke atas gen 16S rRNA. Melalui teknik amplifikasi (ERIC)-PCR, semua strain actinobacteria yang diuji telah direplikasikan kepada tiga kumpulan. Kehadiran gen CYP yang spesifik kepada polyene turut dikaji ke atas sepuluh strain tersebut. Daripada strain-strain tersebut, empat daripadanya (strain H23, RC3, MA04020, dan RHRS) menunjukkan kehadiran fragmen DNA bersaiz 350 pasang bes seperti yang dijangka. Ini menunjukkan bahawa keempat-empat strain tersebut kemungkinan menghasilkan sebatian biosintetik polyene. Analisis turutan gen 16S rRNA menunjukkan bahawa sembilan strain (H23, RHRS, MA04119, MA04020, OPSa, RC3, PDA4, A12 dan OPSa) tergolong dalam genus *Streptomyces*, manakala strain 9 dikenalipasti sebagai *Bevibacterium* sp.

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List of abbreviations

%	percentage
sp.	species
et al.	et alia (~and others)
μm	micrometer
mm	millimeter
rRNA	Ribosomal Ribonucleic Acid
PCR	Polymerase Chain Reaction
bp	base pair
$NaHCO_3$	Sodium bicarbonate
EDTA	Ethylene diaminetetraacetic acid
μl	microliter
TBE	Tris/Borate/EDTA
UV	Ultraviolet
dNTPs	Deoxyribonucleotide triphosphate
ISP	International Streptomyces Project
rpm	revolutions per minute
w/v	weight per volume
mM	millimolar
ºC	degree Celcius
ml	milliliter
μg	microgram
$MgCl_2$	Magnesium chloride
v/v	volume per volume
V	Voltan

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