

## RESEARCH NOTE

### Isolation and characterization of *Streptomyces* spp. from soils

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**Abstract.** The isolation of forty-eight strains of actinomycetes from eight soil samples was done with the conventional dilution plate method on Humic acid-vitamin agar (HVA), Starch Casein agar and Sorenson's agar. HVA was found to be the best isolation medium as more strains of actinomycete could be isolated. Based on the aerial mycelium colour scheme, three colour groups were recognised. Representative isolates from each colour group were subjected to macrorestriction analysis with the pulsed-field gel electrophoresis (PFGE). The different coloured strains exhibited low levels of similarity (Dice coefficient,  $F < 0.50$ ). Acetone extracts of the isolates were screened on mutant yeasts for Mitogen-Activated Protein Kinase (MAPK) Kinase and MAPK Phosphatase inhibitor to find an indirect inhibitor of *ras*. However, no inhibitor was found but seven isolates exhibited toxic effect towards the yeasts. PFGE analysis of these isolates showed that two of isolates were identical. From the phenotypic and genotypic characteristics, the two isolates were shown to be of the same strain.

**Key Words:** morphology, PFGE, soil isolation, *Streptomyces*

Actinomycetes are a phylogenetically defined, metabolically active group of Gram-positive bacteria with DNA rich in G+C (>55 mol %). Actinomycetes, which contain more than 50 genera, are prolific producers of bioactive compounds. Antibiotics such as streptomycin, erythromycin, and vancomycin are produced by members of the genera *Streptomyces*, *Sacharopolyspora* and *Amycolatopsis*, respectively. Proper and accurate classification and identification of actinomycetes are of biotechnological and medical importance. Knowledge of the physiological and cultural properties of actinomycetes is essential for their classification. The objective of this work was to characterize the actinomycetes isolated from soil on the basis of morphological and biochemical properties as well as by pulsed-field gel electrophoresis (PFGE).

Soil samples were collected from 8 sites in Rimba Ilmu, University of Malaya and in Gombak, Kuala Lumpur. A total of 48 actinomycete strains were isolated by the dilution plate method on Humic acid-Vitamin agar, Starch-Casein agar and Sorenson's agar supplemented with cycloheximide and Penicillin G (Labeda and Shearer, 1990). Colouration of the aerial mycelium, substrate mycelium and diffusible

pigment on Oatmeal agar were observed under normal daylight after 14-day-incubation at 28°C. The Methuen Handbook of Colour (Kornep and Wansher, 1963) was used as a standard colour guide. Micromorphology of sporospores was determined by employing the cover slip method (Lechevalier and Lechevalier, 1981).

Submerged fermentation of these isolates were carried out on 2% Mannitol, 2% soybean flour, pH7.2 for 5 days at 28°C, 220rpm (Labeda and Shearer, 1990). Colouration of the culture broth and mycelium were noted. Equal volume of acetone was added to extract secondary metabolites. These extracts were then tested for inhibitory activity against MAPK Kinase and MAPK Phosphatase in mutated yeast strains using the agar diffusion method on glucose and galactose plates.

Representative strains of the colour groupings and seven isolates from the grey colour group were subjected to PFGE analysis. Preparation of DNA for

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