PRODUCTION OF POTENT BIOACTIVE COMPOUNDS BY CERTAIN *Streptomyces* SPECIES

BY

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2004
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APPROVAL SHEET

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Title of Thesis: Production Of Potent Bioactive Compounds By Certain *Streptomyces* Species

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ABSTRACT

Screening, isolation and characterization are still the powerful strategies for searching about potent bioactive compounds of biotechnological importance. Accordingly, from twenty different soil samples collected from different area, 500 actinomycete isolates were recovered and screened for their ability to produce antimicrobial metabolite(s). Only 56 exhibited antimicrobial activities against the tested microorganisms. On the basis of antimicrobial activity, 10 promising isolates that exhibited antagonistic activity against at least one of each of Gram negative, Gram positive and/or filamentous fungi in addition to Candida were selected for further studies. Based on the morphological, cultural, physiological, biochemical and chemotaxonomical characteristics, the numerical identification using the UPGMA program, confirmed that the 10 active strains of *Streptomyces* were assigned to three hierarchical clusters: first cluster consists of 7 strains, 2nd cluster consists of one strain and the 3rd cluster consists of two strains. The most potent strains FHM275 and FHM572 exhibited strong inhibitory effects against the tested multi-drug resistant nosocomial strains Ethyl acetate: chloroform (1:1) proved to be the best solvent system for extracting their bioactive compound(s) from the cultural filtrates. MIC values of crude bioactive compound(s) produced by FHM275 and FHM572 against the tested microorganisms were generally lower than those obtained by reference antibiotics which indicated their antimicrobial potencies. The bioactive crude extracts from the most potent *Streptomycetes* FHM275 and FHM572 showed cytotoxic activities against colon cell line HCT116 and lung cell line A549 but crude extract from FHM572 was more effective against lung cell line A549 than that extracted from FHM275. Phylogenetic analysis of the FHM275 and FHM572 strains based on 16S rRNA gene sequences showed highest similarity (99%) with *Streptomyces fulvissmus* and *St. pratensis* respectively; they were deposited in the GenBank under the accession numbers of KM438035 and KM046933 respectively. The optimum conditions for maximal productivity of bioactive metabolite(s) by *St. fulvissmus* FHM275and *St. pratensis* FHM572 were as follow: 20% v/v loading volume at pH ranged from 7.0 to 7.5, 2% or 1% v/v of 5 or 7- day old spores suspension as inoculum size respectively, incubation at 30°C or 28°C respectively and agitation speed rate of 150 or 180 rpm respectively for 5 days. The highest productivity of bioactive compound(s) was achieved at g/l: starch, 20; KNO_3, 2.0; (C/N ratio= 28.30) and K_2HPO_4, 1.0; $CaCO_3$, 3.0; $MgSO_4.7H_2O$, 0.5; NaCl, 0.5 and $FeSO_4.7H_2O$, 0.01. Using the silica gel column chromatography, the crude compound(s) by FHM275 and FHM572 were purified into three or two fractions, respectively. Fraction F2 from FHM275 and fraction FH2 from FHM572 were the major and the most potent against tested microorganisms including nosocomial pathogens. F2 which obtained from the strain FHM275 did not show any damage to the normal cell line up to 100 µg/ml and showed strong activity and highest selectivity against lung carcinoma (lC_{90} up to 6.25 µg/ml), good activity against colon cancer (lC_{50} of 21. 4 µg/ml). FH2 killed 90% of lung, 50% of breast and colon at 6.25, 11.3 and 14.4 µg/ml, respectively. F2 and FH2 were identified as methyl nonactate or methyl homononactate respectively using spectroscopic techniques (MS, UV, IR, ^1H and ^{13}C NMR).

Key words: *Streptomyces* spp., Antimicrobial activity, Cytotoxic activity, Methyl nonactate, Methyl homononactate, Numerical taxonomy, 16S rRNA, Structure elucidation.

DEDICATION

This Thesis is dedicated to my dear parents: To my father, for his help, patience, continuous encouragement and support; to my mother, for her deep prayers, continuous care and love.

Also I dedicate this work to my dear brothers and beloved twin sister for their love and help.

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