شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

HANAA ALY

شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

HANAA ALY

MARINE *STREPTOMYCETES* AS A SOURCE OF BIOACTIVE COMPOUNDS AGAINST PATHOGENIC BACTERIA: AN EVOLUTIONARY VIEW OF ANTIBIOTIC RESISTANCE

By

AYATOLLAH SAMIR ABDEL-MONIEM EL-ZAYAT

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2009

M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2015

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Agricultural Microbiology)

Department of Agricultural Microbiology
Faculty of Agriculture
Cairo University
EGYPT

2021

Format Reviewer **Vice Dean Graduate Studies**

APPROVAL SHEET

MARINE *STREPTOMYCETES* AS A SOURCE OF BIOACTIVE COMPOUNDS AGAINST PATHOGENIC BACTERIA: AN EVOLUTIONARY VIEW OF ANTIBIOTIC RESISTANCE

Ph.D. Thesis
In
Agric. Sci. (Agricultural Microbiology)

By

AYATOLLAH SAMIR ABDEL-MONIEM EL-ZAYAT

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2009
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2015

APPROVAL COMMITEE

Dr. FATMA IBRAHIM EL-ZAMIK.....
Professor of Microbiology, Fac. Agric., Zagazig University

DR. NABIL ABRAHIM ELSAYED HEGAZI.....
Professor of Microbiology, Fac. Agric., Cairo University

Dr. HAYAM MOHAMED FATHY.....
Associate Professor of Microbiology, Fac. Agric., Cairo University

Dr. FERIAL MOHAMED RASHAD.....
Professor of Microbiology, Fac. Agric., Cairo University

Date: 29/6/2021

SUPERVISION SHEET

MARINE *STREPTOMYCETES* AS A SOURCE OF BIOACTIVE COMPOUNDS AGAINST PATHOGENIC BACTERIA: AN EVOLUTIONARY VIEW OF ANTIBIOTIC RESISTANCE

Ph.D. Thesis
In
Agric. Sci. (Agricultural Microbiology)

By

AYATOLLAH SAMIR ABDEL-MONIEM EL-ZAYAT

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2009
M.Sc. Agric. Sci. (Agric. Microbiology), Fac. Agric., Cairo Univ., 2015

SUPERVISION COMMITEE

Dr. FERIAL MOHAMED RASHAD
Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. HAYAM MOHAMED FATHY
Associate Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. MOHAMED ABDALLAH MOSELHY BESHR
Associate Professor of Agric. Microbiology, Fac. Agric., Cairo University

Dr. SAM BROWN
Associate Professor of Evolutionary Microbiology, school of Biol., Georgia Tech

Name of Candidate: Ayatollah Samir Abdel-Moniem El-zayat **Degree:** Ph.D.
Title of Thesis: Marine *Streptomycetes* as a Source of Bioactive Compounds Against Pathogenic Bacteria: An Evolutionary View of Antibiotic Resistance
Supervisors: Dr. Ferial Mohamed Rashad
Dr. Hayam Mohamed Fathy
Dr. Mohamed Abdallah Moselhy Beshr
Dr. Sam Brown
Department: Agricultural Microbiology **Approval:** 29 / 6 / 2021

ABSTRACT

In response to the challenge and crisis of prevalence of multi-drug resistant pathogens, the major targets of the present study adopted two strategies and conducted in two parts. The major target of the first part was geared to explore a new bioactive metabolite from marine *Streptomyces* 10SAE while the other to steer and slow the evolutionary path of resistance using antibiotic adjuvant. The productivity and potency of the synthesized biomolecule(s) found to be medium and microorganism dependent. The optimum conditions for maximal productivity of bioactive metabolite using starch casein nitrate broth (SCNB) as reference medium simultaneously with shrimp shell (SS) as a monocomponent medium were as follow: 20% v/v loading volume, 2 or 3% v/v of 3-days old spores' suspension as inoculum size, agitation speed 180 rpm, initial pH 6.5-7.5 at 30 - 35°C for 5 days. The highest productivity of bioactive compound(s) was achieved at gl^{-1}: starch, 10; KNO_3, 2.0; casein, 0.3; K_2HPO_4, 2.5; $MgSO_4.7H_2O$, 0.5; $FeSO_4.7H_2O$, 0.01; NaCl, 5.0 and $CaCO_3$, 0.02. SS at 5.0 gl^{-1} proved its significance as a cheap suitable medium be better than or at least on par with SCNB. Antibacterial activity of the petroleum ether extract was more potent than crude filtrate. Minimum inhibition concentration (MIC) values of extract were generally lower than those obtained by reference antibiotics and minimum bactericidal concentration were 1- 4 folds of MIC values. The extract did not show any cytotoxic activity either against human tumor or normal cells up to 100 µg ml^{-1}. All the separated purified fractions using the silica gel column chromatography gave single separated band with similar retention factor (R_f) value of 0.90 on TLC. Based on spectroscopic analyses, the purified bioactive compound was identified according to Scifinder as a novel phthalate derivative, bis (2- ethyl hexadecyl) phthalate with a molecular formula of $C_{44}H_{78}O_4$. Evolution studies revealed that the ancestral *Pseudomonas aeruginosa* PAO1 and PA14 are susceptible to piperacillin (P) alone at 6.25 µg ml^{-1} (MIC); MIC dose led to the rapid evolution of resistance in treatments of P alone within 3 passages; tazobactam (T) at medium and equal ratios led to the reduction in growth at passage 10. Phenotypic characterization using Bayesian model illustrated the robustness of adjuvant in restoring the efficacy of P again when the evolution resulted from P alone. Presence of T in combination with P at the beginning reduces the ability to restore antibiotic effectiveness and thus limits the opportunities for future treatments. Whole genome sequencing using CARD identified different mutations related to efflux pump, antibiotic inactivation, antibiotic target alteration, and β-lactamase expression regulator in PAO1 and PA14. Measuring the emergence of resistance to P quantitatively revealed a sharp decrease in P concentration in all treatments compared to the ancestors as a result of the presence of β-lactamase that inactivates P.

Key words: Marine *Streptomyces*, Antimicrobial activity, Bis (2- ethylhexadecyl) phthalate, MIC, Evolution.

DEDICATION

I dedicate this thesis with my all deepest love and appreciation to all my family especially to my late parents: To my father, for his deep prayers, help, patience, continuous encouragement and support; to my mother, for her deep prayers and love; to my husband and my two sons for their love, patience, care, help and permanent encouragement to be ambitious during my study and for helping me throughout my life.

Also I dedicate this work to my beloved brother for his love and help.

ACKNOWLEDGEMENT

First of all, praise for the Almighty ***ALLAH*** *for his great and continuing help and mercy in all my life.*

I would like to gratefully thank ***Dr. Ferial Rashad****, Professor of Agricultural Microbiology, Cairo University, for her kind supervision, continuous guidance, unlimited assistance, valuable discussion during all stages of fulfillment of the thesis and for her motherly feelings.*

The second part of the experimental work was carried out in cooperation with Brown lab, Georgia Tech, USA during the period 2018-2020. I wish to express my grateful and sincere thanks to ***Dr. Sam Brown,*** *Associate Professor of Evolutionary Microbiology, Georgia Tech for his supervision, guidance, continuous advice, assistance and helpful supervision. My endless thanks to* ***Dr. James Gurney,*** *Post Doc, Georgia Tech for his cooperation, assistance, and continuous help.*

I would like to express my thanks to ***Dr. Hayam Fathy and Dr. Mohamed Moselhy*** *Associate Professors of Agricultural Microbiology, Cairo University for their kind help and assistance.*

Thanks are also extended to ***Dr. Hemaia Motawe,*** *Professor of Phytochemistry, NRC for his assistance in interpretation of spectral data and structure elucidation of isolated compound and* ***Dr. Fatma Motawe,*** *Researcher, NRC for her assistance and continuous help.*

The second part of this work was funded by the Egyptian Ministry of Higher Education.