Control of Biofilm Formation in Fungi Using Ethanol

By

Reham Talaat El Sebaey

B.Sc. Microbiology- Chemistry (2008)

Faculty of Women for Art, Science &Education

Ain-Shams University

National Center for Radiation Research and Technology

A

Thesis Submitted for

Partial fulfillment of the Requirements for

The Degree of Master of Science in Microbiology

Botany Department

Ain –Shams University

Cairo, Egypt

2015

Control of Biofilm Formation in Fungi Using Ethanol

By

Reham Talaat El Sebaey

B.Sc. Microbiology- Chemistry (2008)

Faculty of Women for Art, Science &Education

Ain-Shams University

Supervised By

Dr. Sherif Moussa Husseiny

Assistant Professor of Microbiology

Microbiology Department, Faculty of Women for Art, Science &Education

Ain- Shams University

Prof. Dr. Hussein Abd El Kareem

Professor of Microbiology

Microbiology Department, National Center for Radiation Research and Technology (NCRRT)

Prof. Dr. Ola Mohamed Ahmed Gomaa

Professor of Microbiology

Microbiology Department, National Center for Radiation Research and Technology (NCRRT)

نَرْفَعُ دَرَجَاتٍ مّن نّشَآءُ وَفَوْقَ كُلّ ذِي عِلْمٍ عَلِيم

صدق الله العظيم

سورة يوسف آية (76)

التحكم في تكوين طبقة الغلاف الحيوي(البيوفيلم) فى الفطريات باستخدام الايثانول

رسالة

مقدمة من الطالبة

ريهام طلعت السباعي عبدالجواد

بكالوريوس علوم (2008)

قسم الميكروبيولوجي-كيمياء

كمطلب جزئي

للحصول علي درجة الماجستير

في

الميكروبيولوجي

قسم النبات- كلية البنات للادآب والعلوم و التربية

جامعة عين شمس

القاهرة – مصر

2015

التحكم في تكوين طبقة الغلاف الحيوي (البيوفيلم) فى الفطريات باستخدام الايثانول

رسالة

مقدمة من الطالبة

ريهام طلعت السباعي عبدالجواد

بكالوريوس علوم (2008)

قسم الميكروبيولوجي-كيمياء

تحت اشراف

د. شريف موسي حسيني

استاذ مساعد الميكروبيولوجي- كلية البنات للادآب والعلوم و التربية – جامعة عين شمس

ا.د. حسين عبد الكريم احمد

استاذ الميكروبيولوجي- المركز القومي لبحوث وتكنولوجيا الاشعاع- هيئة الطاقة الذرية

ا.د. علا محمد احمد جمعة

استاذ الميكروبيولوجي- المركز القومي لبحوث وتكنولوجيا الاشعاع- هيئة الطاقة الذرية

قسم النبات- ميكروبيولوجي

كلية البنات للاداب والعلوم والتربية- جامعة عين شمس

رسالة ماجستير

اسم الطالبة: ريهام طلعت السباعي عبد الجواد

عنوان الرسالة: التحكم فى تكوين طبقة الغلاف الحيوى (البيوفيلم) في الفطريات باستخدام الايثانول

لجنه الاشراف:

د/شريف موسى حسيني

أستاذ مساعد بقسم النبات بكليه البنات- جامعه عين شمس

أ.د/حسين عبد الكريم أحمد

أستاذ بقسم الميكروبيولوجى بالمركز القومى لبحوث وتكنولوجيا الاشعاع-هيئه الطاقه الذرية

ا.د./علا محمد أحمد جمعه

أستاذ بقسم الميكروبيولوجى بالمركز القومى لبحوث وتكنولوجيا الاشعاع-هيئه الطاقه الذرية

لجنة التحكيم:

أ.د/ يحيي احمد مهدي الظواطري

أستاذ بقسم النبات بكلية العلوم- جامعة الزقازيق

أ.د/ علي احمد ابراهيم حماد

أستاذ بقسم الميكروبيولوجى بالمركز القومى لبحوث وتكنولوجيا الاشعاع-هيئه الطاقه الذرية

أ.د/ علا محمد احمد جمعة

أستاذ بقسم الميكروبيولوجى بالمركز القومى لبحوث وتكنولوجيا الاشعاع-هيئه الطاقه الذرية

أ.م.د/ شريف موسي حسيني

أستاذ مساعد بقسم الميكروبيولوجى بكليه البنات- جامعه عين شمس

الدراسات العليا تاريخ البحث: / /

ختم الاجازه: اجيزت الرسالة بتاريخ / /

موافقة مجلس الكلية موافقة مجلس الجامعة

/ / / /

شكر

اشكر السادة الاساتذة الذين قاموا بالاشراف و هم:

د. شريف موسي حسيني

استاذ مساعد الميكروبيولوجي- كلية البنات للادآب والعلوم و التربية - جامعة عين شمس

ا.د. حسين عبد الكريم احمد

استاذ الميكروبيولوجي- المركز القومي لبحوث وتكنولوجيا الاشعاع- هيئة الطاقة الذرية

ا.د. علا محمد احمد جمعة

استاذ الميكروبيولوجي- المركز القومي لبحوث وتكنولوجيا الاشعاع- هيئة الطاقة الذرية

و اوجه الشكر لكل من ساهم فى اخراج هذه الرسالة

ACKNOWLEDGEMENT

*First of all, thanks to **God** for the infinite helps and persistent supply with patience and efforts to accomplish this work successfully.*

*I would like to express my deepest sense of gratitude to my supervisor **Dr. Sherif Moussa Husseiny** Assistant Professor of Microbiology Microbiology Department, Faculty of Women for Art, Science & Education Ain- Shams University for his advice, guidance and encouragement as well as his academic experience and great efforts with me.*

*I wish to express my sincere gratitude and appreciation to my supervisor **Prof. Dr. Ola Mohamed Ahmed Gomaa** Professor of Microbiology, Microbiology Department, National Center for Radiation Research and Technology (NCRRT) for suggesting, planning the point of the research and her continuous great efforts throughout my thesis period, as well as her efforts to explain things clearly and simply. She provided good teaching, guidance and valuable helping.*

*I would like to express my special thanks to **Prof. Dr. Hussein Abd El Kareem** Professor of Microbiology, Microbiology Department, National Center for Radiation Research and Technology (NCRRT) for his keen supervision, high experience, great help, facility to all my difficulties and encouragement. He is a great scientist.*

My deepest gratitude to all staff of Microbiology department, National Center for Radiation Research and Technology for their friendship and their advice.

Last, but not least my deep thanks are extended to my family and all my friends, their love, understanding and unfailing support has made everything possible.

I thank all those who, in different ways, have walked beside me along the way; offering support and encouragement, challenging my thinking, and teaching me to consider alternative views.

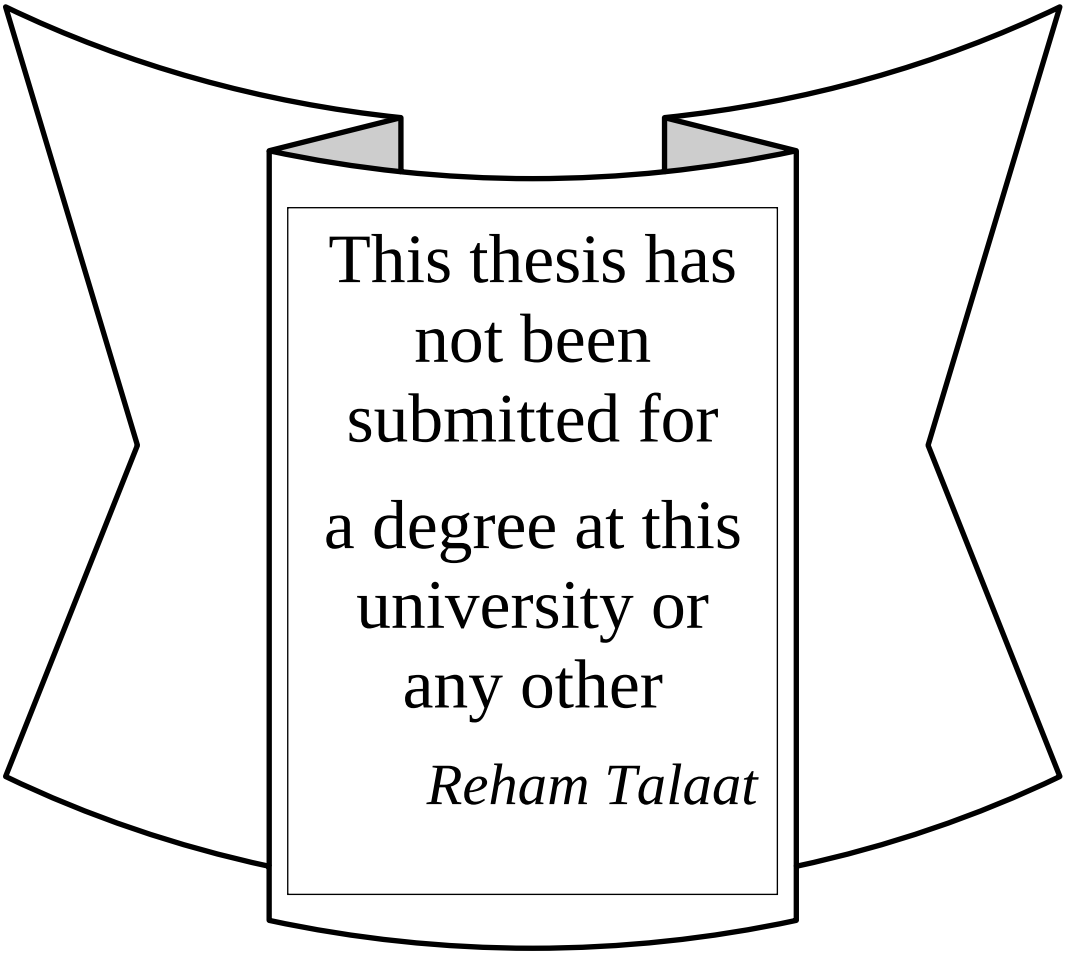
Dedication

My special gratitude to my parents,

Mr. Talaat el sebaey and Mrs. Saneya Mohamed Ali for their forever love, support and their tremendous help. Now, that was your dream, through me. Thank you.

I would like to thank my dear husband, Eng. Haytham alfiqi for his support, encouragements and without his patience; I would not be of success in my life.

Finally, this thesis is dedicated to my daughter Retaj and my future babies Insha'Allah.


This thesis has not been submitted for
a degree at this university or any other
Reham Talaat

Abstract

The use of fungi in biotechnology requires that no cell loss takes place; a maximal level of cell-nutrient interaction is required to achieve efficient performance and avoid cell loss. The main aim of the present study is to use ethanol to control cell-cell and cell-surface adhesion through manipulating cell surface properties. A Fungal isolate with a phenol oxidase activity (43.2 U/ml) was chosen out of twelve isolates belonging to two main genera: *Aspergillus sp.* and *Penicillium sp.* The fungus isolate, assigned as the highest phenol oxidase producer, was morphologically identified as *Penicillium purpurogenum*. *Penicillium purpurogenum* formed a ring around the bottle in static and shaking conditions, therefore, a number of different stress conditions, such as pH, temperature, different nitrogen sources, gamma radiation and ethanol, were employed separately to control biofilm formation in the fungus under study. The fungus was tested for its morphology, mycelial weight, stress response (catalase, lipid peroxidation and red pigment synthesis) and extracellular and surface bound protein and exopolysaccharides. The obtained results correlate the biofilm formation to stress response and surface bound protein. Combining all types of stress did not result in more biofilm formation control; on the contrary, it posed more stress on the fungus and affected the biomass. Ethanol on its own was successively used to control biofilm formation, which was inhibited in the presence of 2.5% v/v ethanol without affecting the growth. The addition of ethanol also increased the intracellular phenol oxidase activity from 43.2 to 228.43 U/ml. Scanning electron microscopy showed that the addition of ethanol resulted in the formation of loose mycelial network as compared to a tight mycelial network in ethanol free cultures. The presence of *Yap1p* gene, the detection of an oxidized form of glutathione (GSSG) and catalase after ethanol addition all suggest that a stress response might be involved in the adhesion process. The process of adhesion could be described as a signaling process and it is affected by the germ tube formation as an initial step in adhesion. The surface charge increased as the ethanol concentration increased confirming that ethanol affects the surface charge. Ethanol also affected the DNA polymorphic profile of DNA rendering the fungus genetically variable. Protein profile showed

polymorphism in surface bound proteins for cultures amended with ethanol as compared to control cultures. Scanning electron microscopy indicated that the adhesion of *Penicillium purpurogenum* on polystyrene surface was decreased after treating the surface with ethanol. The use of gamma irradiation slightly affected the wettability of polystyrene strips at 0.5 and 1 kGy, thus slightly decreasing the adhesion, but was not as effective as using ethanol to control the adhesion. Therefore, ethanol could be employed to control the surface properties of a fungus, and to inhibit biofilm formation to obtain a high surface area for the fungus to be employed in any biotechnological process. Moreover, the addition of ethanol did not affect the fungus in terms of application, as the degrading ability of the fungus under ethanol stress increased when 2.5% v/v ethanol was added to olive mill waste water inoculated with *Penicillium purpurogenum*, but at the same time, the biofilm ring was not observed. The percentage of total phenol removal in olive mill waste water was increased from 37.07 to 42.67 upon the addition of ethanol.

Contents