



# ***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**

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**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 Hussein***

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)

كَبِيرٌ عَظِيمٌ

نَرْفَعُ دَرَجَاتٍ مِّنْ نَّشَأٍ وَفَوْقَ كُلِّ  
ذِي عِلْمٍ عَلِيمٌ

صدق الله العظيم

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

رسالة

مقدمة من الطالبة

ريهام طلعت السباعي عبدالجواد

بكالوريوس علوم (2008)

قسم الميكروبيولوجي-كيمياء

كمطالِب جزئي

للحصول علي درجة الماجستير

في

الميكروبيولوجي

قسم النبات- كلية النبات للآداب والعلوم و التربية

جامعة عين شمس

القاهرة – مصر

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تحت اشراف

**د. شريف موسي حسيني**

استاذ مساعد الميكروبيولوجي- كلية البنات للاداب 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



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لجته الاشراف:

د/شريف موسى حسيني

أستاذ مساعد بقسم النبات بكلية البنات- جامعه عين شمس

أ.د/حسين عبد الكريم أحمد

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

أ.د./علا محمد أحمد جمعه

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

لجنة التحكيم:

أ.د/ يحيى احمد مهدي الظواطري

أستاذ بقسم النبات بكلية العلوم- جامعة الزقازيق

أ.د/ علي احمد ابراهيم حماد

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

أ.د/ علا محمد احمد جمعة

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

أ.م.د/ شريف موسى حسيني

أستاذ مساعد بقسم الميكروبيولوجى بكلية البنات- جامعه عين شمس

تاريخ البحث: / /

الدراسات العليا

اجيزت الرسالة بتاريخ / /

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موافقة مجلس الكلية

/ /

/ /

# شكر

اشكر السادة الاساتذة الذين قاموا بالاشراف و هم:

**د. شريف موسى حسيني**

استاذ مساعد الميكروبيولوجي- كلية النبات للاداب 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

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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.*

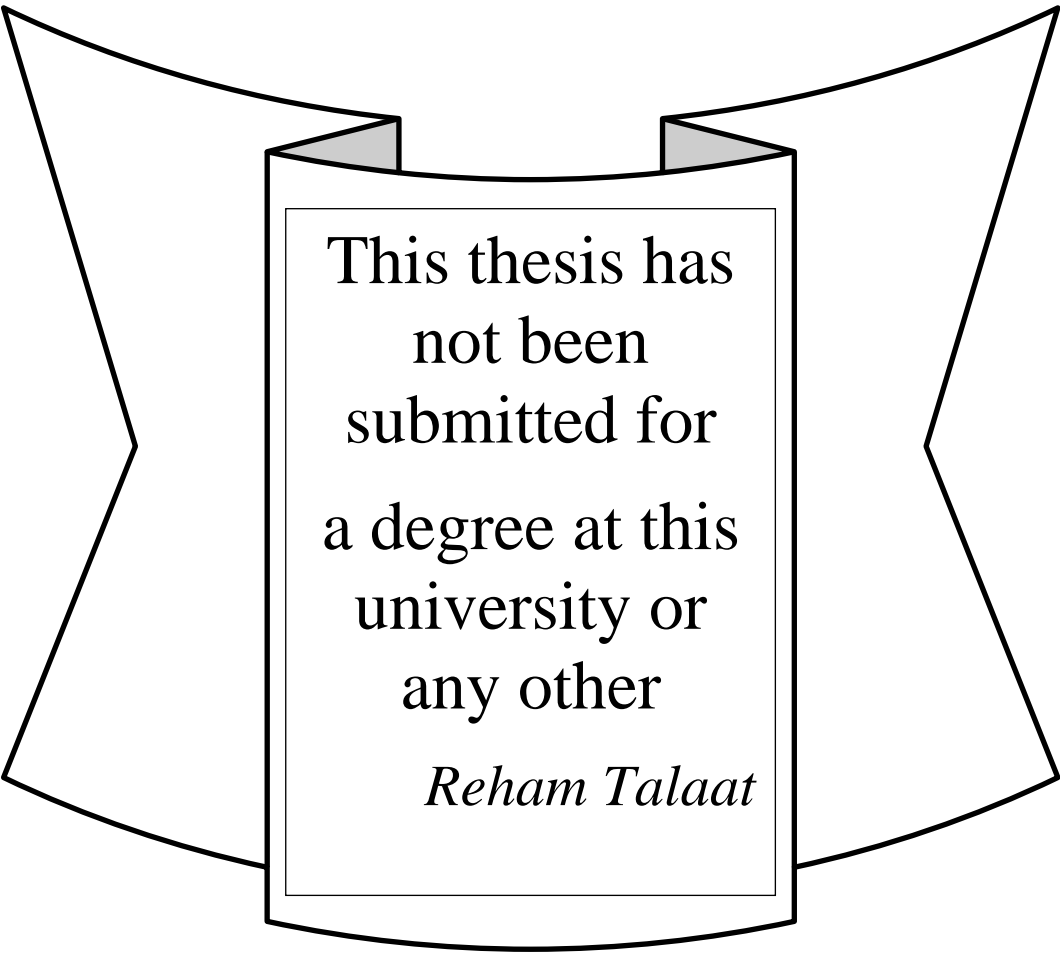
## ***Dedication***

***My special gratitude to my parents,***

***Mr. Talaat el sebaey and Mrs. Saneya  
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your dream, through me. Thank you.***

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would not be of success in my life.***

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

A stylized graphic of an open book. The book is represented by a central rectangular area with a double-line border, flanked by two large, curved, triangular shapes that represent the pages. The central area contains text. The text is centered and reads: "This thesis has not been submitted for a degree at this university or any other". Below this, the name "Reham Talaat" is written in a smaller, italicized font. The entire graphic is set against a plain white background.

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.

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