Production of Chitosan by Some Fungi and Its Industrial Applications

Thesis Submitted for the award of the degree of doctor philosophy in microbiology

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بسم الله الرحمن الرحيم
﴿ ومَا مِن دَابَّةٍ فِي الأرْضِ ولا طَائِرٍ يَطِيرُ
بِجَنَاحَيْهِ إلاَّ أُمَمٌ أمْثَالُكُم مَّا فَرَّطْنَا فِي الكِتَابِ
مِن شَيْءٍ ثُمَّ إلَى رَبِّهِمْ يُحْشَرُونَ(٣٨)والَّذِينَ
كَذَّبُوا بآيَاتِنَا صُمٌّ وبُكْمٌ فِي الظُّلُمَاتِ مَن يَشَأِ
اللَّهُ يُضْلِلْهُ ومَن يَشَأْ يَجْعَلْهُ عَلَى صِرَاطٍ
مُّسْتَقِيمٍ(٣٩)﴾
صدق الله العظيم

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Abbreviation list

AIM	**Alkali-insoluble matter**
CDM	**Cell dry mass**
CH	**chitin**
CHS	**Chitosan**
CHS1	**Chitosan:cell dry mass**
CHS2	**Chitosan:alkali-insoluble matter**
DA	**degree of acetylation**
DD	**Deacetylation degree**
F1	***Rhizopus oryzae* chitosan, grown in PGY**
F2	***Rhizopus oryzae* chitosan, grown in MWB**
FTIR	**Fourier-Transmission Infra Red**
GlcN	**glucosamine**
Gy, Kgy	**Gray, KiloGray; measuring units of gamma radiation**
HMWc	**High molecular weight chitosan**
LMWc	**Low molecular weight chitosan**
MWB	**Molasses wheatbran low cost natural medium**
PGY	**Peptone glucose yeast medium**
TEM	**Transmission electron microscope**
TGA	**Thermogarvimetric analysis**
XRD	**X ray diffractometry**

ABSTRACT

Chitosan production from microorganisms is the main aim of this research. Seventeen fungi were isolated from agricultural soil located in Cairo, Egypt. These isolated fungi were priliminary identified and screened for chitosan content. The best chitosan producer with the highest chitosan content was *Rhizopus oryzae*. The chitinious matter was 255.0 mg/g.The fungus was identified using 18S rRNA revealing it was *Rhizopus oryzae 1.3.32*. Crustacean chitosan was extracted to be compared with the fungal chitosan. It was extracted from shrimp shells with 16% chitosan content. Chitosan samples were characterized by Fourier Transmission Infra Red (FTIR) spectrophotometery, Xray diffiraction (XRD), viscosity analysis and themogravimetry (TGA). One way fermentation experiments were carried out to optimize the environmental conditions including; temperature, agitation, pH, inoculum size, spore age, carbon source, nitrogen source and minerals addition) which gave the maximum chitosan production by *Rhizopus oryzae*. Fungal chitosan was tested for its adsorption ability with Cu^{++} in the form of cupper sulphate compared with the standard and crustacean chitosan. All chitosan samples showed potential ability to adsorb Cu^{++} in aqueous solution. Fungal chitosan showed high spectrum antibacterial activity. The bacteria were *Staphylococcus aureus, Streptococcus epidermidis, Staphylococcus aureus, two isolates of Escherichia coli*1, *Escherichia coli*2 *and Pseudomonas aeruginosa.* In a trial to decrease the costs, a substitution of some components in PGY occurred. Molasses was added to Peptone yeast forming peptone molasses yeast (PMY) medium. Wheat bran was also added to glucose to form wheat bran-glucose (WBG) medium. the medium yielde higher fungal chitosan was PMY since the chitosan was 137.1 mg/g. A mixture of molasses and wheat bran (MWB) medium was tested as a fermentation medium and the chitosan yield was 50.83 mg/g. Chitosan from *Rhizopus*