



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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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿وَمَا مِنْ دَابَّةٍ فِي الْأَرْضِ وَلَا طَائِرٍ يَطِيرُ
بِجَنَاحَيْهِ إِلَّا أُمٌّ أَمْثَلُكُمْ مَا فَرَطْنَا فِي الْكِتَابِ
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اللَّهُ يُضِلَّهُ وَمَنْ يَشَأِ يُجْعَلْهُ عَلَى صِرَاطٍ
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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	<i>Rhizopus oryzae</i> chitosan, grown in PGY
F2	<i>Rhizopus oryzae</i> 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	Thermogravimetric 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 preliminary identified and screened for chitosan content. The best chitosan producer with the highest chitosan content was *Rhizopus oryzae*. The chitinous 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) spectrophotometry, X-ray diffraction (XRD), viscosity analysis and thermogravimetry (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 copper 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 yielded 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*