Faculty of Science
Microbiology Department

Productivity improvement and biochemical characterization of xylanase from rice straw by *Trichoderma*.

Presented by

AHMED MOSELHY ABD EL AZIZ MOSELHY

M.Sc. in Microbiology, Helwan University (2009)

THESIS

Submitted for the Philosophy Degree of Doctor in Science in Microbiology

MICROBIOLOGY DEPARTMENT
FACULTY OF SCIENCE
AIN SHAMS UNIVERSITY

2016

Ain Shams University
Faculty of Science
Microbiology Department

Productivity improvement and biochemical characterization of xylanase from rice straw by *Trichoderma* sp.

T H E S I S

Submitted by

AHMED MOSELHY ABD EL AZIZ MOSELHY
M.Sc. in Microbiology, Helwan University (2009)
Researcher Assistant of Microbiology
Molecular Biology Department
National Research Centre

Submitted for the Philosophy Degree of Doctor in Science in Microbiology

Supervisors

Prof. Dr.
AL ZAHRAA AHMED KARAM EL DIN
Professor of Mycology and medical mycology
Faculty of Science
Ain Shams University

Dr.
EINAS HAMED EL SHATOURY
Assistant professor of Microbiology
Faculty of Science
Ain Shams University

Prof. Dr.
MAGDA ABD- ELNABY MOHAMED
Professor of Biochemistry
Head of Molecular Biology Department
National Research Centre

Prof. Dr.
ABD EL-HADY MAHMOUD GHAZY
Professor of Biochemistry
Molecular Biology Department
National Research Centre

2016

Approval sheet

Thesis

Submitted for the Degree of Doctor of Philosophy in Science in Microbiology

Productivity improvement and biochemical characterization of xylanase from rice straw by *Trichoderma* sp.

By: Ahmed Moselhy Abd El Aziz Moselhy

Examining Committee

Prof. Dr. Mohamed El-Sayed Othman

Professor of Mycology, faculty of science, Helwan University

Prof. Dr. El-Sayed Mohamed El-Mahdy

Professor of Biochemistry, faculty of science, Helwan University

Prof. Dr. Al-Zahraa Ahmed Karam El-Din

Professor of Mycology and medical mycology, Faculty of science, Ain Shams University

Prof. Dr. Magda Abd El-Nabi Mohamed

Professor of Biochemistry, National Research Center

ACKNOWLEDGMENTS

I gratefully acknowledge **Prof. Dr. Al Zahraa Ahmed Karam El-Din** for her advice, supervision, and furthermore, using precious times to read this thesis and gave critical comments about it. I am grateful in every possible way and hope to keep up our collaboration in the future.

I would like to record my gratitude to **Prof. Dr. Magda Abd El-Naby Mohamed Soroor** for her supervision, advice and guidance from my first day in my research career in the *National Research Centre* as well as giving me extraordinary experiences throughout the work. Above all and the most needed, she provided me unflinching encouragement and support in various ways. Crucial contribution made her a backbone of this research and so to this thesis. I am indebted to her more than she knows.

I convey special acknowledgement to **Dr. Abd El-Hady Mahmoud Ghazy** for his unselfish and unfailing support as my dissertation adviser and for his

indispensable help dealing with how I could optimally carry out my research and thesis. I could never have embarked and started all of this without his prior support.

Many thanks go in particular to **Ass. Prof. Dr. Einas Hamed El-Shatoury** for her valuable advice discussion during the writing process.

In addition, I would like to acknowledge **Prof. Dr. Abd El-Nasser Khattab,** professor at Genetics and Cytology Department, NRC. I would like to thank him for being the person who taught me new genetically advanced techniques and how to manage the work in the Genetic experiments.

Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the thesis.

Ahmed Moselhy Abd El-Aziz

CONTENTS

CHAPTER III

ABSTRACT

Name: Ahmed Moselhy Abd El-Aziz Moselhy.

Title of thesis: Productivity improvement and biochemical characterization of xylanase from rice straw by *Trichoderma*.

Trichoderma reesei F418 has a potential for xylanase production using rice straw (RS) as carbon source under solid state fermentation conditions (SSF). A hyperproducing fused isolate *Trichoderma reesei* F418FS was obtained after UV-mutagenesis followed by intra-specific protoplast fusion. The optimized SSF conditions for maximum xylanase production by *T. reesei* F418FS (6875 IU/gDW) was achieved at pH 7.0, 35^{o}C and 90% moisture content at the 5thday of incubation. A major xylanase isoenzyme was purified to homogeneity and has molecular weight of 33 kDa, optimum pH at 5.0, stable at pH ranged from 3.5 to 8.0, optimum temperature for activity at 50^{o}C with activation energy (E_a) 22.93 Kcal mol^{-1} (5.48 KJ mol^{-1}) and stable at temperature up to 70^{o}C. *T. reesei* F418FS xylanase exhibited a high specificity towards xylans with a low activity for cellulosic substrates such as avicel cellulose and carboxymethyl cellulose which concluded that *T. reesei* F418FS xylanase can be described as a cellulase-free xylanase. The Km values for *T. reesei* F418FS xylanase are

2.8, 2.65 and 7.7 mg ml^{-1} for BiWX, BeWX and OSX, respectively. *T. reesei* F418FS xylanase slightly stimulated by monovalent cations K^+ and Li^+, inhibited by Hg^{2+} and also inhibited by Cu^{2+}, confirmed the presence of at least sulfhydryl group in the active site of the enzyme. The inhibitory effect of EDTA suggested that *T. reesei* F418FS xylanase is a metalloprotein. *T. reesei* F418FS xylanase can be described as an acidic cellulase-free xylanase, has a possible application in biobleaching in paper and pulp industry.

Key Words:

Xylanase, *Trichoderma reesei*, UV-mutagenesis, intraspecific protoplast fusion, rice straw, agroindustrial waste, purification and characterization.

PUBLICATIONS

Magda A.M. Soroor, Hoda H. El Hendawy, Abd-Elhady M. Ghazy, Nermeen A. El Semary, Kamal M.A. Khalil, Ahmed M. Abd El Aziz (2009). Characterization of an Alkaline Metalloprotease Secreted by the Entomopathogenic Bacterium *Photorhabdus Sp.* Strain EK1. Research Journal of Agriculture and Biological Science, 5(4): 349-360.

Magda A.M. Soroor, Abd El-Hady M. Ghazy and Ahmed M. Abd El-Aziz (2013). Proteolytic Enzymes Secreted by the Bacterium *Photorhabdus sp*. strain EK1, Symbionts to *Heterorhabditis bacteriophora* EK1. Journal of Applied Sciences Research, 9(8): 4683-4694.

Magda A. M. Soroor, Abd El-hady M. Ghazy, Khattab A. A., Ahmed M. Abd El-Aziz, Einas H. El- Shatoury, Al Zahraa A. Karam El-Din (2014). Enhancement of xylanase production from rice straw by *Trichoderma reesei* through UV- mutagenesis and protoplast fusion. Egyptian Journal of Experimental Biology, 10(2): 125-134.

LIST OF FIGURES