Microbiological and molecular studies on oxalate degrading enzymes isolated from bacteria living in Egyptian fertile soil

Thesis

Submitted for the Fulfillment of the degree Doctor of Philosophy (PhD) in

Microbiology

By

Mona Kilany Abd El Gawad Kilany

(M. Sc. Microbiology 2006)

Microbiology Department
Faculty of Science
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2012

دراسات ميكروبيولوجية وجزيئية على الانزيم المسؤل عن تحليل مادة الاكزلات والمستخلص من البكتريا المعزولة من التربة المصرية

رسالة مقدمة

للحصول على درجة دكتوراه الفلسفة في العلوم (الميكروبيولوجي)

من الطالبة

منى كيلاني عبد الجواد كيلاني (ماجيستير في العلوم / ميكروبيولوجي- ٢٠٠٦)

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Summary

The oxalotrophic bacteria is of great importance in reducing urinary excretion of oxalic acid, since the high oxalate level is one of the important virulence factors for hyperoxaluria, urolithiasis, cardiomyopathy, and renal failure. Oxalate decarboxylase (EC 4.1.1.2) catalyzes the conversion of oxalate to carbon dioxide and formate. The oxalate decarboxylase has commercial uses such as in the brewing industry or for agronomic uses such as to reduce susceptibility of a plant to oxalic acid and phytopathogens, clinical oxalate determination, bioremediators, plant growth promoters, antinematodal and antifungal activity.

In the current work, mesophilic bacteria were isolated from different fertile soil samples obtained from several governorates. Isolation resulted in several isolates. Screening of the bacterial isolates was carried out according to their ability to degrade oxalate. Screening resulted in 16 isolates designated as: SK- 1,SK- 2, SK-3,SOG- 1, SOG- 2, SOG- 3, TG- 1, TG- 2, TG- 3, PG- 1, PG- 2, MG- 1, MG- 2, CG- 1, CG- 2, CG- 3 and SOG- 1. Among the selected isolate 6 isolate showed the highest potential of oxalate degradation.

Characterization of the selected isolate showed that it was highly sensitive to Ofloxacin (5μg), Ceftazidime (30 μg), Claforan cefotaxime (30 μg), Cefoperazone (75 μg), Ciprofloxacin (5 μg), moderately sensitive to Tobramycin (10 μg), Sulphamethxazole trimethoprime (25 μg), Cefepime (30 μg), Erythromycin(15 μg),

APPROVAL SHEET

Title of the The	sis:
Microbiological	and molecular studies on
Oxalate degradir	g enzymes isolated from
Bacteria living in	Egyptian fertile soil
Degree	Doctor of Philosophy (PhD) in
	Microbiology
Name of the stu Kilany	dent: Mona Kilany Abd El Gawad
This thesis for I by:	hD Degree has been approved
1-	
2-	
3-	
Date of examina	tion: / / 2012

This thesis has not been previously submitted for any degree at this or at any other university.

Signature

Mona Kilany Abd El- Gawad Kilany

ACKNOWLEDGEMENT

First and foremost, all thanks are due to **Allah**, the source of all success in my life.

When I sit to write this page numerous faces came across in my mind to whom I am indebted from the beginning of this venture and without all those timely help I couldn't have completed this work.

My deep thanks and greetings are to my **parents**, who grant me their whole life and did their best to help and support me.

First of all, I really find myself luckily to be supervised by **Prof. Dr. Hala M. Abou Shady,** assistant Professor of Microbiology, Microbiology Department, Faculty of Science Ain Shams University. I am really grateful to her sincere and continuous support and encouragement to complete this study and brought this study to the fruition. She earnestly extended the gracious help academically as well as administratively to ensure smooth progress of this work.

No words could express my deep appreciation and gratitude to **Dr. Kadria A. Genedi,** Lecturer of Microbiology, and Microbiology Department National Organization for Drug Control and Research. I am really owed much to her for her continuous support and scientific experience she gave me.

Special thanks are to **Dr. Wafaa F. Ahmed**, Lecturer of Microbiology, Ain Shams University specialized hospital for continuous guidance, fruitful discussion, encouragement, excellent advice.

All my thanks to **Prof. Dr. Essam I. Hassan,** Professor of Immunology and Biotechnology, National Organization for Research & Control of Biologicals, for having seen so wonderfully kind and thoughtful, helping me patiently and skillfully in innumerous ways and great tolerance that support me to finish this work.

I am very grateful to **Dr. Khalid El Gayar**, Lecturer of Biotechnology, VACSERA, for his sincere and grateful cooperation to support this work.

No words suffice to thank **Dr. Hend El Deeb**, Consultant Epidemiologist, GOTHI, for her great help and patience.

I extend my sincere and heartfelt gratitude to **Dr. Mona I. Mabrouk,** Lecturer of Microbiology, Microbiology Department

National Organization for Drug Control and Research for splendid help and valuable co-operation.

List of abbreviations

16S rRNA : Ribosomal 16S ribonucleic acid

API 20NE : Analytical Profile Index

ATCC : American type culture collection

Bcc : Burkholderia cepacia complex

BUG : Biolog reader universal growth

BSA :Bovine serum albumin

CaOx : Calcium oxalate

CCS : Continuous culture system

CF : Cystic fibrosis

Cm : Coniothyrium minitans

E.C. : Enzyme Commission number

EDTA : Ethylene diamine tetra acetic acid

Frc : Formyl-CoA transferase

GIT : Gastrointestinal tract

HPLC : High performance liquid chromatography

kDa. : Kilo Dalton

MLST : Multilocus sequence typing

MnSODase : Manganese dependent oxalate decarboxylase

MnSODase : Manganese-dependent superoxide dismutase

NAD+ : Nicotinamide adenine dinucleotide

NADP+ : Nicotinamide adenine dinucleotide phosphate.

Oxc : Oxalyl-CoA decarboxylase

OxdC : Cytosolic oxalate decarboxylase

OXDC : Oxalate decarboxylase

OXOX : Oxalate oxidase

PBS :Phosphate buffered saline

Abbreviations

PCR : Polymerase chain reaction

PHDC (RT-46 : Philadelphia-District Columbia strain

complex)

pKa : Acid dissociation constant

PPFM : Pink-pigmented facultative methylotrophs

RAPD : Randomly amplified polymorphic DNA typing

recA gene : Protein essential for repair and recombination of

DNA

RFLP : Restriction fragment length polymorphisms

Rnn operons : Ribosomal RNA gene groups

RT-88 complex : Mid-west strain

SCAN- W/A : Microscan WalkAway-96

SDS-PAGE :Sodium dodecyl sulfate polyactylamide gel

electrophoresis

ST : Sequence types

T.S.S. : Trace salts solution

TEMED : N, N, N', N' tetramethylene diamine

TPP : Thiamine pyrophosphate

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