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

Ain Shams University

2012

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2012

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

رسـالة مقدمـة

للحصول على درجة دكتوراه الفلسفة فى العلوم
(الميكروبيولوجى)

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 Thesis:

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Name of the student: Mona Kilany Abd El Gawad Kilany

This thesis for PhD Degree has been approved by:

1-

2-

3-

Date of examination: / / 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

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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

PCR	: Polymerase chain reaction
PHDC (RT-46 complex)	: Philadelphia-District Columbia strain
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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