



Microbiological treatment of selenium containing materials

Thesis

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

By

Ali Mohamed Ali Saeed
(M.Sc. Microbiology, 2010)

Supervisors

Dr. Abd El Moneim M. Osman

*Professor of Mineralogy,
Geology Department,
Faculty of Science,
Ain Shams University*

Dr. Abd El Fattah I. Helal

*Professor of Atomic Physics,
Former Vice Chairman Atomic
Energy Authority*

Dr. Khaled Zakaria El-Baghdady

*Associate Prof. of Microbiology,
Microbiology Department,
Faculty of Science,
Ain Shams University*

Dr. Einas H. El Shatoury

*Associate Prof. of Microbiology,
Microbiology Department,
Faculty of Science,
Ain Shams University*

Dr. Abeer E. M. Zakaria

*Associate Prof. of Microbiology
Microbiology Department,
National Center of Radiation
Research and Technology (NCRRT).*

Microbiology Department,
Faculty of Science,
Ain Shams University.

2015

Approval sheet

Microbiological treatment of selenium containing materials

By

Ali Mohamed Ali Saeed

M.Sc. In Microbiology

Ain Shams University

2010

Supervisors

Approved

Prof. Dr. Abd El Moneim M. Osman

Prof. of Mineralogy,
Faculty of Science,
Ain Shams University.

Prof. Dr. Abd El Fattah I. Helal

Prof. of Atomic Physics
Former Vice Chairman Atomic Energy Authority.

Dr. Khaled Zakaria El-Baghdady

Ass. Prof. of Microbiology,
Faculty of Science,
Ain Shams University.

Dr. Einas H. El Shatoury

Ass. Prof. of Microbiology,
Faculty of Science,
Ain Shams University.

Dr. Abeer E. M. Zakaria

Ass. Prof of Microbiology,
National Center of Radiation
Research and Technology (NCRRT).

Examination committee

Prof. Dr. Zeinab Mohamed Hassan Kheir Allah

Prof. of Microbiology,
Faculty of Science (Girls),
Ain Shams University.

Prof. Dr. Bahgat Mohamed Refaat

Prof. of Microbiology,
Faculty of Science,
El-Azhar University.

Prof. Dr. Abd El Moneim M. Osman

Prof. of Mineralogy,
Faculty of Science,
Ain Shams University.

Dr. Khaled Zakaria El-Baghdady

Ass. Prof. of Microbiology,
Faculty of Science,
Ain Shams University.

Approval date 6/ 2015 University Council approved /2015

**This dissertation has not been previously
submitted for any degree at this or at any
other university**

Ali Mohamed Ali Saeed

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
قَالُوا سُبْحَانَكَ لَا عِلْمَ
لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ
أَنْتَ الْعَلِيمُ الْحَكِيمُ
صَدَقَ اللَّهُ الْعَظِيمُ

(البقرة آية: 32)

ACKNOWLEDGEMENT

*First and foremost, I feel always indebted to
Allah, the most beneficent and merciful*

A great thank to Professor Abd El Moneim M. Osman, Professor of Mineralogy, Faculty of Science, Ain Shams University for his support, encouragement, valuable advices and constant help.

I want to express my thanks to Professor Abd El Fattah I. Helal, Professor of Atomic Physics, Former Vice Chairman Atomic Energy Authority for his continuous helps and encouragement.

I would like to express my deep gratitude and thanks to my dear supervisor Dr. Khaled Zakaria El-Baghdady, Associate Prof. of Microbiology, Faculty of Science, Ain Shams University for his help, encouragement, continuous advice, valuable suggestion in all steps of this thesis and his expert supervision. I am proud to be one of his students and I hope that he is satisfied with me.

A great thank to Dr. Einas H. El Shatoury, Associate Professor of Microbiology, Faculty of Science, Ain Shams University for her continuous helps and

encouragement. She was always patient, perfect in work organization and the best advisor and she is a good leader to our team work.

*I gratefully and sincerely thank my dear supervisor **Dr. Abeer E. M. Zakaria**, Associate Professor of Microbiology, National Center of Radiation Research and Technology (NCRRT) for her help, continuous support, valuable instructions and guidance from the start of the work. She was always patient, perfect in work organization and the best advisor.*

*I would also like to show my gratitude to **Dr. Sahar Tolba**, Associate Professor of microbiology, Faculty of Science, Ain Shams University and **Dr. Mahmoud M. El Mosallamy**, Lecturer of genetics, Faculty of Agriculture, Ain Shams University for their assistance in the molecular techniques.*

*A deep thank to **Microbiology Department** and all **my Colleagues** in Microbiology department for their assistance and support.*

*A very special thanks to **My WIFE** who has stood by me all through my studies and for her constant support and prayers.*

*Deep thanks to **MY DAUGHTERS** for their patience. Great thanks to **My BROTHER** for his support and continuous help from the start of my study.*

*And Finally, My deep appreciation goes to **My ADORABLE PARENTS** who learned me the first of everything in my life and without them, this work would not have seen light.*

CONTENTS

Subject	Page no.
List of tables	
List of figures	
List of photos	
Abbreviations	
Aim of work	
Abstract	1
Introduction	3
Literature Review	5
1-1 Selenium forms and occurrence	6
1-2 Selenium Toxic Effects in the Environment	9
1-3 Uses of Selenium	12
1-4 Applications	16
1-4-1 Manganese electrolysis	16
1-4-2 Glass production	16
1-4-3 Alloys	17
1-4-4 Medical use	17
1-4-5 Other uses	18
1-5 Selenocyanate	19
1-6 Selenium Treatment	20
1-7 Selenite reduction mechanism	26

1-8 Horizontal gene transfer	29
Materials and Methods	
2-1 Materials	31
2-1-1 Soil samples	31
2-1-2 Bacterial isolates	31
2-1-3 Culture media	33
2-1-4 Chemicals	36
2-1-5 Reagents and solutions	37
2-2 Methods	38
2-2-1 Soil samples	38
2-2-2 Isolation of selenite reducing bacteria	38
2-2-3 Preservation and maintenance of the pure bacterial isolates	39
2-2-4 Screening and selection of potent selenite reducing bacterial isolates	39
2-2-5 Detection of elemental selenium production	40
2-2-6 Quantitative measurement of elemental selenium produced by bacterial isolates	40
2-2-7 Preparation of standard curve of selenium	41
2-2-8 Quantitative assay for selenium production	42
2-2-9 Molecular analysis	42
2-2-9-1 Identification of the selenite reducing bacterial isolates by 16S rRNA gene sequencing	42
2-2-9-2 DNA extraction, PCR Amplification of <i>trxB</i> gene	43

2-2-10 Optimization of selenite reduction by bacteria	46
2-2-10-1 Effect of different types and concentrations of electron donor on selenite reduction	46
2-2-10-2 Effect of different concentrations of selenite (electron acceptor) on selenite reduction	47
2-2-10-3 Effect of different incubation periods on selenite reduction	47
2-2-10-4 Effect of different pH on selenite reduction	47
2-2-10-5 Effect of different inoculum sizes on selenite reduction	48
2-2-10-6 Effect of different incubation temperature on selenite reduction	48
2-2-11 Transmission Electron Microscope (TEM)	49
2-2-12 Statistical analysis	49
Results	
3-1 Isolation of selenite reducing bacteria	50
3-2 Preliminary selection for selenite reducing bacteria	51
3-3 Characterization of elemental selenium production	52
3-4 Standard curve of selenium	53
3-5 Quantitative assay for selenium production	54
3-6 Identification of the selenite reducing bacterial isolates by 16S rDNA sequencing	56
3-7 Thioredoxin reductase (<i>trxB</i>) detection	58
3-8 Optimization of selenite reduction by bacterial isolates	61

3-8-1 Study the effect of different types and concentrations of electron donor	61
3-8-2 Effect of different concentrations of selenite (electron acceptor)	64
3-8-3 Effect of different incubation period	66
3-8-4 Effect of different pH	68
3-8-5 Effect of different inoculum sizes	70
3-8-6 Effect of different incubation temperatures	72
3-9 Growth rate of <i>Z. dentrificans</i> Se1 and <i>P. stutzeri</i> Se5	74
3-10 Transmission Electron Microscope	77
Discussion	79
English summary	92
References	96
Arabic summary	

LIST OF TABLES

Table no.	Title	Page no.
1	Soil samples and there localities	31
2	Designed primers for <i>trxB</i> gene	44
3	Color, shape and Gram reaction of the selected isolates	50
4	Quantitative analysis of Se ^o produced by selected Isolates	54
5	Identification and identity of the selected isolates	56
6	pairwise distance of <i>trxB</i> gene	59
7	Effect of different types and concentrations of electron donor on bacterial reduction of selenite	62
8	Effect of different concentrations of selenite on bacterial production of selenium	65
9	Effect of different incubation periods on bacterial reduction of selenite	67
10	Effect of different pH on bacterial reduction of selenite	69
11	Effect of different inoculum sizes on selenite bioreduction	71
12	Effect of different incubation temperatures on reduction of selenite by bacterial isolates	73

LIST OF FIGURES

Figure no.	Title	Page no.
1	Known components of the thioredoxin system (top) and glutaredoxin system (bottom)	28
2	UV–Visible spectrum of Se ⁰ nanoparticles formed by bacteria	52
3	Standard calibration curve of Se ⁰	53
4	Amount of Se ⁰ produced by selected isolates at 30°C for 10 days.	55
5	Neighbor joining phylogenetic tree of 16S rRNA genes	57
6	Neighbor joining phylogenetic tree of <i>trxB</i> genes	60
7	Effect of different types and concentrations of electron donor on bacterial reduction of selenite	63
8	Effect of different concentrations of selenite on bacterial production of selenium	65
9	Effect of different incubation periods on bacterial reduction of selenite	67
10	Effect of different pH on bacterial reduction of selenite	69
11	Effect of different inoculum sizes on selenite bioreduction	71
12	Effect of different incubation temperatures on reduction of selenite by bacterial isolates	73
13	The growth curve of <i>Z. dentrificans</i> Se1 and <i>P. stutzeri</i> Se5	76

LIST OF PHOTOS

Photo no.	Title	Page no.
1	Maps for sampling locations	31
2	Sampling locations by Google Earth	32
3	Representative plates showing the growth of a selenite reducing isolates on NAS medium	51
4	Selenium production capabilities of selected isolates on EBM medium at 30°C for 10 days.	55
5	Transmission Electron Micrographs (TEM) of <i>Z. dentrificans</i> Se1	77
6	Transmission Electron Micrographs (TEM) of <i>P. stutzeri</i> Se5.	78

ABBREVIATIONS

CFU	Colony Forming Units
EBM	Enrichment basal medium
EDTA	Ethylenediaminetetraacetic acid
EPA	Environmental Protection Agency
g	Gram
HGT	Horizontal gene transfer
NAS	Nutrient agar medium supplemented with 5 mM of sodium selenite
mg	milligram
ml	Milliliters
mM	Millimolar
μg	Microgram
μ moles	Micromoles
nm	Nanometer
PCR	Polymerase Chain Reaction
Se⁰	Elemental selenium
Se IV	Selenite
Se VI	Selenate
TBE	Tris/Borate Electrophoresis (buffer)
TE	Tris/EDTA (buffer)
<i>trxB</i>	Thioredoxin reductase type B
TEM	Transmission Electron Microscope