MOLECULAR CHARACTERIZATION OF ISOPRENE SYNTHASE GENE FROM BACTERIA

By

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B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2007

THESIS
Submitted in

MASTER OF SCIENCE

In

Agricultural Sciences
(Genetics)

Department of Genetics
Faculty of Agriculture
Cairo University
EGYPT

2017

APPROVAL SHEET

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Title of Thesis: Molecular characterization of isoprene synthase gene from bacteria
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Department: Genetics **Approval: 23 / 5 / 2017**

ABSTRACT

Isoprene represents a key building block for the production of valuable materials such as latex, synthetic rubber, pharmaceuticals and serves as basis for advanced biofuels. In this study, the isoprene produced by *Bacillus subtilis* ATCC 6633 and *Bacillus stearothermophilus* 12980 ATCC was quantitatively analysed using gas chromatography mass spectrometry (GC-MS). *B. stearothermophilus* 12980 ATCC produced 1.169 mg/ml isoprene at 55^{o}C for 18h incubation and *B. subtilis* 6633 ATCC produced 1.071 mg/ml isoprene at 45^{o}C for 18h incubation. Furthermore, heterologous expression of the Kudzu isoprene synthase (*kIspS*) gene into *B. subtilis* DSM 402, *B. licheniformis* DSM 13 using the pHT01 vector and *E. coli* BL21 (DE3) using the pET28b-C-term to be used as a bench mark. Development of the electroporation protocol for successful tranformations into *Bacillus* spp. Isoprene production was analyzed using Gas Chromatography Flame Ionization Detector (GC-FID). The highest isoprene production was observed by recombinant *B. subtilis* harboring the pHT01-*kIspS* plasmid in which it produced 1275µg/l/OD isoprene, that is three-fold higher than the non recombinant *Bacillus subtilis* (WT) which produced 370 µg/l/OD isoprene, when both incubated at 30℃ for 48 h induced with 0.1 mM IPTG. Additionally, recombinant *B. subtilis* produced five fold higher than the recombinant *B. licheniformis*. Multiple sequnece alignment showed difference in the codon usage analysis between the two *Bacillus* spp. Recombinant *B. subtilis* is considered as a versatile host for heterologous production of isoprene.

Keywords Isoprene, Isoprene synthase, *Bacillus stearothermophilus, Bacillus subtilis, Bacillus licheniformis*

DEDICATION

I dedicate my thesis to whom my heartfelt thanks, to my beloved Dad M. Houssam El-Deen who passed away and supported me through my long journey and to my Mum Azza who have been always my source of inspiration and backbone. Special thanks to my dear brother Lo'ai and my lovely sister Lobna who have never left my side. Sincere appreciation to our new family members my brother Ahmed Alaa and my sister Lobna Fayed for their encouragement and moral support. Special thanks to to my Deutsch Mum Elisabeth Ebenbeck for being kind and helpful during my stay with her in Straubing, Germany.

ACKNOWLEDGEMENT

Special thanks and sincere appreciation to ***Dr. Naglaa A. Abdallah****, Professor of Genetics, Fac. of Agri. Cairo Uni., for her assistance, guidance and support throughout my postgraduate studies, writing of the thesis and research paper publication.*

Sincere appreciation to ***Dr. Haggag S. Zein****, Associate Professor of Genetics, Fac. of Agri. Cairo Uni., for his supervision, and his great efforts in data analysis, writing of the thesis and research publication.*

Also, I would like to thank to ***Dr. Nahed Abdel ghaffar****, head researcher at the microbial molecular biology lab, AGERI, ARC for her supervision, encouragement and support along the period of this work.*

*Additionally, I would like to express my gratitude to the Science and technology development fund - short term fellowships (****STDF-STF****) for their financial support of my proposal entitled "Microbial isoprene production project no.6672" and awarding me a short term fellowship for 4 months to carry out my Master thesis at the Chair of Chemistry and Biogenic Resources, Technical University of Munich, Straubing, Germany.*

I would like to thank all the members of the Chair of Chemistry and Biogenic Resources, TUM for being helpful. Special thanks to ***Dr. Volker Sieber*** *and* ***Dr.-Ing. Jochen Schmid*** *for their contribution to the experimental design and providing me all the materials, supplies and facilities for this work to be done. Special thanks to* ***Michael Loscar*** *for his supervision, planning and direction along the period of this work.*

Many thanks must go to ***Dr. Ali samy Abdelaal*** *Department of Genetics, Faculty of Agriculture, Damietta University for his great efforts in the GC-FID data analysis and his participation in the period of writing and revising the thesis.*

Sincere appreciation to ***Dr. Abdelhadi A. Abdallah****, Associate Professor of Genetics, Fac. of Agri., Cairo Uni., for his great efforts and support during our research article publication process.*

I would like to thank ***Dr. Abdallah Soubhy*** *and* ***Rasha Abdel-majeed*** *Department of Microbilogy, Fac. Of Agri. Cairo Uni. for learning me on Endnote program.*

I am grateful to all of the Genetics Department staff, Fac. Agri. Cairo Uni. I would like also to express my gratitude to all of the ***AGERI*** *staff. Special thanks for the microbial molecular biology lab technician* ***Rabab Saeed*** *for her continuous support. I would like to thank also my colleges* ***Hazem Hassan*** *and* ***Mustafa Fathy*** *for helping me to prepare my samples before my travel to Germany. Sincere appreciation to my colleges* ***Omnia Taha*** *and* ***M. Zamzam*** *for their great efforts and support during the period of writing the thesis and research paper. I would like to thank* ***Miss Ghada*** *at Crystal library for helping me in adjusting the thesis format.*

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