

MOLECULAR CHARACTERIZATION OF ISOPRENE SYNTHASE GENE FROM BACTERIA

By

**LAMIS MUHAMMAD HOSSAM EL-DEEN
HUSSEIN GOMAA**

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

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APPROVAL SHEET

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APPROVAL COMMITTEE

DR. GIHAN MOHAMED HOSNY HUSSEIN.....
Head Research of Plant Genetic Transformation, AGERI, ARC

DR. ABDELHADI ABDALLAH ABDELHADI
Associate Professor of Genetics, Fac. Agric., Cairo University

Dr. NAGLAA ABDELMONEIM ABDALLAH.....
Professor of Genetics, Fac. Agric., Cairo University

Date: 23 /5 / 2017

SUPERVISION SHEET

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SUPERVISION COMMITTEE

Dr. NAGLAA ABDALMONEIM ABDALLAH.....
Professor of Genetics, Fac. Agric., Cairo University

Dr. HAGGAG SALAH ZEIN.....
Associate Professor of Genetics, Fac. Agric., Cairo University

DR. NAHED ABDEL GHAFAR ABDEL AZIZ.....
Head Research of Microbial Molecular Biology, AGERI, ARC

Name of Candidate: Lamis Muhammad Hossam El-Deen Hussein Gomaa
Degree: M.Sc.

Title of Thesis: Molecular characterization of isoprene synthase gene from bacteria

Supervisors: Dr. Naglaa A. Abdallah

Dr. Haggag Salah Zein

Dr. Nahed Abdel Ghaffar Abdel Aziz

Department: Genetics

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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°C for 18h incubation and *B. subtilis* 6633 ATCC produced 1.071 mg/ml isoprene at 45°C for 18h incubation. Furthermore, heterologous expression of the Kudzu isoprene synthase (*klspS*) 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 transformations 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-*klspS* 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°C for 48 h induced with 0.1 mM IPTG. Additionally, recombinant *B. subtilis* produced five fold higher than the recombinant *B. licheniformis*. Multiple sequence 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.

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