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Molecular studies on Isoprene Production from Natural and Recombinant Bacteria

Lamis Gomaa[1]; Haggag S. Zein[2]; Nahed Abdel Ghaffar[1] and Naglaa A. Abdallah[2*]
[1] Agricultural Genetic Engineering Research Institute, ARC, Giza, 12619, Egypt
[2]Department of Genetics, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt
[*]Corresponding author: Senior of Molecular Plant Virology Lab, Agricultural Genetic Engineering Research Ins. (AGERI), Agricultural Research Center (ARC).
Email: naglaa.abdallah@agr.cu.edu.eg

ABSTRACT

The natural volatile hydrocarbon isoprene (2-methyl-l, 3-butadiene) is an essential chemical for polymers, drugs and rubber industry. It is produced naturally by animals, plants and bacteria. The isoprene synthase gene was identified in plants. However, it was not identified in bacteria. Moreover, it was found that *Bacillus* sp. produced the most isoprene compared to other bacteria species. In this work, the quantitative analysis of isoprene produced by *Bacillus subtilis* ATCC 6633 and *Bacillus stearothermophilus* 12980 ATCC was measured using gas chromatography-mass spectrometry (GC-MS) compared to standard isoprene, in which retention time was 3.61 for isoprene standard, 3.69 for *B. stearothermophilus* and 3.68 for *B. subtilis*. Additionally, *Bacillus stearothermophilus* 12980 ATCC produced 1.169 mg/ml isoprene, when incubated at 55^{o}C for 18h and *Bacillus subtilis* 6633 ATCC produced 1.071 mg/ml isoprene when incubated at 45^{o}C for 18h. The synthetic kudzu isoprene synthase gene was used to transform the *E. coli* DH5α cells. SDS-PAGE of proteins from the transformed *E. coli* cells showed a band in the predicted size of the *kIspS* protein at 65 Kd. In conclusion, from the previous work further studies might develop recombinant bacteria as a biological source capable of isoprene production for chemical feedstock and rubber synthesis.

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

INTRODUCTION

Isoprene is known to be produced from the two five-carbon (C5) building units isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP). The Isoprene synthase utilize dimethylallyl diphosphate (DMAPP) as the substrate (Withers *et al.*, 2007). The isopentenyl diphosphate (IPP) and the dimethylallyl diphosphate (DMAPP) were known to be produced by either pathway: the mevalonate (MVA) pathway or the1-deoxy-D-xylulose-5-phosphate (DXP) pathway. Additionally, the MVA-independent has been identified as an alternative pathway for isoprene production in bacteria, algae and plants which is known as the methylerythritol 4-phosphate (MEP) pathway or the non mevalonate pathway (Rodríguez-Concepción and Boronat, 2012). Isoprenoids were found to be used as flavors, neutraceuticals, polymers, and drugs (Withers *et al.*, 2007). Enzymes of the MEP pathway were found to be a promising targets for the development of drugs targeting infectious diseases such as malaria and tuberculosis, due to the occurrence of this pathway in pathogenic prokaryotes and its absence in human metabolic pathways (Gräwert *et al.*, 2011). Isoprene is naturally released by various herbaceous plants. However, these plants are unsuitable for isoprene production due to isoprene volatility as well as low solar energy transfer efficiency (Melis, 2009; Lindberg *et al.*, 2010). The MEP pathway for isoprenoid bioproduction is essential in many eubacteria including the major human pathogen, *Mycobacterium tuberculosis*, as well as *apicomplexan* protozoa including the *Plasmodium* sp (Gräwert *et al.*, 2011). Just recently, the seven recombinant enzymes from the MVA biosynthetic pathway have been heterologously expressed in *Synechocystis* sp (Bentley *et al.*, 2014). In addition, isoprene has been successfully produced in *Synechocystis* sp PCC 6803 by heterologous expression of the isoprene synthase (IspS) from *Pueraria montana* which is also known as kudzu [GenBank Accession no. AY316691] (Lindberg *et al.*, 2010). Isoprene synthase from different plant species has been identified (Silver and Fall, 1991). The isoprene synthase gene from poplar was successfully isolated and heterologously expressed in *E. coli* (Miller *et al.*, 2001). Development of large bacterial isoprene production process could supply an efficient isoprene feedstock for chemicals, pharmaceuticals and synthetic rubber. It was found that *B. subtilis* produces isoprene through three diverse peaks that correspond to the three stages of growth; first the glucose catabolism and acetoin secretion, second acetoin re-assimilation from the medium and third the early stages of sporulation (Wagner *et al.*, 1999). *Bacillus* is considered as the most bacteria producing isoprene when compared to other bacterial species (Kuzma *et al.*, 1995). However, the isoprene synthase gene sequence was not identified in bacteria. The alignment analysis from the GenBank database sequence alignment results showed no homology sequence between the isoprene synthase from plants and any sequence in the *B. subtilis* genes (Julsing *et al.*, 2007). This study aimed to measure the produced isoprene by *B. subtilis* 6633 ATCC and *B. stearothermophilus* 12980 ATCC using gas chromatography-mass spectrometry, in addition to cloning of the synthetic Kudzu isoprene synthase gene into *E. coli* DH5α cells.

MATERIALS AND METHODS

Chemicals, Isoprene, 3-methyl-2-buten-l-ol was obtained from Sigma Aldrich Chemical Co. and acetonitrile solvent was used. Organisms, Bacteria obtained from Microbiological Resources Center (Mircen) and both *Bacillus* strains are American Type Culture Collection (ATCC). *E. coli* DH5α obtained from Novagen. Media, Luria-Bertani (LB) growth medium was used.

Quantitative analysis procedure of isoprene produced by *Bacillus* spp. using gas chromatography-mass spectrometry (GC-MS)

B. subtilis 6633 ATCC, *B. stearothermophilus* 12980 ATCC were inoculated into 5 ml LB and grown for 18 h at temperature of 45^{o}C for *B. subtilis* 6633 ATCC and 55^{o}C for *B. stearothermophilus* 12980 ATCC with shaking at 150 rpm.1 ml of each culture (900 µl solvent +100 µl bacterial culture supernatant) injected onto a DB-1 column (1 µm film thickness, 0.25 mm diameter, 30 m long) connected to a 7000 Agilent mass selective detector (electron ionization, operated in total ion mode). The temperature program for each GC-MS run included a 2 min hold at 20^{o}C followed by a warming rate of 2^{o}C per min up till 40^{o}C and then with a rate15^{o}C per min to 55^{o}C. Quantitative analysis of the produced Isoprene by *B. subtilis* 6633 ATCC and *Bacillus stearothermophilus* 12980 ATCC using Gas Chromatography-Mass Spectrometry was done at the Regional Center for food and feed Institute, Agricultural Research Center.

Retention times, gas chromatography and mass spectrum parameters

Mass spectra and peak retention times gained from bacterial head pace were compared with the retention time and mass spectrum of an authenticated isoprene standard for positive detection of bacterial isoprene production. GC parameters; in which the temperature of the inlet was 130^{o}C as well as split mode ratio was 40:1.

Mass spectrum parameters; source temperature was 250^{o}C and selective ion mass (SIM) used were 39, 53, 67 and 68.

Transformation of the synthetic *kIspS* into *E. coli* DH5α

The synthetic Kudzu isoprene synthase from pBA2(S)kIKmA2 (Figure 1) was provided by Anastasios Melis (Lindberg *et al.*, 2010). A double enzyme digestion for pBA2(S)kIKmA2plasmid (Figure 2) was performed using *Nde* I and *Bam* HI to confirm that the plasmid is harbouring the Kudzu isoprene synthase gene of 1.7 kb. Then after to amplify the *kIspS* insert, primers used were forward primer *Nde*I-*kIspS* 5'-AACACATATGATGCCATGGATTTGTGCTACGAG C-3' and reverse primer *Not*I-*kIspS* 5'-ATCCGCGGC-CGCTTACACGTACATTAGTTGATTGATTGG-3'.
The amplification reaction was performed using PCR with an initial cycle at 95^{0}c for 5min, followed by 30 cycles 95^{0}c for 30 sec, 58^{0}c for 30^{0}c sec, 72^{0}c for 2 min and a final extension at 72^{0}c or 5 min. Then, PCR was cleaned up and the fragment was digested with *Nco* I and *Not* I restriction enzymes, ligated at the corresponding sites with pET-28b forming the new plasmid pET28b-*kIspS*. Transformation of the pET28b plasmid and the recombinant plasmid pET28b-*kIspS* into *E. coli* DH5α was carried out using heat shock method (Mamiatis *et al.*, 1985). PCR colony technique was carried out for screening of *E. coli* cells containing the pET28b-*kIspS*.

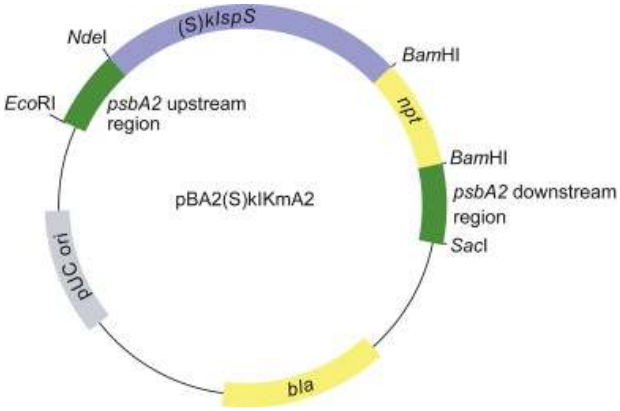

Figure 1. The pBA2kIKmA2 plasmid (Addgene plasmid #39213). Gene/insert name: Kudzu Isoprene Synthase (*kIspS*). Insert size: 1719 bp (1.7kb). Plasmid size: 6449 bp.

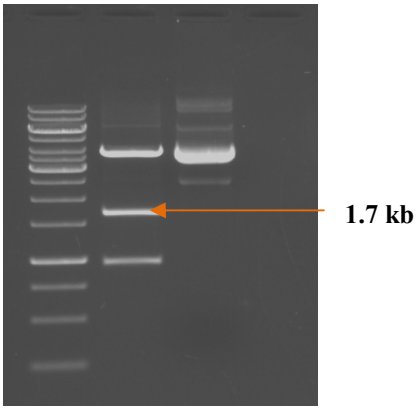

Figure 2. pBA2kIKmA2 plasmid double digestion by *Nde* I and *Bam* HI. Marker: GeneRuler 1 kb DNA ladder. Lane 1: the vector is double digested by *Nde* I and *Bam* HI. In which the upstream fragment is from psbA2 upstream region till psbA2 downstream region. The middle fragment is the *kIspS* (1.7 kb). The downstream fragment: *npt*, which is conferring resistance to kanamycin (~812 bp). Lane 2: vector without digestion (6449 bp).

SDS-PAGE analysis

E. coli DH5α cells containing the pET28b plasmid as well as cells containing the recombinant pET28b-*kIspS* were grown in LB (Luria Bertoni) medium at 37°C, 150 rpm for 4h with and without 0.1mM IPTG induction. All cells were harvested by centrifugation at 4,580×*g* for 20 min. Total soluble proteins of crude extracts were separated on 8–12% SDS-PAGE (Laemmli, 1970). Samples were prepared in which the sample should be 5 times the 1 X loading buffer (50 mM Tris-Cl (pH 6.8), 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol), boiled for 5 min at 95°C, centrifuge 10 min at 13,000 rpm, then load 10µl /well. Voltage was adjusted as 30 ml ampere/gel for 50 min.

RESULTS AND DISCUSSION

Quantitative analysis of the naturally produced isoprene by *Bacillus subtilis* and *Bacillus stearothermophilus*

Previous studies demonstrated that *B. subtilis* bears an isoprene synthase activity which utilizes the dimethylallyl diphosphate (DMAPP) as a substrate for isoprene production (Sivy *et al.*, 2002). Additionally, the isoprene synthase activity was optimal at pH 6.2 as well as it requires low levels of divalent ions and it was found to be separated from the chloroplast isoprene synthase. Moreover, various *B. subtilis* genes involved in the MEP pathway were determined by studying mutant's isoprene emission (Julsing *et al.*, 2007). In which the five genes, *IspC*, *IspD*, *IspE*, *IspF*, and *IspH*, were determined to be essential for the isoprene production in *Bacillus*. However, gene knockout of the sixth gene *IspG*, did not cause a decrease in isoprene production (Julsing *et al.*, 2007). In this study isoprene produced by *B. subtilis* 6633 ATCC and *B. stearothermophilus* 12980 ATCC was analyzed using gas chromatography-mass spectrometry, in which retention time was 3.61 for isoprene standard (Figure 3), 3.69 for *B. stearothermophilus* (Figure 4), and 3.68 for *B. subtilis* (Figure 5). On one hand, *B. stearothermophilus* 12980 ATCC produced 1.169 mg/ml isoprene, when incubated at 55°C for 18h. On the other hand, *B. subtilis* 6633 ATCC produced 1.071 mg/ml isoprene when incubated at 45°C for 18h. It was demonstrated that heat induces isoprene production in bacteria (Xue and Ahring, 2011), in which isoprene increases at temperature ranging between 25°C - 45°C as well as decreases until it reaches zero at 65°C, moreover optimum bacterial isoprene production was found to be obtained at temperature of 45°C (Kuzma *et al.*, 1995). Production of isopentenol was determined in *E. coli* by the dephosphorylation of IPP and DMAPP (Connor and Atsumi, 2010).

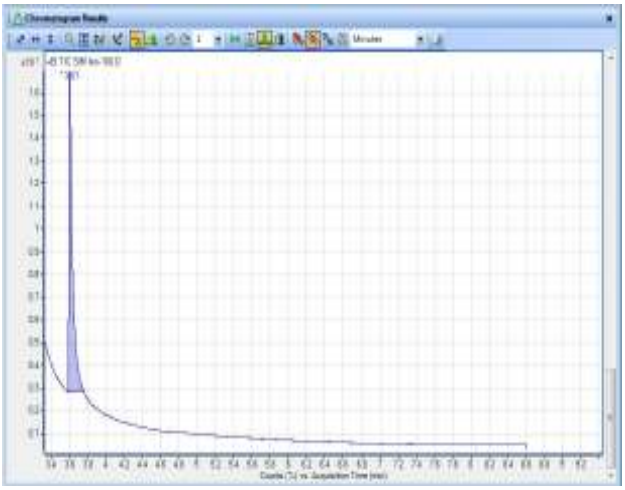

Figure 3. Isoprene standard, in which retention time was 3.61. Mass spectrum parameters; source temperature was 250°C and selective ion mass (SIM) were 39, 53, 67 and 68.

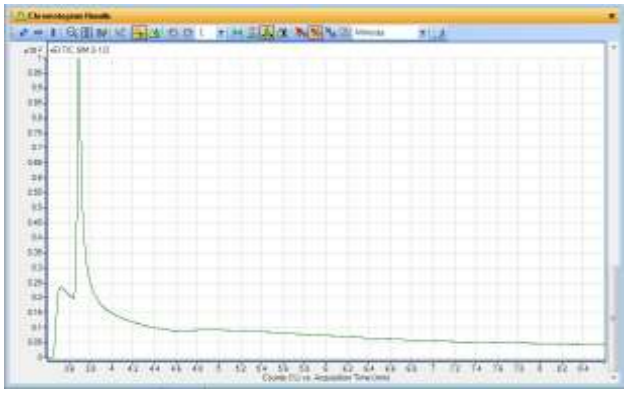

Figure 4.Quantitative analysis of isoprene produced by *B. stearothermophilus*12980 ATCC using GC-MS. In which *B. stearothermophilus* produced 1.169 mg/ml isoprene during incubation at 55°C for 18h and retention time was 3.69.

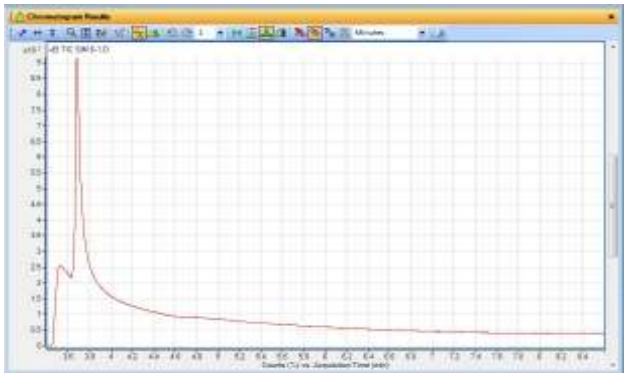

Figure 5. Quantitative analysis of isoprene produced by *B. subtilis* 6633 ATCC using GC-MS. In which *B. subtilis* produced 1.071 mg/ml isoprene when incubated at 45°C for 18h and retention time was 3.68.

Table 1:

Sample	Isoprene concentration
Bacillus stearothermophilus	1.169 mg/ml
Bacillus subtilis	1.071 mg/ml

Detection of recombinant pET28b-*kIspS* plasmid in *E. coli* DH5α

Detection for the positive colonies using colony PCR showed successful transformation of the pET28b-*kIspS* into *E. coli* DH5α (Figure 6), the positive transformed colonies appeared at the expected size of the insert *kIspS* (1.7 kb). Previously poplar isoprene synthase gene was isolated and transformed into *E. coli* (Miller *et al.*, 2001). Later on, studies revealed that *E. coli* showed highest growth rate when glucose is utilized as an organic carbon source in the media (Zurbriggen *et al.*, 2012).

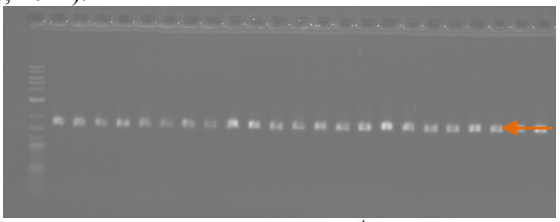

Figure 6. Colony PCR results. 1st lane: 2-Log DNA Ladder (0.1-10.0 kb). The rest of the lanes are for positive colonies harbouring pET28b-*kIspS*.

SDS-PAGE of the pET28b-*kIspS* in *E. coli*

A band appeared in the predicted size of the *kIspS* protein at 65 Kd for the SDS-PAGE of pET28b-*kIspS* in *E. coli* as well as the 0.1 mM IPTG induction enhances the isoprene production (Figure 7). Isoprene production assays demonstrated that *kIspS* expression in *E. coli* best activity was obtained at 37°C for 6h by 0.1 mM IPTG induction (Zurbriggen *et al.*, 2012).

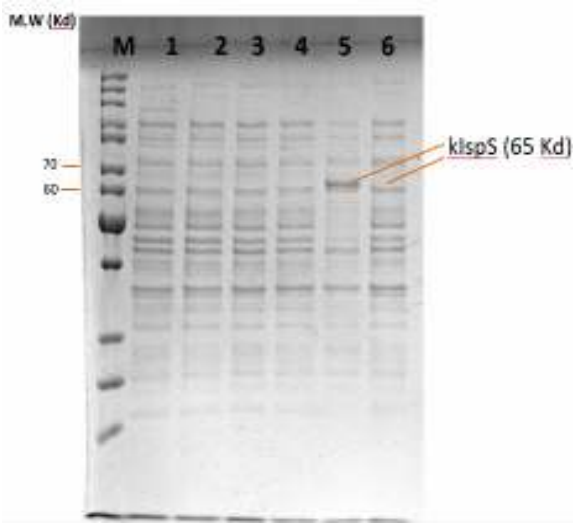

Figure 7. SDS-PAGE of pET28b-*kIspS* in *E. coli* for 4h incubation at 37°C. *kIspS* protein presented at 65 Kd with 0.1M IPTG. Marker: Page Ruler unstained protein ladder product, lane 1: *E. coli* 0.1 mM IPTG induced, lane 2: *E. coli* non IPTG induced, lane 3: *E. coli* harbouring pET28b 0.1 mM IPTG induced, lane 4: *E. coli* harbouring pET28b non IPTG induced, lane 5: *E. coli* harbouring pET28b-*kIspS* 0.1 mM IPTG induced, lane 6: *E. coli* harbouring pET28b-*kIspS* non IPTG induced.

Previous studies demonstrated that *Synechocystis* PCC6803 and *E. coli* are responsive strains for heterologous transformation by the IspS gene, in which they express and store the isoprene protein into their cytosol (Lindberg *et al.*, 2010). Isoprene synthase activity was assayed by (Lehning *et al.*, 1999) in which the isoprene synthase is at a steady state at higher temperatures and the exponentially rate is increased to the maximum between 40°C to 42°C (Sanadze and Kalandaze, 1966; Loreto and Sharkey, 1990; Monson *et al.*, 1992; Sharkey and Loreto, 1993; Sharkey *et al.*, 1996; Singsaas and Sharkey, 1998, 2000). The Km value of the recombinant *kIspS* enzyme was identified to be 2.5 mM, whereas the enzymatic activity Vmax was 4.1 $\mu mol.mg^{-1}.min^{-1}$, while the kcat was 4.4 s−1. Therefore, the kcat/Km ratio was described to be 1,760:1. The Vmax in the previous study was determined to be 52-fold higher than Sharkey study (Sharkey *et al.*, 2005). The isoprene synthase proteins as well as genes have been characterized from various *Populus* species e.g. aspen, *Poplar alba* (Silver and Fall, 1995; Miller *et al.*, 2001; Sasaki *et al.*, 2005; Sharkey *et al.*, 2005; Fortunati *et al.*, 2008; Vickers *et al.*, 2010; Chotani *et al.*, 2013; Beatty *et al.*, 2014). In addition to, the isoprene synthase from *Pueraria montana* (Kudzu), *Pueraria lobata* (Sharkey *et al.*, 2005; Beatty *et al.*, 2014; Hayashi *et al.*, 2015). Additionally, the isoprene synthase from *Myrtus communis* with the common name myrtle and *Mucuna bracteata* (Hayashi *et al.*, 2015). In poplar and other plants, isoprene synthase is found in three to four copies; these genes are regulated differentially (Vickers *et al.*, 2010).

CONCLUSION

There is a need to develop renewable fuels and chemicals from sustainable carbon alternatives. Enabling large bacterial isoprene production process could supply an efficient isoprene, in which further studies might develop recombinant bacteria as a biological source capable of isoprene production for pharmaceuticals production, chemical feedstock and rubber synthesis. The MEP pathway awaits detailed characterization in which it is considered as a promising pathway for providing new drugs and herbicides.

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REFERENCES

Beatty MK, Hayes K, Hou Z, Meyer DJ, Nannapaneni K, Rife CL, Wells DH, Zastrow-hayes GM (2014) Legume isoprene synthase for production of isoprene. U.S. Patent No. 8,895,277

Bentley FK, Zurbriggen A, Melis A (2014) Heterologous expression of the mevalonic acid pathway in cyanobacteria enhances endogenous carbon partitioning to isoprene. Mol plant 7 (1):71-86

Chotani GK, Nielsen A, Sanford KJ (2013) Reduction of carbon dioxide emission during isoprene production by fermentation. U.S. Patent No. 8,470,581

Connor, R. M, Atsumi S (2010) Synthetic biology guides biofuel production. BioMed Res. Int. 2010: 9 pages

Fortunati A, Barta C, Brilli F, Centritto M, Zimmer I, Schnitzler JP, Loreto F (2008) Isoprene emission is not temperature-dependent during and after severe drought-stress: a physiological and biochemical analysis. Plant J 55(4):687-697

Gräwert T, Groll M, Rohdich F, Bacher A, Eisenreich W (2011) Biochemistry of the non-mevalonate isoprenoid pathway. Cell Mol Life Sci 68 (23):3797-3814

Hayashi Y, Harada M, Takaoka S, Fukushima Y, Yokoyama K, Nishio Y, Tajima Y, Mihara Y, Nakata K (2015) Isoprene synthase and gene encoding the same, and method for producing isoprene monomer. U.S. Patent No. 8,962,296

Julsing MK, Rijpkema M, Woerdenbag HJ, Quax WJ, Kayser O (2007) Functional analysis of genes involved in the biosynthesis of isoprene in *Bacillus subtilis*. Appl microbiol Biotechnol 75(6):1377-1384

Kuzma J, Nemecek-Marshall, M., Pollock, W. H., Fall R (1995) Bacteria produce the volatile hydrocarbon isoprene. Curr Microbiol30(2): 97-103

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lehning, A., Zimmer, I., Steinbrecher, R., Brüggemann, N., & SCHNITZLER, J. P. (1999). Isoprene synthase activity and its relation to isoprene emission in Quercus robur L. leaves. Plant Cell Environ22(5):495-504

Lindberg P, Park S, Melis A (2010) Engineering a platform for photosynthetic isoprene production in cyanobacteria, using Synechocystis as the model organism. Metab Eng12(1):70-79

Loreto, F., & Sharkey, T. D. (1990) A gas-exchange study of photosynthesis and isoprene emission inQuercus rubra L. Planta 182(4):523-531

Mamiatis T, Fritsch Et, Sambrook J, Engel J (1982) Molecular cloning–A laboratory manual. New York: Cold Spring Harbor Laboratory

Melis, A. (2009) Solar energy conversion efficiencies in photosynthesis: minimizing the chlorophyll antennae to maximize efficiency. Plant sci 177(4):272-280

Miller, Barbara, Oschinski C, Zimmer W (2001) First isolation of an isoprene synthase gene from poplar and successful expression of the gene in Escherichia coli. Planta 213(3):483-487

Monson, R. K., Jaeger, C. H., Adams, W. W., Driggers, E. M., Silver, G. M., & Fall, R. (1992) Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. Plant Physiol 98(3):1175-1180

Rodríguez-Concepción M, Boronat A (2012) Isoprenoid biosynthesis in prokaryotic organisms. In Isoprenoid Synthesis in Plants and Microorganisms. Springer; 1-16

Sanadze G, Kalandaze A (1966) Light and temperature curves of the evolution of C5H8. Soviet Plant Physiol 13:458-461

Sasaki, Kanako, Ohara K, Yazaki K (2005) Gene expression and characterization of isoprene synthase from Populus alba. FEBS letters 579(11):2514-2518

Sharkey, Thomas D., et al (2005) Evolution of the isoprene biosynthetic pathway in kudzu. Plant Physiol 137(2):700-712

Sharkey, T. D., & Loreto, F. (1993). Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia 95(3):328-333

Sharkey, T. D., Singsaas, E. L., Vanderveer, P. J., & Geron, C. (1996). Field measurements of isoprene emission from trees in response to temperature and light. Tree Physiol 16(7):649-654

Silver, G. M., & Fall, R. (1991). Enzymatic synthesis of isoprene from dimethylallyl diphosphate in aspen leaf extracts. Plant Physiol 97(4):1588-1591

Silver GM, Fall R (1995) Characterization of aspen isoprene synthase, an enzyme responsible for leaf isoprene emission to the atmosphere. J Biol Chem 270(22):13010-13016

Singsaas, E., & Sharkey, T. (1998) The regulation of isoprene emission responses to rapid leaf temperature fluctuations. Plant Cell Environ 21(11):1181-1188

Singsaas, E., & Sharkey, T. (2000) The effects of high temperature on isoprene synthesis in oak leaves. Plant Cell Environ 23(7):751-757

Sivy, L. T, Shirk MC, Fall R (2002) Isoprene synthase activity parallels fluctuations of isoprene release during growth of *Bacillus subtilis*. Biochem Biophys Res Commun 294(1):71-75

Vickers CE, Possell M, Hewitt CN, Mullineaux PM (2010) Genetic structure and regulation of isoprene synthase in Poplar (Populus spp.). Plant mol biol 73(4-5):547-558

Wagner WP, Nemecek-Marshall M, Fall R (1999) Three distinct phases of isoprene formation during growth and sporulation of Bacillus subtilis. J bacteriol 181 (15):4700-4703

Withers ST, Gottlieb SS, Lieu B, Newman JD, Keasling JD (2007) Identification of isopentenol biosynthetic genes from *Bacillus subtilis* by a screening method based on isoprenoid precursor toxicity. Appl Environ Microbiol73(19):6277-6283

Xue, Junfeng, Ahring BK (2011) Enhancing isoprene production by genetic modification of the 1-deoxy-d-xylulose-5-phosphate pathway in *Bacillus subtilis*. Appl Environ Microbiol77.7:2399-2405

Zurbriggen, Andreas, Kirst H, Melis A (2012) Isoprene production via the mevalonic acid pathway in *Escherichia coli* (Bacteria). BioEnergy Res5.4:814-828

دراسات جزيئية علي إنتاج الأيزوبرين في البكتريا الطبيعية والمعدلة وراثياً

لميس جمعة[1]، حجاج صلاح زين[2]، ناهد عبد الغفار[1]، نجلاء عبد الله[2]

[1]معهد بحوث الهندسة الوراثية الزراعية، مركز البحوث الزراعية، الجيزة، 12619، مصر

[2]قسم الوراثة، كلية الزراعة، جامعة القاهرة، الجيزة، ١٢٦١٣، مصر

تعتبر مادة الأيزوبرين هيدروكربون (وهي مادة طبيعية متطايرة) أساسية لإنتاج الكيماويات والأدوية والمطاط الصناعي. ويمكن إستخراجها من النباتات والحيوانات والبكتريا. وقد تم تعريف جين *isoprene synthase* في النباتات ، ولكن لم يتم تعريفه في البكتريا. وتعتبر *Bacillus subtilis* أكثر أنواع البكتريا إنتاجاً للأيزوبرين. تم قياس الأيزوبرين المستخرج طبيعيا من كل من *Bacillus subtilis* ATCC 6633 و *Bacillus stearothermophilus* ATCC 12980 عن طريق التحليل الكمي بإستخدام (GC-MS)، مقارنة بمادة الأيزوبرين الكيمائية كمعيار للقياس. وتم الحصول علي النتائج الآتية لوقت الإحتفاظ أو البقاء :3.61 لمادة الأيزوبرين الكيمائية كمعيار للقياس و 3.69 للـ *B. stearothermophilus* و 3.68 للـ *B. subtilis* . وتم تحضين *B. stearothermophilus* لمدة ١٨ ساعة عند درجة حرارة 55°C بحيث أنتجت 1.169 mg/ml أيزوبرين، وعندما تم تحضين *B. subtilis* لمدة ١٨ ساعة عند درجة حرارة ٤٥°C فقد أنتجت 1.071 mg/ml أيزوبرين. كما قد تم استنساخ جين *isoprene synthase* المصنع من نبات ال Kuzdu وذلك لتعبير الجين في الـ *E. coli* عن طريق بلازميد pET28b. ولتأكيد تعبير الجين في الـ *E. coli* تم تحليل البروتينات عن طريق SDS-PAGE ، بحيث تم الحصول علي band عند الحجم المتوقع للبروتين الناتج عن جين *isoprene synthase* وهو 65 Kd.ولذا فمن المتوقع مستقبلا إستنساخ بكتريا كمصدر حيوي لإنتاج الأيزوبرين وذلك مساهمة من أجل الحصول علي الكيماويات والمطاط الصناعي.