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MDPI

Supplementary Materials

Geranyl Diphosphate Synthase (CrtE) Inhibition Using Alendronate Enhances Isoprene Production in Recombinant *Synechococcus elongatus* UTEX 2973: A Step towards Isoprene Biorefinery

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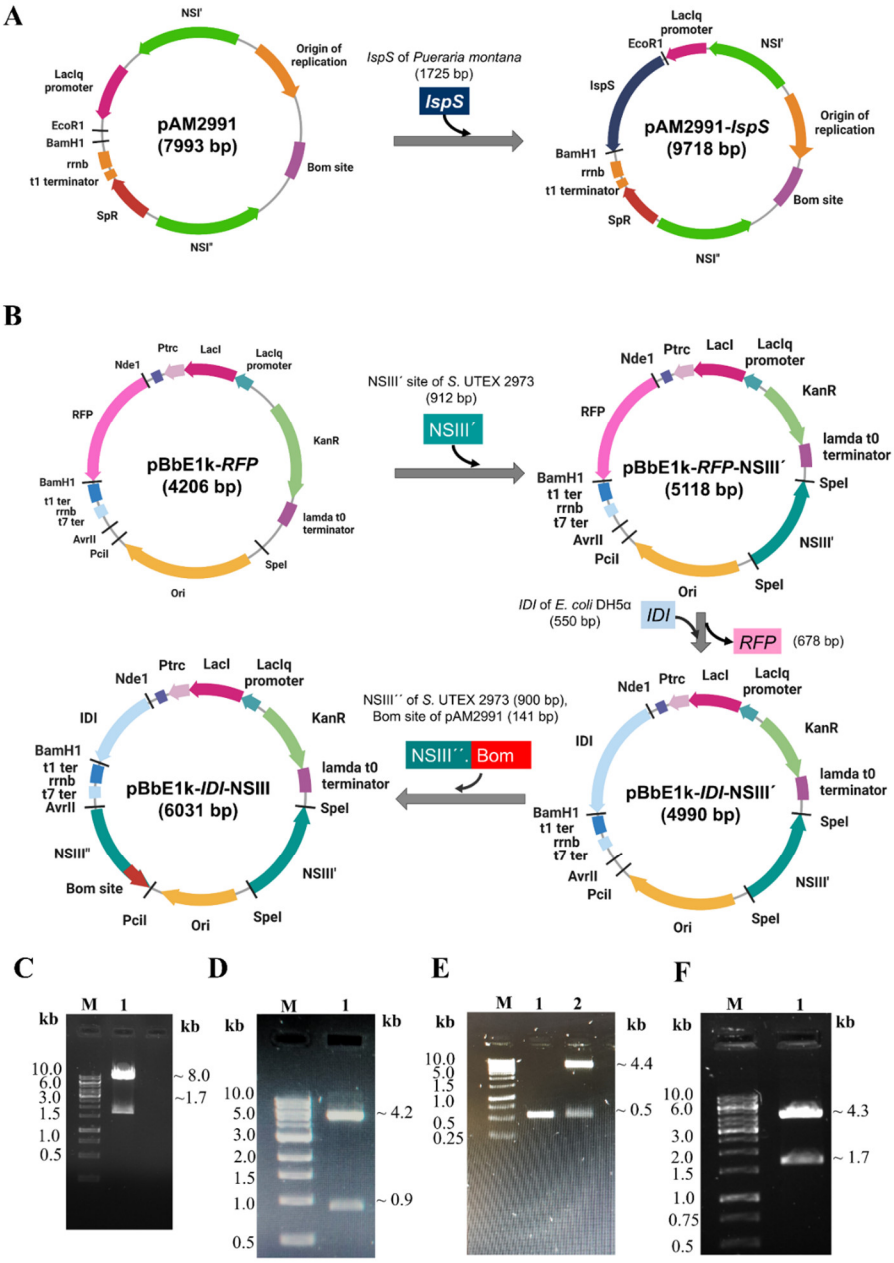
Supplementary Table S1. List of primers used.

Name	Sequence (5′-3′)	Tm (°C)
IspS FP	CGGAATTCATGCCCTGGCGTGTAATCTGTG	64.4
IspS RP	CGGGATCCCCTCTAGATTACACGTACATTAATTG	63.2
NSI′ FP	GACTAGTCAGCTTAGTCCTGCGCAATCT	51.8
NSI′ RP	GACTAGTCGAAATGTTCTGGACTTGCAGC	52.4
NSI′′ FP	CCTAGGTGAAACAAACCACGGGCA	48.0
NSI′′ RP	CCTAGGGACACCAAAATCACCACG	48.0
NSIII′ FP	GACTAGTCTCGAGATCAGCCAGCTC	57.0
NSIII′ RP	GACTAGTCGACCGACCGATCAACCA	58.0
NSIII′′ FP	ATA CCTAGG GACAAGCCGGGGCAG	62.0
NSIII′′ RP	ATTCTGTGGATAACCGTATTACCGCCTTTGACAGTCGGCGTCACGG	51.1
bom FP	CAAAGGCGGTAATACGGTTATCCACAGAATGCGTCGGTACTGGGTC	51.1
bom RP	AGAACATGTGGACTACGCCATAAAAGAGG	58.0
IDI FP	GCATGACAT ATGCAAACGGAACACGTC	59.7
IDI RP	CGGGATCCTTATTTAAGCTGGGTAAATGCAG	61.7
IspS rt FP	TCGGTGGTGGACTGAAATG	51.1

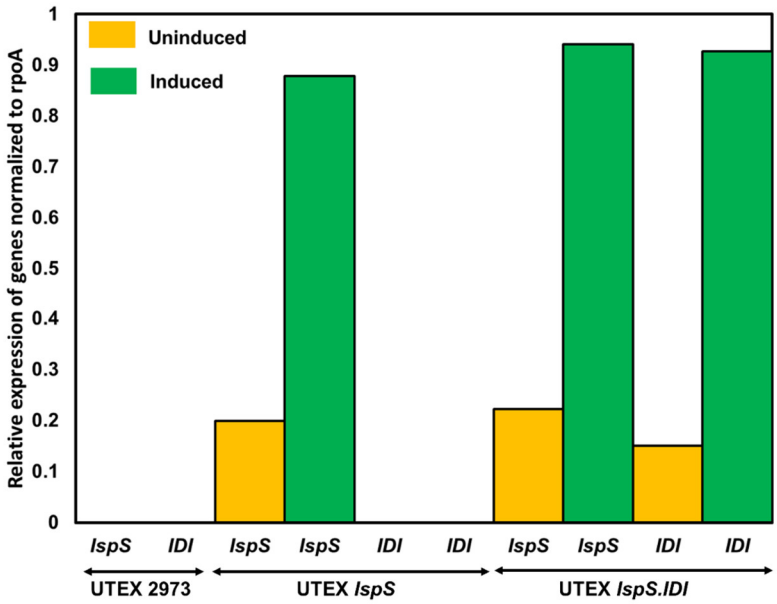
IspS rt RP	GTCACTGCCTTACGACACT	51.1
IDI rt FP	GCTGTTTAATGCCAAAGGAC	49.7
IDI rt RP	GATCACTGCGTCTTCGTTG	51.1
rpoA FP	GACATCTTGCTCAACGTCC	51.1
rpoA RP	CTTCAACTTCAGGGCCAAAG	51.8

Supplementary Table S2. Amplification of gene/DNA segment by PCR.

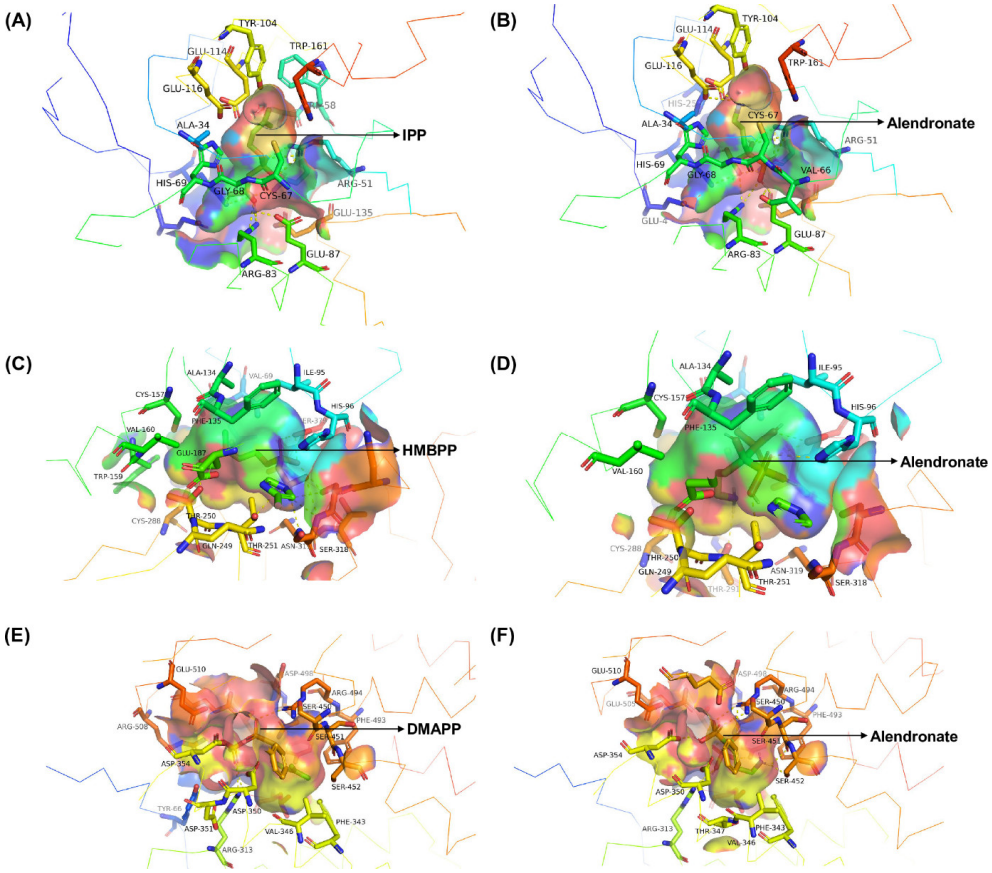
Gene/DNA segment	Amplification conditions
IspS	Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, **annealing at 57 °C for 20 sec, extension at 72 °C for 2 min** and final elongation at 72 °C for 10 min
IDI	Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, **annealing at 55 °C for 20 sec, extension at 72 °C for 1 min** and final elongation at 72 °C for 10 min
NSIII′; NSIII′′; NSIII′′.bom	Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, **annealing at 55 °C for 20 sec, extension at 72 °C for 1.5 min** and final elongation at 72 °C for 10 min
bom	Initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 sec, **annealing at 55 °C for 20 sec, extension at 72 °C for 30 sec** and final elongation at 72 °C for 10 min
Semiquantitative PCR of IspS/IDI/rpoA	Initial denaturation of 95 °C for 5 min, **followed by 35 cycles of denaturation at 94 °C for 1 min, annealing 20 sec at gene-specific temperatures (Supplementary Table S1), extension at 72 °C for 30 sec** with a final elongation at 72 °C for 10 min
Verification of genomic DNA integration	Initial denaturation of 95 °C for 5 min, **followed by 30 cycles of denaturation at 94 °C for 1 min, annealing 30 sec at gene-specific temperatures (Supplementary Table S1), extension at 72 °C (time according to amplicon size at rate 1 kb/min)** with a final elongation at 72 °C for 10 min



Supplementary Figure S1. Plasmid constructs preparation and digestion verification of inserts. (A) Schematic representation of pAM2991-*IspS* construct preparation (B) Scheme of sequential addition of DNA inserts to form BbE1k-*IDI*-NSIII plasmid construct (C) Digestion of pAM299-*IspS* to verify *IspS* insert by EcoRI and BamHI, M- molecular marker, 1- plasmid digest. (D) Digestion of construct pBbE1k-*RFP*-NSIII´ to verify NSIII´ insert by SpeI enzyme, M- molecular marker, 1- plasmid digest. (E) Digestion of construct pBbE1k-*IDI*-NSIII´ to verify *IDI* insert by NdeI and BamHI enzymes, M- molecular marker, 1- *IDI* gene (positive control), 2- plasmid digest. (F) Digestion of construct pBbE1k-*IDI*-NSIII to verify NSIII´´ insert by NdeI (an additional restriction site of NdeI is present in bom sequence), M- molecular marker, 1- plasmid digest.



Supplementary Figure S2. Densitometric analysis of semi-quantitative RT-PCR to test the expression levels of *IspS,* and *IDI* genes in uninduced and induced (1mM IPTG) conditions using ImageJ 1.53t software. *rpoA* gene was used as an internal control to normalize the expression level.



Supplementary Figure S3. Lowest energy docked poses of IDI, IspH, and IspS enzymes with their natural substrates and alendronate inhibitor. (A) Docking poses of IDI with IPP and (B) with alendronate (C) Docking pose of IspH with HMBPP and (D) with alendronate (E) Docking poses of IspS with DMAPP and (F) with alendronate. The substrates and alendronate have been labelled and shown as sticks.