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Appendix A

BG-11 media preparation for cyanobacterial cultivation

BG-11 medium is a widely used nutrient solution for the growth and maintenance of cyanobacteria. Firstly, the stock solution of the components required is made. Table A1 lists the components of BG-11 and the amount required to make stock solutions. From the stock solutions appropriate volume of the stock is taken, and deionized water is to make up the desired volume of BG-11. The media is mixed thoroughly and autoclaved at 121 °C for 15-20 minutes. The media is then cooled down to room temperature and components to be added after autoclaving are added under sterile conditions.

Table A1 BG-11 media composition

Stock No.	**Component**	**Amount for stock solution (gm/L)**	**Volume needed for BG-11 (ml/L)**
1	Sodium Nitrate	150 g	10 ml
2	Magnesium sulphate heptahydrate	75 g	1ml
3	Calcium chloride dihydrate	36 g	1ml
4	Sodium carbonate	20 g	1ml
Add after autoclaving			
5	Potassium phosphate dibasic trihydrate	40 g	1ml
6	Citric acid with Ferric ammonium citrate	6 g each	1ml
7	EDTA	1 g	1ml
Trace metals			
8	Boric acid	2.8 g	1ml
	Manganese chloride tetrahydrate	1.81 g	1ml
	Zinc sulphate heptahydrate	0.22 g	1ml
	Sodium molybdate dihydrate	0.39 g	1ml
	Copper sulphate pentahydrate	0.079g	1ml
	Cobalt nitrate hexahydrate	0.0494 g	1ml

Appendix B

Dry cell weight (DCW) calibration curve

To establish a dry cell weight (DCW) calibration curve for cyanobacteria, cultures were grown until they reached the exponential phase. Subsequently, aliquots of the cell culture, varying in optical density (OD), were prepared and centrifuged at 5000g for 10 minutes to pellet the cells. After pelleting, cultures were washed twice with distilled water to eliminate residual media and then resuspended in 5 mL of distilled water. These resuspended cultures were transferred into pre-weighed aluminium foil cups and subjected to drying in a hot air oven at 60°C for 6 hours. Upon drying, the cups containing the dried biomass were reweighed to obtain the final biomass weight by subtracting the initial weight of the cups. Finally, a calibration curve was constructed by plotting the dry cell weight against the corresponding optical density values. Careful attention was given to maintaining sterile conditions throughout the procedure to prevent contamination, and precise measurements were ensured for accurate results.

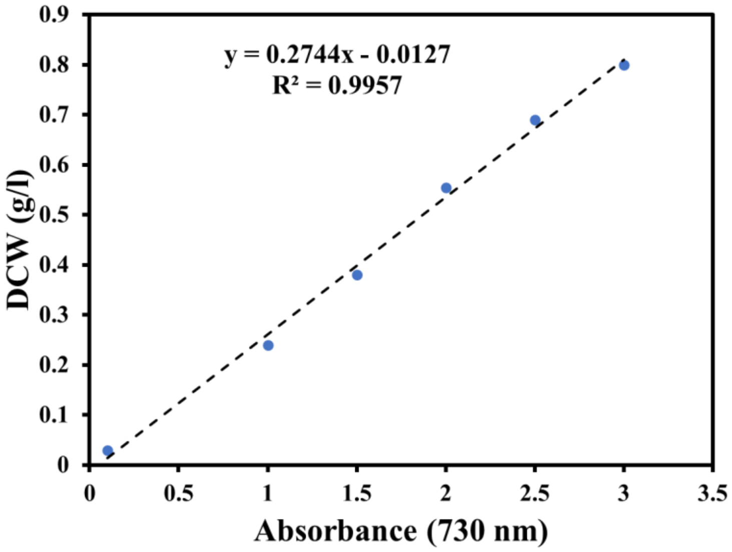

Fig. B1. Dry cell weight calibration curve for *Synechococcus elongatus* UTEX 2973.

Appendix C

Protein calibration curve through Bradford assay

The protein quantification assay, employing the Bradford method, estimates total protein concentration by exploiting the interaction between positively charged amino acids (such as arginine, lysine, and histidine) in proteins and the negatively charged Coomassie dye under acidic conditions. This interaction leads to a colour shift from brown to blue, enabling the quantification of protein content. Initially, a stock solution of BSA at 0.2 mg/ml concentration was prepared. Six test tubes were labelled as blank, 1, 2, 3, 4, and 5. Subsequently, volumes of the stock solution ranging from 50 to 250 μl are added into tubes 1 to 5 respectively, while the blank tube receives 2000 μl of sodium phosphate buffer (0.01 M) to maintain uniform volume across all tubes. Protein samples were diluted in a 1:1 ratio (dilution factor = 2) prior to the assay by adding buffer, and 50 μl aliquots from all samples were taken with volumes adjusted to 2000 μl. Following this, 5 ml of Bradford reagent was added to all samples and blank tubes, and they were then incubated in dark for 10 minutes. Finally, the absorbance of each solution was measured at 595 nm to determine protein concentration.

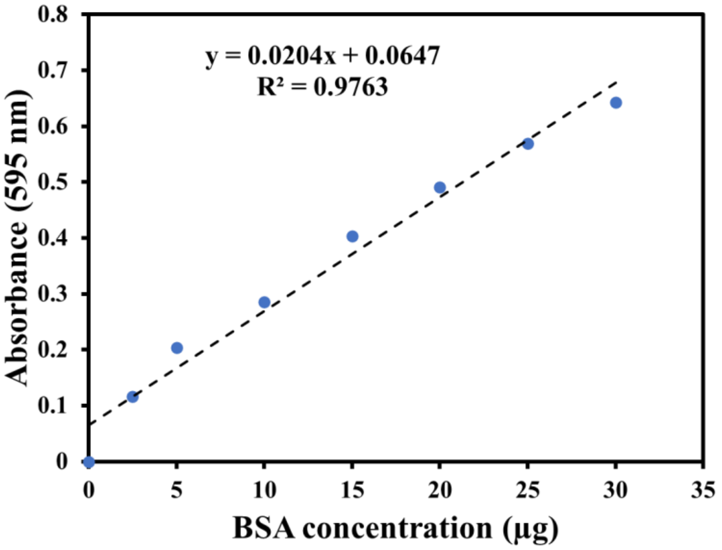

Fig. C1. BSA standard curve for protein estimation.

Appendix D

Farnesene calibration curve

Since the isopropyl myristate (IM) is being used in the experiments as an overlay to trap the synthesized farnesene, dilutions of pure farnesene are made in IM. Farnesene, Biofene (CAS# 18794–84-8) was provided by Amyris Inc., USA. Different dilutions in the range of 0.1 µg/µl to 1.0 µg/µl were made, and GC-FID analysis was done to make the area vs concentration curve. The initial oven temperature of the GC-FID was set at 60 °C for one minute, which was then ramped up to 230 °C at a rate of 15 °C/min and held for 5 min. Subsequently, the temperature was further increased to 250 °C at a rate of 25 °C/min and held for 15 min. The injector and detector temperature were maintained at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1.5 ml/min.

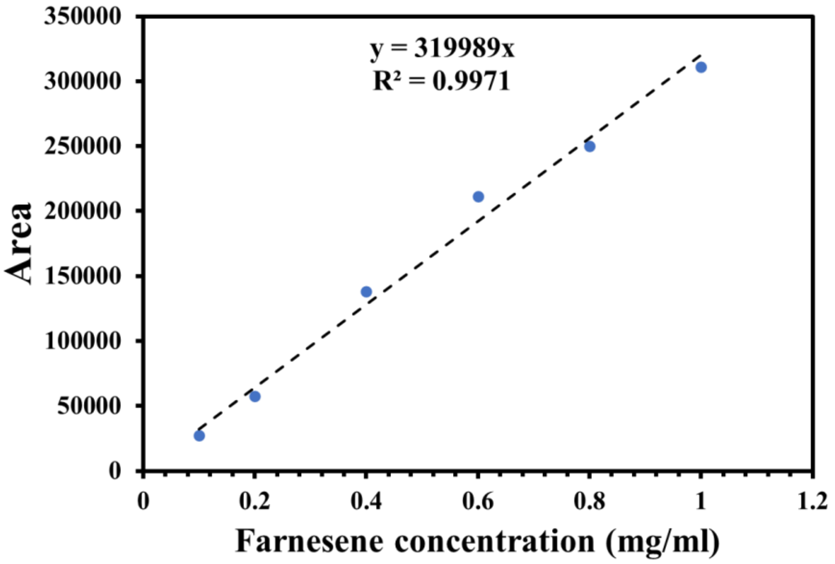

Fig. D1. Farnesene calibration curve.

Appendix E

RNA isolation and cDNA preparation

Some preparations must be made prior to RNA extraction. Firstly 0.1% DEPC water was made by adding DEPC to autoclaved double distilled water. The water was vortexed overnight to ensure proper dissolving in the dark. The next day, PCR tubes, microcentrifuge tubes, different pipette tips, mortar pestle, and spatula were soaked in DEPC water overnight in the dark. The next day, DEPC water was carefully decanted (can be reused 2-3 times), and the soaked items were dried in a hot air oven. The soaked items were double autoclaved and again dried in a hot air oven.

The RNA isolation was done by using a Qiagen RNeasy mini kit. For this purpose, the cyanobacterial cells were harvested by centrifugation at 4 °C. The cells were homogenized with the help of liquid nitrogen in a pre-cooled mortar pestle. The samples were kept on ice till further use. 350 µl of RLT buffer was added to each sample. The lysate was centrifuged at high speed for 3 minutes. The supernatant was removed and added to a fresh microcentrifuge tube. To the supernatant, 1 volume (~ 400 µl) of 70% molecular grade ethanol was added. The mixture was then transferred to the RNeasy mini spin column and centrifuged at high speed for a minute. On column DNase treatment was given by adding 80 µl DNaseI mix. This was incubated for 15 minutes at room temperature. After that, 350 µl of RW1 buffer was added to the column and centrifuged. The flow-through was discarded. Again, 600 µl RW1 buffer was added, centrifuged, and flow-through was discarded. 500 µl of RPE buffer was added, and centrifuged and flow-through were discarded. The column was centrifuged for an additional 2 minutes to discard any remaining RPE buffer. The RNeasy spin column was placed in a fresh 1.5 ml microcentrifuge tube. 40 µl of RNase-free water was directly added to the spin column and was centrifuged to collect the RNA. A nanodrop reading was taken to ensure the purity of the RNA.

The extracted RNA was used for cDNA preparation using Promega GoScript Reverse Transcription System. The RNA and random primers were combined in the following manner:

Component	**Volume**
RNA	up to 5 µg
Random primers	1 µl
Nuclease free water	To make up the volume
Total	10 µl

The above mixture was kept in a pre-heated heating block at 70 °C for 5 minutes. The mixture was chilled immediately for at least 5 minutes and spin down briefly. The mixture was kept on ice till further use.

Further reverse transcription reaction mixture was made in the following manner:

Component	**Volume**
5 X reaction buffer	4 µl
$MgCl_2$	1.2 µl
PCR nucleotide mix	1 µl
RNasin ribonuclease inhibitor	0.2 µl
Go script Reverse Transcriptase	1 µl
Nuclease free water	0.6 µl
Total	8 µl

To the above mix 10 µl of RNA and primer mix was added. The tubes were placed at 25 °C for 5 minutes to anneal. After that the it was placed at 42 °C for an hour for extension. Finally, the inactivation of reverse transcriptase was done at 70 °C for 15 minutes. The cDNA made was stored till further use.

Appendix F

pAM2991-*AFS* sequence

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Highlighted in pink is the bom site
Highlighted in grey is NSI" region
Highlighted in yellow is the *AFS* gene
Highlighted in green is NSI' region

Appendix G

pBbE1cNSII-*dxs* sequence

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Highlighted in yellow is the *dxs* gene
Highlighted in grey is NSII" region
Highlighted in pink is the bom site
Highlighted in green is NSII' region

Appendix H

pBbE1kNSIII-*idispA* sequence

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agccatccagtttactttgcagggcttcccaaccttaccagagggcgccccagctggcaattcc

Highlighted in yellow is the *idispA* fused gene
Highlighted in grey is NSIII" region
Highlighted in pink is the bom site
Highlighted in green is NSIII' region

Appendix I

List of Publications

From this work

1. **Akhil Rautela**, Indrajeet Yadav, Agendra Gangwar, Rishika Chatterjee and Sanjay Kumar (2024) “Photosynthetic production of α-farnesene by engineered *Synechococcus elongatus* UTEX 2973 from carbon dioxide.” Bioresource Technology, 396, 130432.
2. **Akhil Rautela**, Rishika Chatterjee, Indrajeet Yadav and Sanjay Kumar (2024) “A comprehensive review on engineered microbial production of farnesene for versatile applications.” Journal of Environmental Chemical Engineering, 12, 112398.
3. **Akhil Rautela** and Sanjay Kumar (2022) “Engineering plant family TPS into cyanobacterial host for terpenoids production.” Plant Cell Reports, 41, 1791-1803.
4. **Akhil Rautela**, Indrajeet Yadav, Agendra Gangwar, Rishika Chatterjee and Sanjay Kumar “Techno-economic analysis and life cycle assessment of sustainable farnesene production by genetically engineered cyanobacteria utilizing carbon dioxide: A step towards commercial viability” (Under review)

Others

5. Agendra Gangwar, Shweta Rawat, **Akhil Rautela**, Indrajeet Yadav, Anushka Singh and Sanjay Kumar (2024) “Current advances in produced water treatment technologies: A perspective of techno-economic analysis and life cycle assessment”. Environment, Development and Sustainability, 1-35.
6. Indrajeet Yadav, **Akhil Rautela**, Agendra Gangwar, Lokesh Wagadre, Shweta Rawat and Sanjay Kumar (2023) “Enhancement of isoprene production in engineered *Synechococcus elongatus* UTEX 2973 by metabolic pathway inhibition and machine learning-based optimization strategy.” Bioresource Technology, 387, 129677.
7. Shweta Rawat[#], **Akhil Rautela**[#], Indrajeet Yadav, Sibashis Misra and Sanjay Kumar (2023) “A comprehensive review on enhanced biohydrogen production: pretreatment, applied strategies, techno-Economic assessment, and future perspective.” BioEnergy Research, 1-24. ([#]Equal authorship)
8. Indrajeet Yadav, **Akhil Rautela**, Agendra Gangwar, Vigya Kesari, Aditya K. Padhi and Sanjay Kumar (2023) “Geranyl diphosphate synthase (CrtE) inhibition using

alendronate enhances isoprene production in recombinant *Synechococcus elongatus* UTEX 2973: A step towards isoprene biorefinery." Fermentation, 9, 217.

9. Indrajeet Yadav, **Akhil Rautela** and Sanjay Kumar (2021) "Approaches in the photosynthetic production of sustainable fuels by cyanobacteria using tools of synthetic biology." World Journal of Microbiology and Biotechnology, 37, 1-17.
10. Piyush Kumar, **Akhil Rautela**, Vigya Kesari, David Szlag, Judy Westrick and Sanjay Kumar (2020) "Recent developments in the methods of quantitative analysis of microcystins." Journal of Biochemical and Molecular Toxicology, 34.

Book chapters

1. **Akhil Rautela**, Shweta Rawat, Indrajeet Yadav, Agendra Gangwar and Sanjay Kumar (2023) "Process integration opportunities applied to microalgae biomass production." In Microalgae-Based Systems: Process Integration and Process Intensification Approaches, pp. 183-210. De Gruyter.
2. Indrajeet Yadav, **Akhil Rautela**, Shweta Rawat, Ajay Kumar Namdeo and Sanjay Kumar (2023) "Metabolic engineering of yeast for advanced biofuel production." In Advances in Yeast Biotechnology for Biofuels and Sustainability, pp. 73-97. Elsevier.
3. **Akhil Rautela** and Sanjay Kumar (2021) "Engineered microorganisms for production of biocommodities." In Biomolecular Engineering Solutions for Renewable Specialty Chemicals: Microorganisms, Products, and Processes, pp. 1-48. Wiley.
4. Jyoti Rani, Indrajeet Yadav, **Akhil Rautela** and Sanjay Kumar (2020) "Biovalorization of winery industry waste to produce value-added products." In Biovalorisation of Wastes to Renewable Chemicals and Biofuels, pp. 63-85. Elsevier.

Appendix J

Conferences/workshops

1. BioSangam 2022: Emerging trends in biotechnology, March 10-12, 2022, organized by the Department of Biotechnology, Motilal Nehru Institute of Technology Allahabad, Prayagraj, India.
2. International Lectures Workshop on Recent advances in Interdisciplinary Chemical Sciences (RAICS-2022) organized by Mahila Mahavidyalay, BHU, Varanasi, India.
3. BioSangam 2024: 6th International Conference on Bio-Technological Intervention for Health, Agriculture and Circular Economy, Feb 23-25, 2024, organized by the Department of Biotechnology, Motilal Nehru Institute of Technology Allahabad, Prayagraj, India.

Certificate

BioSangam 2024

February 23 - 25, 2024

THIS IS TO CERTIFY THAT

(Prof./Dr./Mr./Mrs./Ms.) Akhil Rautela

has won **Young Scientist Award** for his/her research presentation titled

Photosynthetic Production of Farnesene by Engineered Cyanobacteria

in **BioSangam 2024: 6th International Conference on Bio-Technological Intervention for Health, Agriculture and Circular Economy.**

Organized By
Department of Biotechnology
Motilal Nehru National Institute of Technology
Allahabad, Prayagraj - 211004, India

Sponsored By

Dr. Sangeeta Negi
Convener

Received the **Young Scientist Award** for the oral presentation on “Photosynthetic production of farnesene by engineered cyanobacteria” at the 6th International Conference on Bio-Technological Intervention for Health, Agriculture and Circular Economy, organized by the Department of Biotechnology, Motilal Nehru Institute of Technology Allahabad, Prayagraj, India.