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Metabolic Engineering of Algae for Sustainable Linalool Production: A Biotechnological Perspective

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Abstract

Linalool, a monoterpenoid alcohol widely prized for its aromatic properties, finds extensive applications across the flavor, fragrance, and pharmaceutical industries. Traditionally sourced from plant essential oils, its production is often constrained by low yields, seasonal variability, and the environmental impact of conventional agriculture. Metabolic engineering of microalgae offers a compelling and sustainable alternative. Leveraging their photosynthetic capabilities, rapid growth rates, and ability to thrive on non-arable land, algae present an eco-friendly bioproduction platform. This review delves into the current biotechnological strategies employed for enhancing linalool synthesis in algae, highlighting the critical metabolic pathways, engineering approaches, and the existing challenges that need to be addressed for commercial viability.

Keywords: Algal biotechnology, renewable resources, metabolic engineering, bioproduction

Introduction

Linalool ($C_{10}H_{18}O$) is a naturally occurring acyclic monoterpenoid alcohol, known for its pleasant floral scent and bioactive properties such as antimicrobial, anti-inflammatory, and anxiolytic effects (Peana et al., 2002). It is commonly found in the essential oils of plants like lavender, coriander, and basil. Due to increasing demand in perfumery, pharmaceuticals, and green pesticides, sustainable production routes are urgently needed (Beekwilder et al., 2014).

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is a chiral monoterpene alcohol that serves as a key ingredient in numerous products due to its characteristic floral and woody scent. Its demand is steadily increasing, driven by consumer preferences for natural ingredients. Current industrial production heavily relies on extraction from aromatic plants such as lavender (*Lavandula angustifolia*), coriander (*Coriandrum sativum*), and basil (*Ocimum basilicum*) (Bakkali *et al*., 2008). However, these methods are often resource-intensive, environmentally burdensome, and susceptible to geopolitical and climatic fluctuations.

Microalgae, as photosynthetic microorganisms, offer a sustainable solution for producing high-value compounds. They efficiently convert atmospheric CO_2 into biomass using solar energy, thus contributing to carbon capture. Their cultivation does not compete with food crops for land or freshwater,

and they can be grown in diverse environments, including saline or wastewater. These attributes position microalgae as an ideal platform for the biotechnological production of various chemicals, including terpenoids (Georgianna *et al.,* 2012). Metabolic engineering allows for the redirection of algal metabolic flux towards the synthesis of desired compounds, making it a powerful tool for sustainable linalool production.

The limitations of plant extraction—low yield, seasonal variation, and land usage—have encouraged research into microbial and algal biosynthesis (Vickers et al., 2017). Algae, particularly cyanobacteria and microalgae like *Chlamydomonas reinhardtii*, provide an ideal platform due to their photoautotrophic nature and compatibility with genetic engineering (Halfmann et al., 2014).

Linalool Biosynthesis: A Metabolic Overview

Linalool is synthesized via the isoprenoid pathway, which proceeds through two major routes:

- **Mevalonate (MVA) Pathway** – Common in eukaryotes, archaea, and some bacteria.
- **Methylerythritol phosphate (MEP) Pathway** – Predominant in algae, cyanobacteria, and higher plants.

In both pathways, the key precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are condensed to form geranyl pyrophosphate (GPP), which is then converted to linalool by linalool synthase (LIS) (Kirby & Keasling, 2009).

Terpenoid biosynthesis in plants and algae primarily proceeds via two distinct pathways: the mevalonate (MVA) pathway and the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway. In microalgae, the MEP pathway is the dominant route for producing plastid-derived terpenoids, including those that serve as precursors for linalool (Lichtenthaler, H. K. (1999).

The MEP pathway, localized in the chloroplasts, synthesizes the universal five-carbon isoprenoid building blocks: isopentenyl pyrophosphate (IPP) and its isomer, dimethylallyl pyrophosphate (DMAPP). These precursors are then condensed to form longer isoprenoid chains.

1. **IPP and DMAPP Synthesis:** Pyruvate and glyceraldehyde-3-phosphate are condensed and processed through a series of enzymatic reactions catalyzed by enzymes such as 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), ultimately yielding IPP and DMAPP (Lois *et al*., 2000).
2. **Geranyl Pyrophosphate (GPP) Formation:** IPP and DMAPP are condensed by geranyl diphosphate synthase (GPPS) to form the ten-carbon monoterpene precursor, geranyl pyrophosphate (GPP).
3. **Linalool Synthesis:** The final and crucial step involves the conversion of GPP to linalool, catalyzed by linalool synthase (LIS). This enzyme is not naturally present in most algal species, necessitating its heterologous expression for *de novo* linalool production.

Metabolic Engineering Strategies for Linalool Production

The overarching goal of metabolically engineering algae for linalool production is to increase the availability of precursors (IPP and DMAPP) and introduce or enhance the activity of LIS.

Enhancing Precursor Supply (MEP Pathway Optimization)

(i) Overexpression of Rate-Limiting Enzymes: The DXS enzyme is often considered a major flux-controlling step in the MEP pathway. Overexpressing the native or a heterologous DXS gene can significantly boost the supply of IPP and DMAPP, thus "pushing" more carbon flux towards terpenoid synthesis.

(ii) Blocking Competing Pathways: In some cases, downregulating or knocking out metabolic pathways that divert IPP/DMAPP towards other undesirable products (e.g., carotenoids or chlorophylls) can redirect precursors specifically towards linalool biosynthesis (Ma *et al*., 2016).

Heterologous Expression of Linalool Synthase (LIS)

(i) **Gene Introduction:** The primary strategy involves introducing a plant-derived LIS gene into the algal genome. LIS genes from various plants, such as *Ocimum basilicum* (basil), *Cinnamomum camphora* (camphor tree), or *Clarkia breweri*, have been successfully expressed in microbial hosts (Degenhardt *et al.,* 2000).

(ii) **Codon Optimization and Chloroplast Targeting:** To ensure efficient expression in the algal system, the heterologous LIS gene is typically codon-optimized to match the algal host's codon usage bias. Furthermore, the LIS enzyme is often engineered with a chloroplast transit peptide to ensure its localization within the chloroplast, where the MEP pathway precursors are readily available (Lauersen *et al*., 2015).

Subcellular Compartmentalization and Enzyme Scaffolding:

(i) **Chloroplast Engineering:** Targeting the entire linalool biosynthetic pathway (from MEP precursors to LIS) within the chloroplast can create a metabolically channeled environment, minimizing diffusion losses and maximizing enzymatic efficiency (Ramesh, S., & Abuelnaga, H. (2022).

(ii) **Enzyme Scaffolding:** Synthetic biology approaches, such as enzyme scaffolding, can physically link sequential enzymes in a metabolic pathway. This increases the local concentration of intermediates and reduces their degradation or diffusion away from the active sites, potentially enhancing linalool yield (Dueber *et al.,* 2010).

Strain Selection and Cultivation Optimization:

(i) **Model Algae:** *Chlamydomonas reinhardtii* is a widely used model microalga due to its well-characterized genetics, ease of transformation, and established molecular tools. Other industrially relevant strains like *Phaeodactylum tricornutum* or *Nannochloropsis oculata* are also being explored (Potvin, G., & Zhang, Z. (2010).

(ii) **Bioprocess Optimization:** Cultivation conditions (e.g., light intensity, CO□ supply, nutrient availability, temperature) play a crucial role in overall biomass accumulation and product yield. Two-stage cultivation strategies, where cells are first grown under optimal conditions for biomass and then shifted to conditions favoring product accumulation, can be beneficial (Hannon *et al.,* 2010).

Algae as Biofactories for Terpenoids

Algae are emerging as green cell factories for bio-based chemicals due to their ability to:

- Grow on non-arable land and wastewaters
- Use sunlight and CO□ for growth
- Be engineered for high-value compound production (Rosenberg et al., 2008)

Species like *Synechocystis sp. PCC 6803* and *Chlamydomonas reinhardtii* have been successfully modified for isoprenoid production including linalool (Lindberg et al., 2010).

Metabolic Engineering Strategies

Pathway Engineering

Integration of heterologous linalool synthase genes (e.g., from *Clarkia breweri* or *Arabidopsis thaliana*) into algal genomes has enabled production of linalool from CO□ and sunlight (Aharoni et al., 2003).

Precursor Supply Enhancement

Overexpression of rate-limiting enzymes such as DXS (1-deoxy-D-xylulose-5-phosphate synthase) and IDI (isopentenyl diphosphate isomerase) improves flux toward GPP (Ajikumar et al., 2010).

Cofactor and Energy Optimization

Efficient linalool synthesis requires a steady supply of NADPH and ATP. Engineering NADPH regeneration systems and optimizing light conditions can enhance productivity (Zhou et al., 2016).

Synthetic Biology and CRISPR Applications

Recent advances in synthetic biology have enabled modular design and rapid prototyping of engineered algal strains. CRISPR/Cas systems offer precise gene editing to knock out competing pathways and enhance flux toward linalool (Wang et al., 2016).

Use of regulatory elements (promoters, riboswitches, terminators) and biosensors allows fine-tuning of expression systems in algal hosts (Ruffing, 2014).

Cultivation and Bioprocess Strategies

For industrial application, cultivation systems (e.g., photobioreactors, open ponds) must be optimized for growth and linalool accumulation. Strategies include:

- Two-phase cultivation (growth phase and production phase)
- In situ product removal using solvents or adsorbents to reduce toxicity (Jongedijk et al., 2016)

Waste-to-Value Integration

Glycerol, a biodiesel by-product, has been explored as a low-cost carbon source for linalool production in engineered cyanobacteria (Li et al., 2020). This approach supports circular bioeconomy goals by valorizing industrial waste.

Challenges and Future Directions

Despite the significant progress in metabolically engineering algae, several hurdles remain for the commercial production of linalool:

- **Low Linalool Titer and Yield:** Current yields of linalool in engineered algae are often low, limiting their economic viability compared to traditional extraction or other microbial platforms (e.g., yeast). This can be attributed to the complex regulatory networks in algae, metabolic burden from heterologous protein expression, and potential toxicity of linalool to algal cells at high concentrations [13].
- **Genetic Tool Development:** While *C. reinhardtii* has robust genetic tools, many other industrial algal strains lack efficient and stable transformation systems, limiting the breadth of engineering possibilities.
- **Product Recovery and Purity:** Linalool is a volatile compound, and its *in situ* recovery from aqueous algal cultures can be challenging. Efficient and cost-effective downstream processing technologies for separation and purification are essential. Strategies such as **two-phase bioreactors**, where an organic solvent layer extracts linalool as it's produced, can mitigate product loss and reduce cellular toxicity [14].
- **Scalability and Bioreactor Design:** Moving from laboratory-scale proof-of-concept to large-scale industrial production requires optimized photobioreactor designs that can provide uniform light distribution, efficient CO□ transfer, and robust mixing, especially in regions with high solar irradiance like Virudunagar, Tamil Nadu.

Future directions should focus on:

- **Advanced Genome Editing:** Utilizing CRISPR-Cas9 technology for precise gene editing and multiplex engineering to simultaneously modify multiple genes involved in the MEP pathway and LIS expression [15].
- **Synthetic Biology Approaches:** Designing and implementing synthetic genetic circuits for inducible gene expression, allowing for temporal control over linalool production to separate growth and production phases.
- **Systems Biology Integration:** Employing omics technologies (genomics, transcriptomics, proteomics, metabolomics) to gain a comprehensive understanding of algal metabolism and identify new targets for engineering.
- **Process Intensification:** Developing integrated bioprocesses that combine efficient cultivation, *in-situ* product recovery, and streamlined downstream purification to enhance overall productivity and reduce costs.

Conclusion

Metabolic engineering of algae holds immense promise for the sustainable production of linalool. By harnessing the power of photosynthesis and applying advanced biotechnological tools, we can develop eco-friendly and economically viable platforms. Addressing the current challenges through innovative genetic engineering, synthetic biology, and bioprocess optimization will be key to unlocking the full potential of algae as microbial cell factories for valuable terpenoids like linalool, paving the way for a greener bio-based economy.

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