Simplified Harvesting Achieved by Cyanobacterial Cell Surface Engineering Economically Enables Machine Learning-informed Semi-continuous Cultivation for Sustainable Biofuel Production

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Background

Algae-based biofuel production is a promising solution to meet the increasing fuel demands and to slow down greenhouse gas accumulation. Despite the significance, its application and commercialization are still hindered by some technical barriers, including the growth limitation result from mutual shading and costly harvesting. We developed a machine learning- informed semi-continuous cultivation (MISC) system to successfully reduce the mutual shading during the cyanobacterial cultivation and achieved sustainable optimal growth. However, the MISC requires frequent harvesting, so it is economically infeasible unless a simplified and low-cost harvesting method is developed. Traditional harvesting methods such as centrifugation, chemical flocculation, and filtration are too expansive to fit the MISC[1,2]. Sedimentation is a promising method for low-cost harvesting, but it is slow and strain-specific[1,2]. As the settling rate is directly relevant to the particle size[2], we thereby aimed to enhance cell aggregation to enable/ enhance cell sedimentation. To achieve this, we took advantage of the natural hydrophobic effects and manipulated the cyanobacterial cell surface hydrophobicity with a synthetic biology design.

Methods

We selected *Synechococcus elongatus* UTEX 2973 (UTEX 2973)[3] as the model strain in this study, primarily due to its relatively smooth cell surface (loss of function in pilus assembly result from the mutation of pilN[4]) and high potential in biofuel production[5]. To increase the cell surface hydrophobicity of UTEX 2973 cells, we engineered the strain to produce limonene, a strong hydrophobic hydrocarbon that had been proven to be excreted from cyanobacterial cells[6]. The limonene synthesis is driven by a light-inducible promoter, psbA[6].

Results

We first verified the limonene producing strain, L524, by PCR, qPCR, and measuring limonene productivity. Limonene synthase and its expression were only detected in L524, and L524 shows limonene productivity at ~5mg/L/day. Bacterial adherence to hydrocarbons (BATH) assay suggests that cell hydrophobicity of L524 is higher than UTEX 2973 wildtype (WT). To further understand the mechanisms of the cell hydrophobicity changes, we observed L524 and WT under transmission electron microscopy (TEM). Putative limonene droplets were found on L524 cells but not on WT, suggesting the hydrophobicity increase in L524 could result from the limonene accumulation on the cell surface. The aggregation assay shows ~90% of L524 cells were aggregated, but no aggregation was found in WT, indicating the increased cell hydrophobicity successfully drives cell aggregation. The aggregation was observed to drive cell sedimentation, which could potentially be used for biomass harvesting. More importantly, the aggregation-enabled sedimentation is very fast, as 85% of cells were settled within 30 minutes, and the solid content from AES output reached ~14%, which is comparable to output from centrifugation.

Conclusion

In this study, we developed a simple, fast, and low-cost harvesting method, the AES, by taking advantage of the natural hydrophobic effects and manipulating the cyanobacterial cell surface hydrophobicity with a synthetic biology design. The AES achieves a high recovery rate and high solid content in the outputs. More importantly, its low-cost economically enabled the implementation of the machine learning-informed semi-continuous cultivation system, which ultimately addressed the technical barriers in algal-based biofuel production.

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