One-pot bioconversion of algae biomass into terpenes for advanced biofuels and bioproducts

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Abstract

Under robust algae growth conditions, algal carbohydrates and proteins typically comprise up to ~ 80% of the ash-free dry weight of microalgae biomass. Therefore, production of algal biofuel through comprehensive utilization of all algal components and the addition of high energy density fuel compounds with “fit for purpose” properties or high-value bioproducts will both diminish the process cost and improve the overall process feasibility. In this study, we firstly demonstrated the concept of a "one-pot" bioconversion of algal carbohydrate and protein into value-added terpene compounds as advanced biofuel and high value bioproducts to improve the overall process feasibility through the development of engineered microbial consortium. The consortium for caryophyllene production yielded the highest titer of total terpene, up to 507.4 mg/L, including 471 mg/L of sesquiterpene, 36.4 mg/L of monoterpene, and 124.4 mg/L of caryophyllene on algal hydrolysate from *Nannochloropsis sp*. Additionally, the consortium expressing chamigrene synthase produced 187 mg/L total terpene including 87 mg/L of monoterpene, 100 mg/L of sesquiterpene, and 62 mg/L chamigrene on hydrolysate from benthic polyculture biomass. Compared to the yields of terpene extracted from plant tissue, both consortia increased the terpene yield about 3~40 times, which makes it a promising alternative pathway for terpene production.

Key words: One-pot conversion, terpene, microbial consortium, algal biofuel, caryophyllene, chamigrene

Introduction

Rising demand for transportation fuels and the concerns with fossil fuel derived environmental pollution as well as the green-house gas emission derived climate change have resulted in the compelling need for alternative, sustainable energy sources(1). Algae-based biofuels have been considered one of the promising alternatives to fossil fuels as they can overcome some of these issues (2-4). The current state-of-the-art of algal biofuel technologies have primarily focused on biodiesel production through prompting high algal lipid yields under the nutrient stress conditions. There has been less emphasis on using algae-based carbohydrates and proteins as carbon sources for the fermentative production of liquid fuel compounds or other high-value bioproducts(5-7).

Terpenes are a group of natural products with over 55,000 structurally similar chemical compounds. Compared to biodiesel and other short- and medium-chain alcohols, these molecules contain near zero oxygen content, have various biological functionalities (8-12) and have high energy density, making them particularly attractive candidates as “drop-in” fuel candidates for aviation fuels(13-18). In this study, we demonstrated the concept of "one-pot" bioconversion of algal carbohydrates and proteins into terpenes as advanced biofuel compounds and the high value bioproducts (figure 1) through the development of engineered microbial bioconversion consortium.

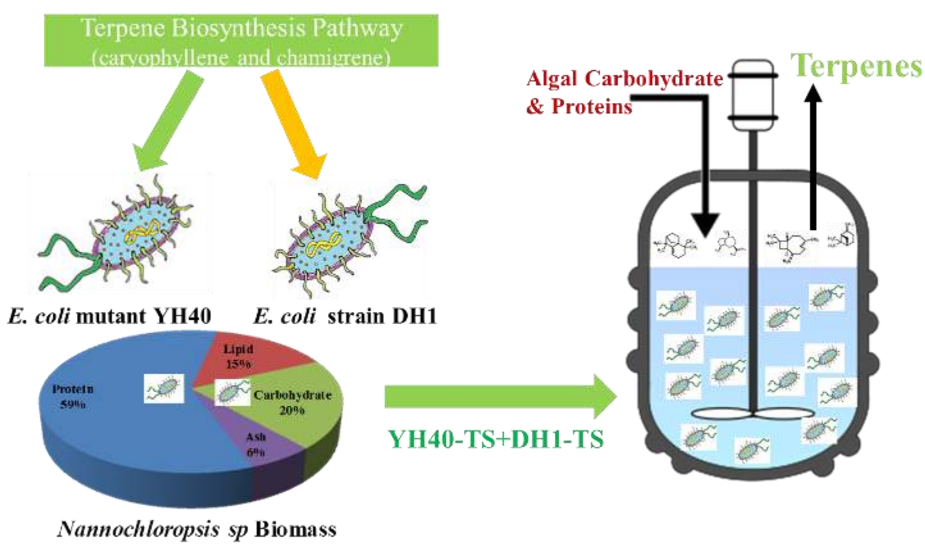

Figure 1.

Results and Discussion

Caryophyllene and chamigrene, natural bicyclic sesquiterpene (C15) compounds, are common components present in the essential oils from various plants (19-22). A recent study suggested that the blending of hydrogenated sesquiterpanes (in particular carophyllanes), which have a moderate cetane number and only moderately high viscosity, with synthetic branched paraffins to raise cetane and reduce viscosity, could produce biosynthetic fuels that meet applicable jet fuel and diesel specifications(23). Therefore, caryophyllene and its isomers have been deemed to be among the top three most promising candidates for jet fuel with high energy density (24). In our previous study, we discovered and functionally characterized caryophyllene and chamigrene synthases from endophytes(25). Furthermore, we demonstrated the feasibility of bioconversion of algal protein into terpene through terpene biosynthesis reconstruction into mutant *E. coli* strain YH40. Based on the previous studies, we developed a synthetic microbial consortium and investigated the production of caryophyllene, chamigrene, and other terpene products in one-pot fermentation using algal hydrolysate of microalgae monocultures from strain *Nannochloropsis sp* as well as natural benthic algal assemblages cultivated from wastewater. To achieve this, the terpene biosynthesis pathway was reconstructed into *E. coli* strain YH40(7), designated for the conversion of algal protein into caryophyllene or chamigrene, and into *E. coli* strain DH1, designated for the conversion of algal carbohydrate into caryophyllene or chamigrene, respectively, as described in previous studies(15). The caryophyllene and chamigrene yields were investigated under three different combinations of inoculum YH40-CI4A-CS/DH1-CI4A-CS at ratios of 2:1, 1:1, 0.5:1 as well as the single strainsYH40-CI4A-CS or DH1-CI4A-CS alone. As shown in figure 2 (B), when co-culture of the two strains containing caryophyllene synthases were grown on algal hydrolysate from *Nannochloropsis sp*, at an inoculum ratio 1:1 (consortia R1) the consortia produced the highest titer of total terpene, up to 507.4 mg/L, including 471 mg/L of sesquiterpene, 36.4 mg/L of monoterpene as well as 124.4 mg/L of caryophyllene. Correspondingly, the consortia R1 consumed the highest amount of algal carbohydrates and proteins, which accounted for 48.2% of total algal carbohydrates and 36% of total algal proteins in the media, figure 2(C). Compared to the consortia R1, the consortia R2 and R0.5 consumed a significantly lower fraction of the total algal biomass, with correspondingly lower concentrations of terpenes. The strain YH40-CI4A-CS alone produced the least amount of total terpene (274.7 mg/L), sesquiterpene (232.1 mg/L) and caryophyllene (14.4 mg/L) while DH1-CI4A-CS yielded 30% higher sesquiterpene and total terpene than strain YH40-CI4A-CS as well as 4 times higher titer of caryophyllene (75.2 mg/L). Compositional analysis of the *Nannochloropsis sp*. biomass indicated that the biomass was 20% carbohydrates and 58% protein (data not shown). Based on this data, the highest terpene yield that was achieved corresponded to ~42 mg total terpene/ g algae from consortia R0.5 with 37.4 mg sesquiterpene/ g algae and 6.6 mg caryophyllene/ g algae, as shown in figure 2(D).

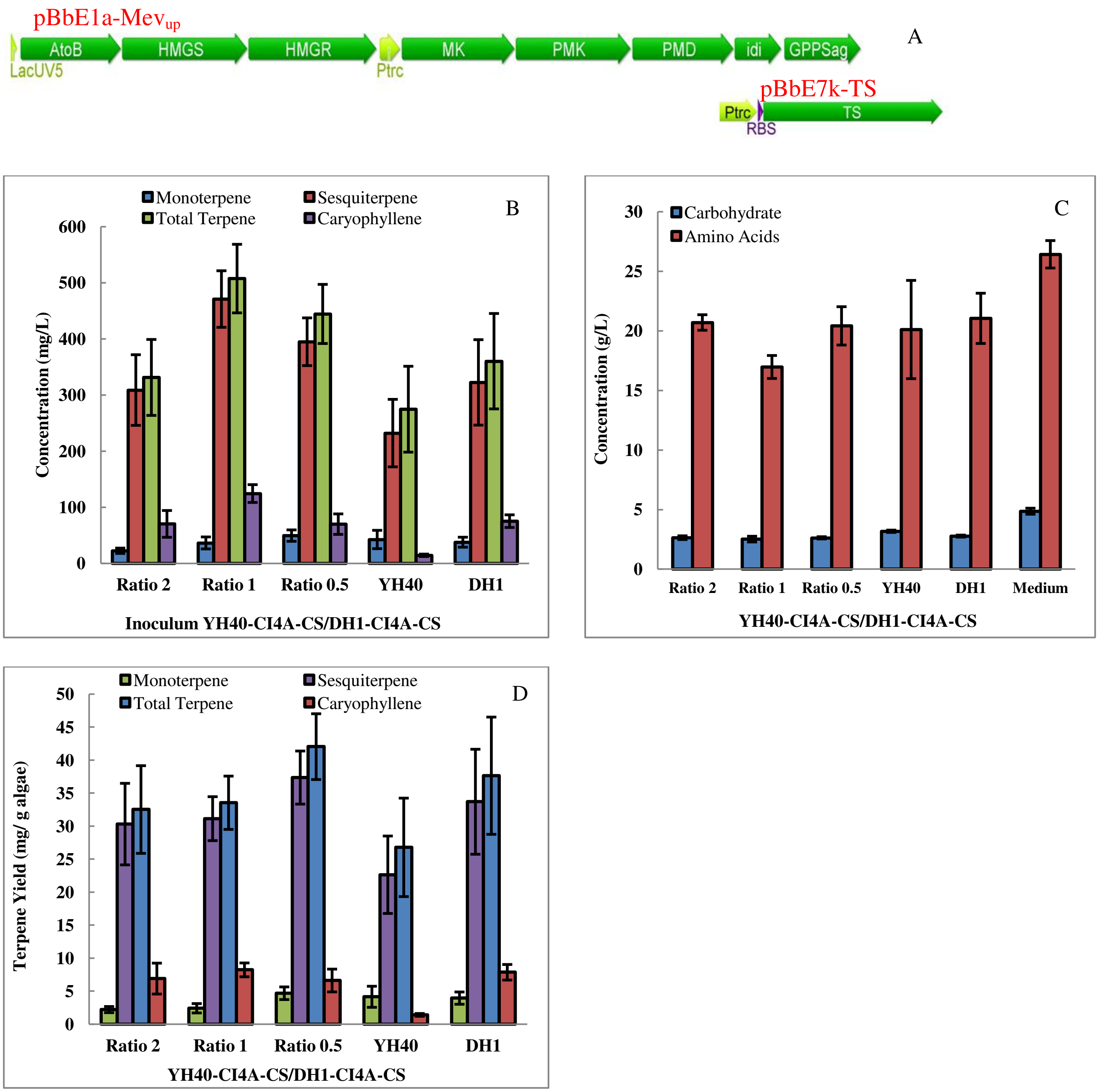

Figure 2.

For co-culture of the two engineered strains containing chamigrene on the hydrolysate of benthic algal assemblages, the experimental results showed that the terpene yield reached 187 mg/L total terpene at the 2:1 ratio (YH40-CI4A-CPS/DH1-CI4A-CPS), including 87 mg/L of monoterpene and 100 mg/L of sesquiterpene, and chamigrene was the major product accumulated up to 62 mg/L. The synthetic microbial consortia produced similar total terpene at the 1:1 and 0.5:1 ratios (YH40-CI4A-CPS/DH1-CI4A-CPS), which were ~150 mg/L of total terpene. The microbial consortium at ratio1 yielded the highest concentration of sesquiterpene (113 mg/L) as well as chamigrene (80 mg/L) among three consortia, while the monoterpene yield was the lowest (34.5 mg/L). The strains YH40-TS and DH1-TS alone produced only 26 and 43 mg/L of total terpene, respectively, indicating relatively inefficient bioconversion of algal biomass. Compared to a single bioconversion strain, the synthetic microbial consortia produced 2.5-6.2 times higher total terpene concentration, suggesting that both algal carbohydrate and protein can be more effectively converted in the single-pot process. In terms of algal carbohydrate and amino acid consumption, none of the synthetic consortia were able to completely consume the algal carbohydrates and amino acids. The 2:1 consortium ratio utilized the highest amount of algal biomass, corresponding to 36.8% of total carbohydrates and 31.3% of algal amino acids. The other two consortia ratios consumed similar amount of the total carbohydrates and algal amino acids, which were 10-15% less than the 2:1 consortium. Strain YH40—CI4A-CPS utilized approximately half of the algal amino acids in the medium but algal carbohydrate consumption was minimal (3.8% of total carbohydrate). Strain DH1-TS consumed both algal carbohydrates (37.8 % of total carbohydrate) and amino acids (23.3% of algal amino acids) in the medium. Compositional analysis indicated that carbohydrate and protein accounts for 74.2% of the mixed benthic biomass ash free dry weight (HydroMentia, Inc). Based on these data, the 2:1 consortium ratio produced the highest terpene yield at 30.5 mg terpene/ g algae while the 1:1 and 1:2 consortium ratios yielded 27.0 and 28.5 mg terpene/ g algae, respectively. The strain YH40—CI4A-CPS only produced 3.3 mg terpene/ g algae, which was lower than 8.7 mg terpene/ g algae yielded by strain DH1-CI4A-CPS, as shown in figure 3 (C). Compared to total terpene yield produced from the benthic polyculture biomass in our previous study, the consortium employing *Nannochloropsis sp*. monoculture produced more than 2- fold higher titer of total terpene. In the consortium used for bioconversion of the benthic polyculture biomass, the chamigrene synthase (JGI protein ID 322581) gene was expressed as the last enzyme in the terpene biosynthesis pathway. Compared to the multiple sesquiterpene produced by caryophyllene synthase in this study, chamigrene synthase only produces a single sesquiterpene (chamigrene) with a limited number of monoterpenes(15), which was likely a reason for the higher yield of total terpene from *Nannochloropsis sp*. Furthermore, the ash content of the benthic polyculture was more than 50% of total biomass, compared to 5.9% of *nannochloropsis sp*. (data not shown). The higher ash content of the benthic polyculture biomass resulted in higher ionic strength in the final algal hydrolysates (fermentation medium), which retarded the cell growth and compromised the terpene yield. Additionally, according to techno-economic analysis of the current state-of-the-art technologies for essential oil production, which are mainly based on water/solvent extraction, the extraction yield of essential oil ranged from 0.1% to 1% of plant tissue, corresponding to 1 mg-10 mg essential oil/ g plant tissue(26, 27) based on the relatively low concentration of essential oils in plant tissue(28). Compared to the extraction yield of essential oil from plant tissue, the engineered strains in this study increased the terpene yield about 3~40 times, which makes it a promising alternative pathway for terpene production.

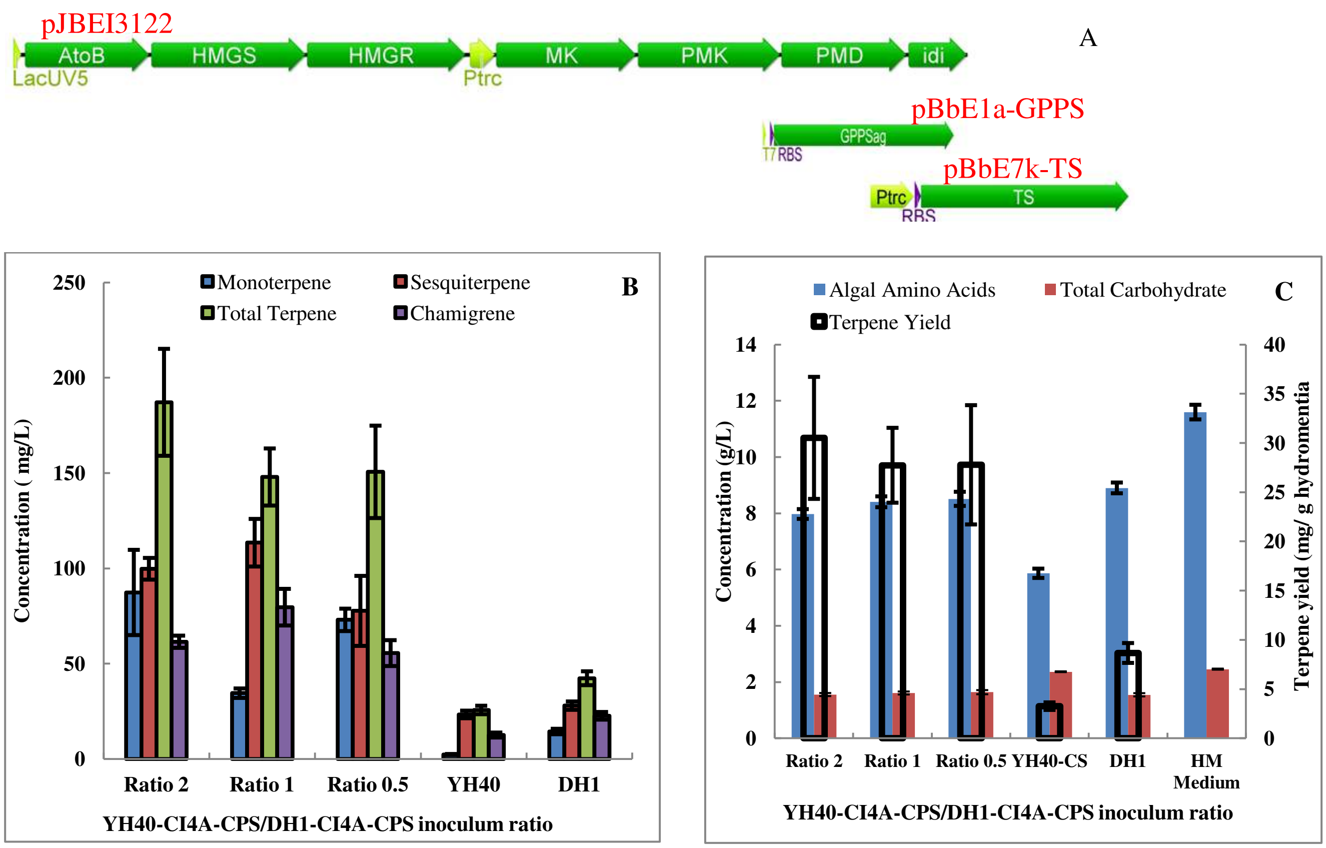

Figure 3

Conclusion

Algae-based biofuels production has primarily focused on biodiesel production through transesterification of algal lipids. Under robust algal biomass accumulation conditions, carbohydrate and proteins typically comprise up to ~80% of the ash-free dry weight of algae biomass. Therefore, a comprehensive process for bioconversion of algal carbohydrates and proteins to high energy density fuels and value-added bioproducts should significantly improve the algal fuel process feasibility. In this study, we demonstrated simultaneous bioconversion of algal carbohydrates and proteins to terpenes which are attractive candidates for high energy density aviation fuels and other intermediate to high value bio-based chemicals applications. Using an engineered microbial consortium, greater than 30% of the carbohydrates and proteins from both a wastewater-based mixed algal feedstock and monoculture of strain *Nannochloropsis sp* were converted to terpenes, including both monoterpenes and sesquiterpenes. This microbial consortium concept for comprehensive utilization of algal biomass offers a versatile path forward for the production of fuels and active bioproducts from algae.

Material and Methods

Strains and Plasmids

The *E.coli* strain DH1 was obtained from Joint BioEnergy Institute (JBEI). The mutant *E.coli* strain YH40 (BW25113/F' [traD36, proAB+, lacIqZΔM15]ΔglnA, ΔgdhAΔluxSΔlsrA) was generously provided by Professor James C Liao from University of California, Los Angeles (UCLA). The plasmid pBbE1a-MEV$_{up}$ containing the terpene biosynthesis pathway(29), the plasmid pBbE1a-GPPS, and pBbE7k-TS were constructed in our previous study(15, 25). The plasmids containing the whole terpene biosynthesis pathway were co-transformed into strains DH1 and YH40, respectively.

Terpene production from a microbial consortium on algal hydrolysates

Algal biomass samples from both sources were pretreated according to protocols from the National Renewable Energy Laboratories and hydrolyzed with 2 mg/mL Pronase (Promega, CA) following the manufacturer's protocol. The pretreated and hydrolyzed algal biomass was sterilized through filtration. *E. coli* strains DH1 and YH40 each containing the terpene biosynthesis pathway were cultured into 15ml of LB medium as described in the previous study. The overnight cultures were centrifuged and the cell pellets were re-suspended into 4 ml of pretreated algal hydrolysate. Various ratios (2:1, 1:1, 1:2) of engineered YH40 to DH1 were inoculated into the algal hydrolysate at a final concentration of 10% v/v. The culture were incubated at 37˚C, 220 rpm and induced with 1 mM IPTG once the OD reached 0.8. The flasks were cap-sealed and cultured for another 72 hours at 25˚C, 180 rpm for terpene production. Analytical samples were taken at the initial and end point of fermentation. The concentrations of total carbohydrate and amino acids were determined according to the established colorimetric protocols. The terpene profile and concentration was determined as described in the previous study(15, 25).

Experimental replication and statistical treatment

All the fermentation experiments were performed in triplicate. The data presented in the figures were the mean values and the errors were calculated as the standard deviation of the triplicates.

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Caption of Figures

Figure 1: Cartoon depiction of “one-pot” bioconversion of algal hydrolysate into terpenes.

Figure 2: Comprehensive conversion of algal carbohydrates and proteins into caryophyllene and other terpenes using a synthetic microbial consortium on algal hydrolysate of *Nannochloropsis sp*. A: Caryophyllene biosynthesis pathway construct, B: concentration of caryophyllene and other terpenes, C: algal carbohydrate and protein consumption of the microbial consortia, D: caryophyllene and other terpene yields based on the substrate consumption.

Figure 3: Comprehensive conversion of algal carbohydrate and protein into chamigrene and other terpenes using a synthetic microbial consortium on algal hydrolysate of benthic polyculture biomass. A: Chamigrene biosynthesis pathway construct, B: concentration of chamigrene and other terpenes, C: algal carbohydrate and protein consumption and total terpene yields based on the substrate consumption.

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Contributions

W.W.H conceived and designed the study, performed the experiments, collected and analyzed the data, wrote and revised the manuscript. R.W.D supervised the study, and revised the manuscript. All authors read and approved the manuscript.