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Combustion and emissions characterization of terpenes with a view to their biological production in cyanobacteria

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HIGHLIGHTS

- Twelve terpenes were tested as pure fuels in a direct injection diesel engine.
- The terpenes are potential products of a genetically engineered cyanobacteria.
- Molecular structure of the terpenes impacted significantly on ignition quality.
- Four terpenes were tested as diesel and gasoline extenders up to 60% terpene.
- Terpene toxicity to the suggested producing cyanobacteria was also assessed.

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ABSTRACT

In developing future fuels there is an opportunity to make use of advances in many fields of science and engineering to ensure that such fuels are sustainable in both production and utilization. One such advance is the use of synthetic biology to re-engineer photosynthetic micro-organisms such that they are able to produce novel hydrocarbons directly from CO_2. Terpenes are a class of hydrocarbons that can be produced biologically and have potential as liquid transport fuels. This paper presents experimental studies on a compression ignition engine and spark ignition engine in which the combustion and emissions of 12 different terpenes that could potentially be produced by cyanobacteria were assessed as single components and blends with fossil diesel and fossil gasoline. The 12 terpenes were chosen to explore how small changes to the molecular structure of geraniol (a terpene most easily produced by cyanobacteria) impact on combustion and emissions. Furthermore, the toxicity of some of the best performing terpenes were assessed using the model cyanobacterium *Synechocystis* sp. PCC6803 (hereafter, *Synechocystis*) as a prelude to a metabolic engineering programme. The compression ignition engine tests were carried out at constant injection timing and constant ignition timing, and the spark ignition engine tests were conducted at a constant spark timing and a constant lambda value of 1. Of the terpenes tested in the compression ignition engine, geranial and farnesene were found to be the best performing single component fuels in terms of combustion and emissions. In blends with fossil diesel, the presence of geranial or farnesene did not have a significant effect on combustion phasing up to a terpene content of 20% (wt/wt), though levels of NOx and CO did increase. In the spark ignition engine experiments of terpene and fossil gasoline blends, citronellene and linalool were found to be soluble in fossil gasoline and combusted in a steady manner up to a terpene content of 45% and 65% (wt/wt) respectively. Of those terpenes with the most potential as either diesel or gasoline fuels, geraniol and geranial were found to be the most toxic to *Synechocystis*, with farnesene and linalool less toxic and citronellene having no detrimental effect. Addition of *n*-dodecane to the cultures was found to ameliorate the toxic effects of all five terpenes.

1. Introduction

The advent of genetically engineered micro-organisms is an exciting opportunity to explore novel and sustainable methods of bio-fuel production [1,2]. While the introduction of novel biosynthesis pathways into micro-organisms is a field still very much in its infancy, un-modified micro-organisms have already seen considerable utilization in industrial applications [3]. Yeasts have been utilized for several decades in the large scale production of bio-ethanol via fermentation of sugars [4]. More recently, the use of algal lipids for the production of bio-diesel via transesterification of fatty

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Nomenclature

NOx	nitrogen oxides
CO_2	carbon dioxide
IQT	ignition quality testing
CFR	cooperative fuels research
DCN	derived cetane number
CO	carbon monoxide
THC	total hydrocarbons
CAD	crank angle degree
PID	proportional integral derivative
DAQ	data acquisition
O_2	oxygen
SOI	start of injection
BTDC	before top-dead-centre
TDC	top-dead-centre
SOC	start of combustion
SOC2	start of 2nd phase of combustion and point between SOC and the time of peak heat release rate at which d(tan^{-1}(dHRR/dCAD) is at a minimum and dHRR is positive.
IMEP	indicated mean effective pressure
FAME	fatty acid methyl ester
RON	research octane number
E. coli	*Escherichia coli*
DNC	did not combust
Dp	Mean particle diameter

acids with an alcohol is attracting significant interest [5–8], with favorable comparisons to other lipid sources [9,10]. However, while the feedstock is renewable, the production of algal bio-diesel via lipid extraction and transesterification does not yet represent a low-carbon route to replace fossil fuels. It is also, at present, generally considered economically non-viable without either government subsidy or a decline in global oil resources and a concurrent escalation in the price of crude fossil oil [11–13]. This economic non-viability can in part be attributed to the large energy cost associated with the harvesting and drying of algal cells that grow in aqueous media at densities typically less than 10 g/L [14] and is currently considered to be a major limitation of biochemical production from photosynthetic organisms [15]. Therefore, the possibility of engineering a microalga in such a way so as to negate this prohibitive energy cost is highly attractive.

There has been considerable progress in recent years in the use of synthetic biology approaches to re-engineer metabolic pathways in heterotrophic bacteria such as *Escherichia coli* to produce a range of novel hydrocarbons that could be used as fungible fuel molecules [1]. These candidate biofuels include alkanes, alkenes, alcohols and terpenoids. Furthermore, efflux systems engineered into the bacteria should allow for the excretion of the product into the medium, thereby avoiding toxic build-up in the cell and circumventing the need for cell harvesting and extraction [16]. If similar metabolic engineering approaches can be applied to photosynthetic micro-organisms such as eukaryotic microalga or cyanobacteria, then an efficient biological system for direct light-driven conversion of CO_2 into advanced biofuels could be envisaged. Of the two groups of organisms, cyanobacteria are the more attractive as a chassis for such metabolic engineering, not least because species can be found that are fast-growing and tolerant to a range of abiotic stresses (high temperature, high light, high salt, etc.), and their simpler genetics and physiology make them easier to engineer [17]. Indeed, promising initial work has been reported in which the model species, *Synechocystis* or other genetically tractable species have been engineered to produce a range of bio-industrial compounds including biofuels [18].

A particularly attractive target for biofuel engineering in cyanobacteria is the terpenoid (or isoprenoid) pathway, which is responsible for the biosynthesis of a diverse range of compounds using

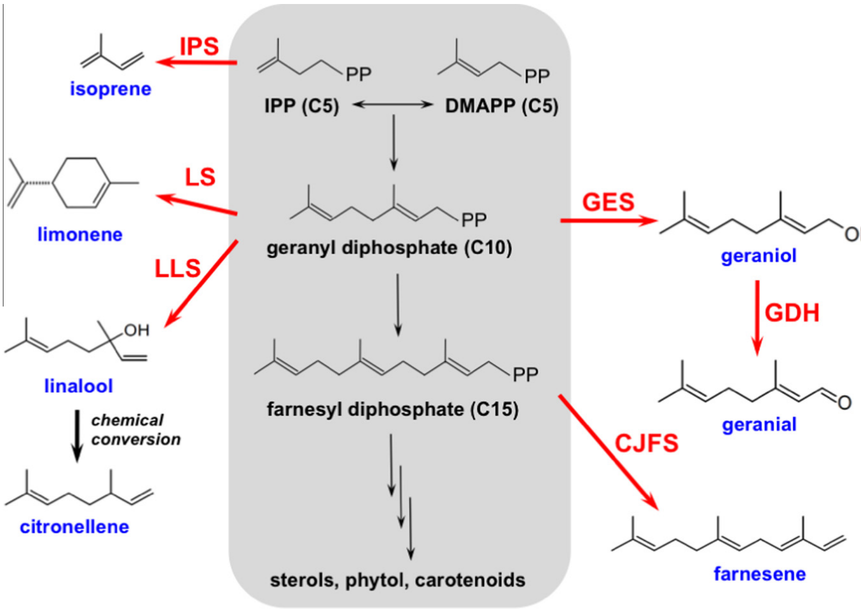

Fig. 1. The isoprenoid pathway (highlighted in gray) and examples of possible pathways (red arrows) that could be introduced to produce novel hydrocarbons (blue). IPS: isoprene synthase; LS: limonene synthase; LLS: linalool synthase; GES: geraniol synthase; geraniol dehydrogenase; CJFS: (*E*)-b-farnesene synthase.