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Heterologous Expression of the Mevalonic Acid Pathway in Cyanobacteria Enhances Endogenous Carbon Partitioning to Isoprene

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ABSTRACT Heterologous expression of the isoprene synthase gene in the cyanobacterium *Synechocystis* PCC 6803 conferred upon these microorganisms the property of photosynthetic isoprene (C_5H_8) hydrocarbons production. Continuous production of isoprene from CO_2 and H_2O was achieved in the light, occurring via the endogenous methylerythritol-phosphate (MEP) pathway, in tandem with the growth of *Synechocystis.* This work addressed the issue of photosynthetic carbon partitioning between isoprene and biomass in *Synechocystis.* Evidence is presented to show heterologous genomic integration and cellular expression of the mevalonic acid (MVA) pathway genes in *Synechocystis* endowing a non-native pathway for carbon flux amplification to isopentenyl-diphosphate (IPP) and dimethylallyl-diphosphate (DMAPP) precursors of isoprene. Heterologous expression of the isoprene synthase in combination with the MVA pathway enzymes resulted in photosynthetic isoprene yield improvement by approximately 2.5-fold, compared with that measured in cyanobacteria transformed with the isoprene synthase gene only. These results suggest that the MVA pathway introduces a bypass in the flux of endogenous cellular substrate in *Synechocystis* to IPP and DMAPP, overcoming flux limitations of the native MEP pathway. The work employed a novel chromosomal integration and expression of synthetic gene operons in *Synechocystis*, comprising up to four genes under the control of a single promoter, and expressing three operons simultaneously. This is the first time an entire biosynthetic pathway with seven recombinant enzymes has been heterologously expressed in a photosynthetic microorganism. It constitutes contribution to the genetic engineering toolkit of photosynthetic microorganisms and a paradigm in the pursuit of photosynthetic approaches for the renewable generation of high-impact products.

Key words: biofuels; cyanobacteria; isoprene; isoprene synthase; metabolic engineering; photosynthesis; *Synechocystis*.

INTRODUCTION

Terpenoids are the largest family of naturally occurring products (more than 40 000 different molecules have been described) (Keeling and Bohlmann, 2012), and have diverse chemical properties that find application in the pharmaceutical, neutraceutical, cosmetic, and food industries (Bohlmann and Keeling, 2008). Terpenoids have potential to be also developed as biofuels (Lindberg et al., 2010; Peralta-Yahya et al., 2011). Accordingly, synthetic biologists have devoted considerable attention in recent years to the terpenoid biosynthetic pathways with the aim of increasing metabolic flux to ultimately increase terpenoid product yield in engineered microbes (Martin et al., 2003; Leonard et al., 2010; Zurbriggen et al., 2012; Bentley et al., 2013). One of the major challenges is the ability to experimentally control cellular carbon partitioning, in this case to channel a greater proportion of the endogenous substrate towards the terpenoid pathway without inducing negative consequences on cell fitness or metabolism (Melis, 2013). Here, we report a genetic engineering strategy to increased photosynthetic carbon partitioning towards the terpenoid pathway in a cyanobacterial strain endowed with the ability to produce and release isoprene (C_5H_8) hydrocarbons (Lindberg et al., 2010; Bentley and Melis, 2012). This was achieved upon the heterologous expression of the mevalonic acid (MVA) pathway in *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) to increase the pool of isopentenyl-diphosphate (IPP) and dimethylallyl-diphosphate

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(DMAPP), the prenyl diphosphate precursors to isoprene and all other terpenoids.

There are two independent and distinct terpenoid biosynthetic pathways leading to the production of IPP and DMAPP (Figure 1). The methylerythritol-phosphate (MEP) pathway is of prokaryotic bacterial origin and present in most (but not all) bacteria, cyanobacteria, green microalgae, and plant plastids (Rohmer et al., 1996; Rohmer, 1999; Lange et al., 2000; Lichtenthaler, 2000; Lee and Schmidt-Dannert, 2002). The MEP pathway utilizes glyceraldehyde-3-phosphate (G3P) and pyruvate as primary feedstock molecules. The mevalonic acid (MVA) pathway is of archael/eukaryotic origin, and utilizes acetyl-CoA as the primary precursor (Miziorko, 2011). Most organisms also contain an IPP isomerase that catalyzes

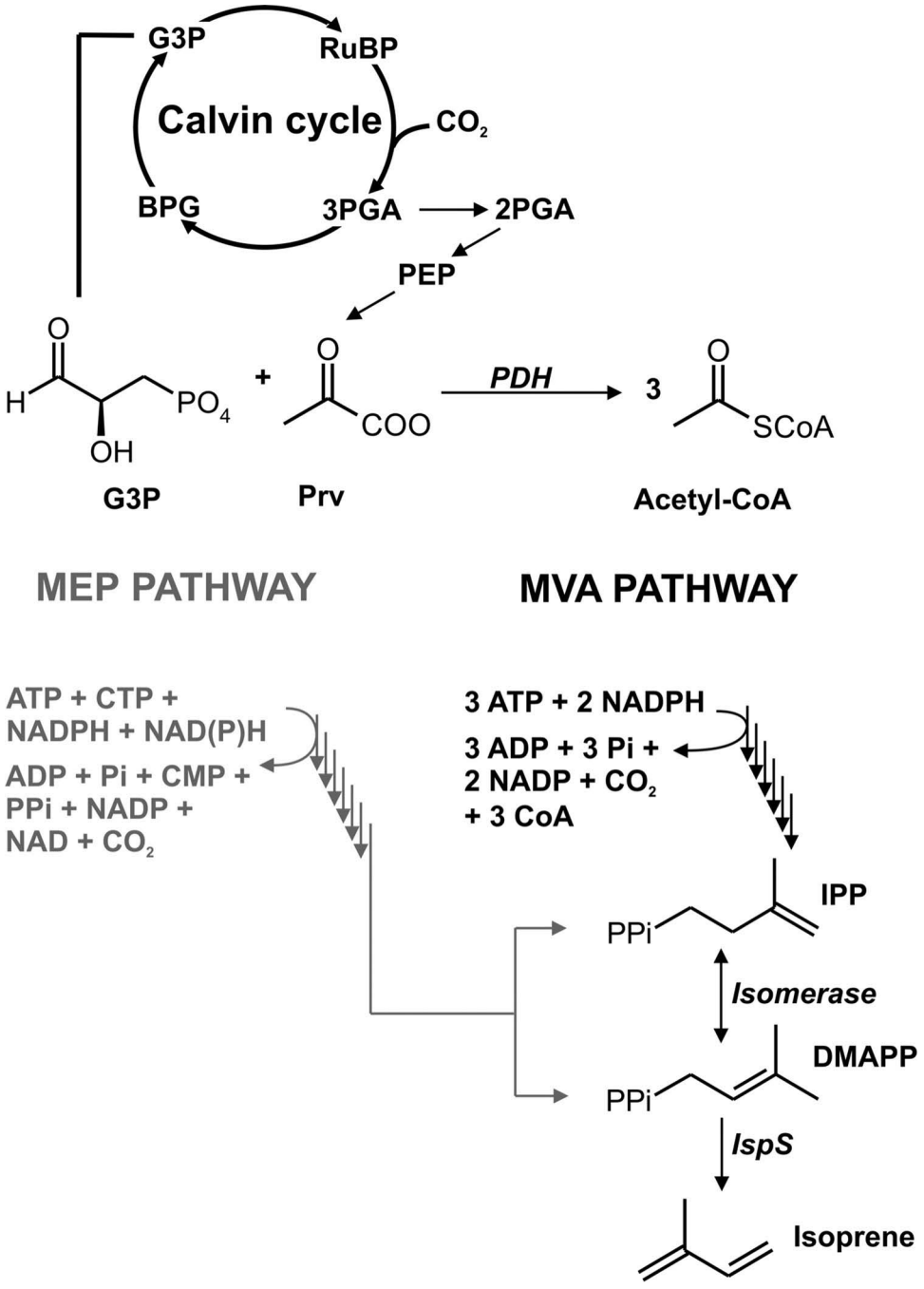

Figure 1. Photosynthetic Carbon Flux towards the Production of Isoprene.

G3P, glyceraldehyde-3-phosphate; RuBP, ribulose-1,5-bisphosphate; 3PGA, 3-phospho-glyceric acid; BPG, 1,3-biphosphoglycerate, 2PGA, 3-phospho-glycerate; PEP, phosphoenolpyruvate, Prv, pyruvate; PDH, pyruvate dehydrogenase; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate.

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