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Exploring the photosynthetic production capacity of sucrose by cyanobacteria

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ABSTRACT

Because cyanobacteria are photosynthetic, fast-growing microorganisms that can accumulate sucrose under salt stress, they have a potential application as a sugar source for the biomass-derived production of renewable fuels and chemicals. In the present study, the production of sucrose by the cyanobacteria *Synechocystis* sp. PCC6803, *Synechococcus elongatus* PCC7942, and *Anabaena* sp. PCC7120 was examined. The three species displayed different growth curves and intracellular sucrose accumulation rates in response to NaCl. *Synechocystis* sp. PCC6803 was used to examine the impact of modifying the metabolic pathway on the levels of sucrose production. The co-overexpression of *sps* (*slr0045*), *spp* (*slr0953*), and *ugp* (*slr0207*) lead to a 2-fold increase in intracellular sucrose accumulation, whereas knockout of *ggpS* (*sll1566*) resulted in a 1.5-fold increase in the production of this sugar. When combined, these genetic modifications resulted in a fourfold increase in intracellular sucrose accumulation. To explore methods for optimizing the transport of the intracellular sucrose to the growth medium, the acid-wash technique and the CscB (sucrose permease)-dependent export method were evaluated using *Synechocystis* sp. PCC6803. Whereas the acid-wash technique proved to be effective, the CscB-dependent export method was not effective. Taken together, these results suggest that using genetic engineering, photosynthetic cyanobacteria can be optimized for efficient sucrose production.

1. Introduction

The production of biofuels and biochemicals from renewable biomass feedstocks results in less carbon dioxide emission compared to utilizing fossil resources to produce fuels and chemicals. Plants rich in different types of sugars, such as sucrose, starch and cellulose, constitute major biomass feedstocks. Sugarcane is the main resource, providing 70% of the world's sugars (Lakshmanan et al., 2005). The major product derived from sugarcane is sucrose, a disaccharide composed of glucose and fructose monosaccharides. Sucrose can be directly fermented by yeast to produce ethanol. Sugarcane-derived ethanol has been successfully commercialized in Brazil, however the requirement of a tropical or subtropical planting region has limited the development of the sugarcane ethanol industry in other areas of the world. Starchy plants, such as corn, produce a polysaccharide consisting of glucose units linked by α-glycosidic bonds that can be easily cleaved to yield glucose. Therefore, it is relatively easy to industrialize the production of corn ethanol. However, corn ethanol production competes for agricultural resources, making it non-sustainable. Cellulosic biomasses, such as agricultural wastes (e.g., corn straw) and energy grasses (e.g., *Miscanthus*) are rich in cellulose, which is a major component of plant cell walls. Cellulose is a polysaccharide consisting of glucose units linked by β-glycosidic bonds. Although the wide abundance of cellulose makes it an attractive biofuel source, challenges remain to be overcome in its use for generating ethanol in a cost-competitive way. The structural nature of cellulose, which comprises a network of hydrogen bonds leading to a microcrystalline structure, makes it a difficult material to degrade. Innovative and advanced technologies for the pretreatment of lignocellulosic biomasses with low energy consumption and enzymatic hydrolysis of cellulose with low processing costs must be developed for cellulosic ethanol to become competitive with fossil fuels. Thus, the development of sustainable and efficient technologies for the production of sugars, which can be converted to fuels and chemicals through microbial fermentation or chemical catalysis, is very important.

Cyanobacteria are photosynthetic bacteria found in diverse environments including freshwater, oceans and terrestrial habitats. Because they are able to synthesize and accumulate sucrose, they are a potential source of fermentable sugar for biofuel production.

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Indeed, cyanobacteria have recently drawn attention as promising agents for the production of biofuel and biochemicals (Lan and Liao, 2011; Lindberg et al., 2010; Wang et al., 2013; Zhou et al., 2012). Furthermore, sucrose can be fermented directly, thus avoiding the costly pretreatments required for cellulosic biomasses.

Recently, a soluble sugar production rate of 14 mg L^{-1} h^{-1} was achieved in the *Synechococcus* sp. PCC 7002 *glgA-I glgA-II* mutant strain under salt shock (Xu et al., 2012). A sucrose production rate of 36.1 mg L^{-1} h^{-1} was achieved in *Synechococcus elongatus* PCC7942 (Ducat et al., 2012) by combining the induced overexpression of the *Escherichia coli* gene *cscB,* which encodes a proton and sucrose symporter, with the deletion of the ADP-glucose pyrophosphorylase gene (*agp*) and the sucrose-hydrolyzing enzyme gene (*inv*). Thus, the potential for sucrose production in genetically engineered photosynthetic bacteria is estimated to be higher than that observed for sugarcane (Ducat et al., 2012).

Sucrose accumulation in cyanobacteria occurs as a cellular response to salt stress, which triggers the enhancement of activation transport systems (salt-out or salt-in) and the accumulation of osmoprotective compounds (Hagemann, 2011; Reed and Stewart, 1985). Cyanobacterial species can be divided into three subgroups according to their salt tolerance abilities and the types of osmoprotective compounds they produce. Species with a low salt tolerance (maximum of 0.7 M NaCl) synthesize sucrose or trehalose. Species with moderate salt tolerance (maximum of 1.8 M NaCl) accumulate glucosylglycerol (GG), whereas high salt tolerance (maximum of 3.0 M NaCl) is observed in species that produce glycinebetaine or glutamate betaine (Mackay et al., 1984; Reed et al., 1984; Reed and Stewart, 1985). Based on their sucrose biosynthetic pathways and salt tolerance levels, the cyanobacterial species *Synechocystis* sp. PCC6803 (*Synechocystis*), *S. elongatus* PCC7942 (*S. elongatus*), and *Anabaena* sp. PCC7120 (*Anabaena*) were initially selected for this effort to determine the sucrose production levels and for genetic engineering aimed at generating an efficient sugar-producing platform.

The sucrose biosynthetic pathways of *Synechocystis*, *S. elongatus* and *Anabaena* are depicted in Fig. 1. The basic mechanisms of cyanobacterial salt acclimation have been extensively studied in the model organism (Hagemann, 2011). Many proteins related both to this process and to sucrose biosynthesis have been identified and investigated (Desplats et al., 2005; Ferjani et al., 2003; Karandashova et al., 2002; Miao et al., 2003), paving the way for further genetic modifications. *Synechocystis* is also the most salt-tolerant strain in the group mentioned above and, in theory, it could possibly accumulate the highest concentrations of osmoprotective compounds. Therefore, we chose *Synechocystis* to conduct further investigations of methods to optimize the photosynthetic production of sucrose.

In the studies described below, ion-exchange chromatography was used to monitor sucrose accumulation in wild-type *Anabaena*, *S. elongatus* and *Synechocystis*, and GG accumulation in wild-type *Synechocystis* exposed to different salt concentrations. The sucrose production capacity of *Synechocystis* was systematically explored in this study in the context of intracellular metabolic pathway modifications and sucrose secretion. The intracellular metabolic pathway modifications include the co-overexpression of *sps* (sucrose phosphate synthase, *slr0045*), *spp* (sucrose phosphate phosphatase, *slr0953*) and *ugp* (UDP-glucose pyrophosphorylase, *slr0207*), and the knockout of *ggpS* (glucosylglycerol phosphate synthase, *sll1566*). The sucrose secretion rates were evaluated using the acid-wash technique and the CscB-dependent export method. The results indicated that the three species possess different sucrose-accumulation capacities. In the case of the genetically engineered *Synechocystis*, both co-overexpression of *sps, spp* and *ugp* and *ggpS* knockout significantly increased sucrose accumulation; however, the sucrose secretion efficiency is not as high as that reported for *S. elongatus* PCC7942 (Ducat et al., 2012).

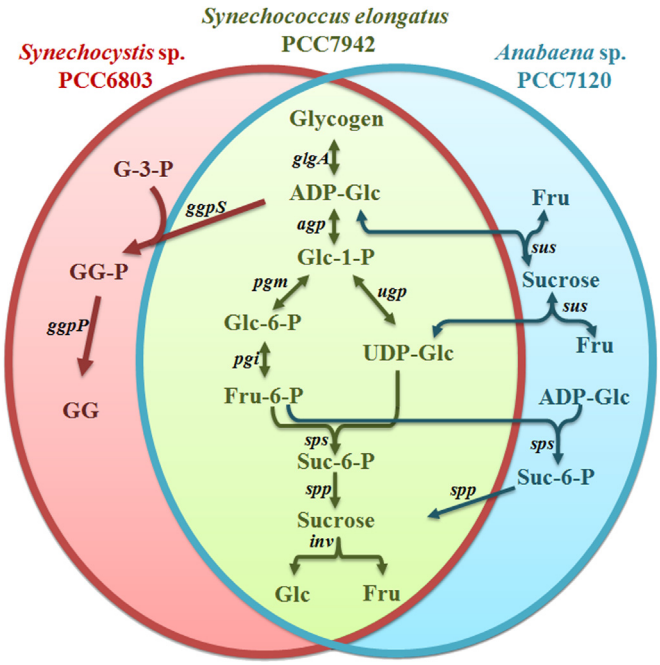

Fig. 1. The biosynthetic pathways for sucrose production in *Synechocystis*, *S. elongatus*, and *Anabaena*. Abbreviations: ADP-Glc, ADP-glucose; Glc-1-P, glucose-1-phosphate; Glc-6-P, glucose-6-phosphate; Fru-6-P, fructose-6-phosphate; UDP-Glc, UDP-glucose; Suc-6-P, sucrose-6-phosphate; Glc, glucose; Fru, fructose; G-3-P, glycerol-3-phosphate; GG-P, glycosylglycerol-phosphate; GG, glycosylglycerol; *glgA*, glycogen synthase; *agp*, ADP-glucose pyrophosphorylase; *pgm*, phosphoglucomutase; *pgi*, glucose-6-phosphate isomerase; *ugp*, UDP-glucose pyrophosphorylase; *sps*, sucrose phosphate synthase; *spp*, sucrose phosphate phosphatase; *inv*, invertase; *sus*, sucrose synthase; *ggpS*, glucosylglycerol phosphate synthase; *ggpP*, glucosylglycerol phosphate phosphatase.

2. Materials and methods

2.1. Chemicals and reagents

Glycosylglycerolwas obtained from the Bitop Company (Germany) and sucrose from Aladdin-Reagent (China). Other chemicals were obtained from the Sinopharm Chemical Reagent company (China). Taq DNA polymerases and all of the restriction endonucleases were purchased from Fermentas (Canada) or Takara (Japan). The kits used for molecular cloning were purchased from either Omega (U.S.A.) or Takara (Japan).

2.2. Strains and plasmid construction

The plasmids and bacterial species used are listed in Tables 1 and 2, respectively. The plasmids listed in Table 1 were used to create the clones listed in Table 2. *E. coli* DH5α was used as the host for the construction of vectors. *Synechocystis*, *Anabaena*, and *S. elongatus* were obtained from Prof. Xudong Xu of the Institute of Hydrobiology, Chinese Academy of Sciences. In this study, *sps*, *spp*, and *ugp* were amplified from the genomic DNA of *Synechocystis* by PCR and cloned into the NdeI/XhoI site of pXT37b, resulting in pWD15, pWD16, and pWD17, respectively. pWD18 and pWD19 were constructed using fusion PCR to ligate *sps* and *spp*, or *sps*, *spp*, and *ugp*, respectively, which were cloned into NdeI/XhoI site of pXT37b. pWD8 and pWD12 were constructed by inserting the corresponding gene knockout cassette into the T-cloning site of the pMD18-T vector. Each gene knockout cassette was constructed by ligating a