



Engineered *Enterobacter aerogenes* for efficient utilization of sugarcane molasses in 2,3-butanediol production



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HIGHLIGHTS

- A sucrose regulator was deleted from the genome of *Enterobacter aerogenes*.
- The deletion mutant utilized sugarcane molasses much efficiently for 2,3-butanediol production.
- The mutant had higher priority to sucrose than fructose among the sugars in molasses.
- With fed-batch fermentation, 98.69 g/L of 2,3-butanediol was produced in 36 h.

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ABSTRACT

Sugarcane molasses is considered to be a good carbon source for biorefinery due to its high sugar content and low price. Sucrose occupies more than half of the sugar in the molasses. *Enterobacter aerogenes* is a good host strain for 2,3-butanediol production, but its utilization of sucrose is not very efficient. To improve sucrose utilization in *E. aerogenes*, a sucrose regulator (ScrR) was disrupted from the genomic DNA. The deletion mutation increased the sucrose consumption rate significantly when sucrose or sugarcane molasses was used as a carbon source. The 2,3-butanediol production from sugarcane molasses by the mutant was enhanced by 60% in batch fermentation compared to that by the wild type strain. In fed-batch fermentation, 98.69 g/L of 2,3-butanediol production was achieved at 36 h.

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1. Introduction

The development of the biorefinery industry has enabled and improved the eco-friendly production of several industrial chemicals with a reduced dependency on petroleum resources. As an example, the microbial production of 2,3-butanediol as a platform chemical has attracted great interests, due to its large potential in industrial applications (Ji et al., 2011). Therefore, several bacterial species, such as *Klebsiella pneumonia* (Ma et al., 2009), *Serratia marcescens* (Zhang et al., 2010) and *Enterobacter aerogenes* (Jung et al., 2012), have been developed as industrial strains for 2,3-butanediol production.

The price of the carbon source accounts for a large portion of the cost of microbial 2,3-butanediol production. Therefore, there have been many efforts to find cheap substrates. Sun et al. (2009) reported that *Klebsiella pneumoniae* produced 91.63 g/L of 2,3-

butanediol from pretreated Jerusalem artichoke tubers by fed-batch simultaneous saccharification and fermentation (SSF). Wang et al. (2010) used corncob molasses, a waste byproduct in xylitol production, for 2,3-butanediol production, where 78.9 g/L of 2,3-butanediol was produced by *K. pneumonia* after 61 h of fed-batch fermentation. Jiang et al. (2012) tried to produce 2,3-butanediol from acid hydrolysates of jatropha hulls, where a two-step hydrolysis was applied to effectively hydrolyze the jatropha hulls, and 31.41 g/L of 2,3-butanediol was achieved by *Klebsiella oxytoca*.

Among the inexpensive substrates from agriculture residues, sugarcane molasses is considered to be a suitable fermentative carbon source due to its low-price and rich sugar composition (Akaraonye et al., 2012). In 2008, the USDA reported that the price of sugarcane molasses was less than \$0.50/kg (Chan et al., 2012). Sugarcane molasses contains a few mixed sugars, a dominant amount of sucrose, and similar amounts of glucose and fructose (Akaraonye et al., 2012). Therefore, the efficient utilization of sucrose is necessary in order to maximize the use of sugarcane molasses. A sucrose utilization pathway has been studied in enteric bacteria. As shown

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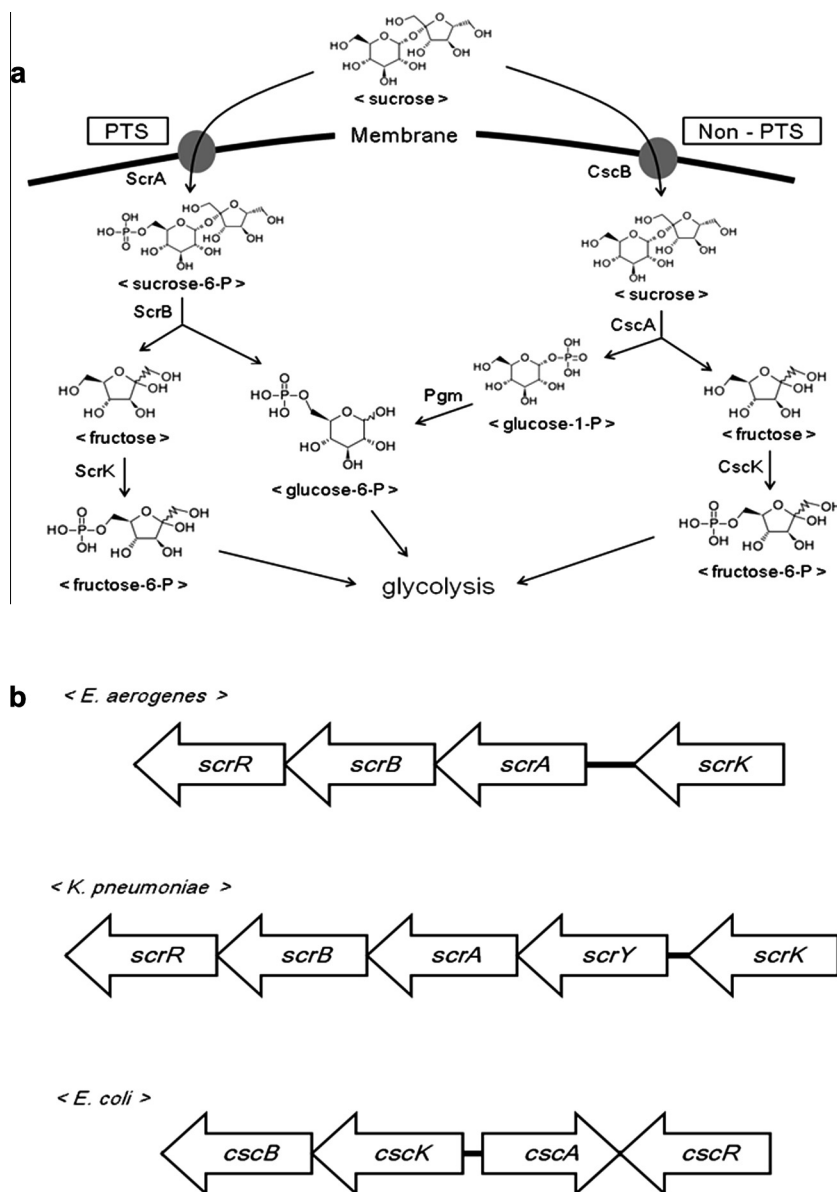


Fig. 1. (a) Bacterial sucrose catabolic pathways with PTS and non-PTS sugar transporters and (b) the corresponding gene arrangements in the genome of three enteric bacteria. ScrA, EII transport protein for sucrose; CscB, sucrose-specific permease; ScrB and CscA, sucrose-6-phosphate hydrolase; ScrK and CscK, fructokinase; Pgm, phosphoglucomutase; ScrY, outer membrane sucrose porin; ScrR and CscR, repressors of the sucrose metabolic genes.

in Fig. 1a, the transport and catabolism of sucrose can be classified into two routes: the phosphotransferase system (PTS) and non-PTS (Reid and Abratt, 2005). A PTS-dependent transporter imports sucrose with phosphorylation by EII^{scr}, which was characterized in *K. pneumonia* as *scr* operon (Sprenger and Lengeler, 1988). Meanwhile, a non-PTS permease transfer of sucrose into the cell without chemical modification has been determined in *Escherichia coli* as *csc* operon (Bockmann et al., 1992). In *K. pneumonia*, the operon consisted of five open reading frames, encoding fructokinase (ScrK), sucrose-specific outer membrane porin (ScrY), PTS EII transport protein (ScrA), sucrose-6-phosphate hydrolase (ScrB) and sucrose dependent regulator (ScrR) (Fig. 1b). The transcription of *scr* operon was repressed by ScrR (Sprenger and Lengeler, 1988). However, the effect of *scrR* mutation on the utilization of sugarcane molasses has not been reported yet.

E. aerogenes is able to produce significant amounts of 2,3-butanediol from a wide range of carbon sources and has high growth rate. Furthermore, the *E. aerogenes* genome sequence has been completed recently (Shin et al., 2012) and the method of tar-

get gene disruption was developed successfully (Jung et al., 2012). In this study, the deletion of the *scrR* gene in *E. aerogenes* was attempted for the efficient utilization of sucrose and sugarcane molasses. The mutant showed much higher efficiency in sucrose utilization and increased 2,3-butanediol production with sugarcane molasses as a carbon source. The result assured that the sugarcane molasses can be a promising carbon source to produce economical 2,3-butanediol with the engineered strain.

2. Methods

2.1. Strain development

All *E. aerogenes* strains were derived from a wild type strain, KCTC 2190. Previously, a λ Red recombination method for *E. aerogenes* was used for deleting a lactate dehydrogenase (LdhA) from KCTC 2190, generating EMY-01 (Jung et al., 2012). In this study, the gene encoding sucrose regulator of the LacI family, ScrR, was

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