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Utilization of economical substrate-derived carbohydrates by solventogenic clostridia: pathway dissection, regulation and engineering

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Solventogenic clostridia can produce acetone, butanol and ethanol (ABE) by using different carbohydrates. For economical reasons, the utilization of cheap and renewable biomass in clostridia-based ABE fermentation has recently attracted increasing interests. With the study of molecular microbiology and development of genetic tools, the understanding of carbohydrate metabolism in clostridia has increased in recent years. Here, we review the pioneering work in this field, with a focus on dissecting the pathways and describing the regulation of the metabolism of economical substrate-derived carbohydrates by clostridia. Recent progress in the metabolic engineering of carbohydrate utilization pathways is also described.

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Introduction: general feature of carbohydrate metabolism by solventogenic clostridia

The use of carbohydrates derived from various low-cost raw feedstocks for the sustainable and economical production of chemicals and biofuels has attracted much interest over the past few years [1]. The cheap biomass mainly includes agricultural residues and industrial solid or liquid wastes [2••,3]. Because the sources of these carbohydrates are so diverse, microbes with a high level of substrate adaptability that can tolerate toxic inhibitors in industrial media are sought after for industrial-level fermentation.

Clostridia-based ABE (acetone–butanol–ethanol) production has a long history and was once one of the largest fermentation industries [4]. According to previous studies [5–7], these *Clostridium* strains capable of producing ABE belong to four species: *C. acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum* and *C. saccharoperbutylacetonicum*. However, ABE fermentation is currently uneconomically unfavorable and unable to compete with petrochemical processes [8]. Thus, a new generation of ABE solvent-producing processes is required to utilize those cheaper, low-grade substrates other than traditional cereal materials [2••]. The ability of clostridia to metabolize a wide range of carbohydrates offers the potential to revive ABE fermentation (Table 1).

Most biomass waste, particularly lignocellulosic biomass, is a complex mixture of different sugars. In sugar uptake by bacteria, the phosphoenolpyruvate (PEP)-dependent phosphotransferase system (PTS) normally plays an important role, which consists of two proteins (i.e. enzyme I and HPr) and a range of Enzymes II that are responsible for concomitant sugar translocation and phosphorylation [9]. Although *Clostridium* species display good substrate adaptability, the ability of these low-GC content, Gram-positive bacteria to utilize some carbohydrates is still lower than desired [10]. Even the utilization of preferable sugars (e.g. glucose) by clostridia requires further improvements in productivity and substrate conversion to achieve the levels of some other industrial organisms, such as *Saccharomyces cerevisiae*.

Several genomes of solventogenic clostridia have been sequenced to date, such as *C. acetobutylicum* ATCC 824 [11], *C. acetobutylicum* DSM 1731 [12], *C. acetobutylicum* EA2018 [13] and *C. beijerinckii* NCIMB 8052 (http://www.ncbi.nlm.nih.gov/genome/1166?project_id=58137). These data, together with the analyses that followed, have been used to dissect the carbohydrate utilization pathways in clostridia [13–15]. This review focuses on the recent research progresses in dissecting these pathways and defining the relevant regulation mechanism of carbohydrate metabolism in solventogenic clostridia. A general scheme for these results is then offered. Additionally, the derivative strategies that were used to engineer clostridia to improve substrate utilization are discussed.

Clostridia metabolism of substrate-related carbohydrates

Agricultural residues and industrial by-products are the primary components of cheap, low-grade biomass. The

Table 1

Economical substrates and the clostridia capable of utilizing these substrates

Substrates	Primary sugar components	*Clostridium* strains used	Refs
Crop stalks and straw	Glucose, xylose, arabinose, mannose and galactose	*C. beijerinckii* *C. saccharoperbutylacetonicum* *C. acetobutylicum*	[59–61]
Rice bran	Glucose and fructose	*C. saccharoperbutylacetonicum* *C. beijerinckii*	[62,63]
Wheat bran	Glucose, xylose and arabinose	*C. beijerinckii*	[64]
Corncob	Xylose, glucose and arabinose	*C. beijerinckii*	[65]
Corn fiber	glucose, xylose, galactose, and arabinose	*C. acetobutylicum*	[66]
Molasses and maize stalk juice	Sucrose, fructose and glucose	*C. beijerinckii* *C. saccharobutylicum*	[40,67,68]
Cheese whey	Lactose	*C. acetobutylicum*	[69]
Corn steep liquid	Glucose	*C. acetobutylicum*	[70]

carbohydrates derived from this biomass include hexose sugars (glucose, fructose, galactose and mannose), pentose sugars (xylose and arabinose) and disaccharides (sucrose and lactose). Among these carbohydrates, glucose, xylose, arabinose and mannose and galactose are derived from lignocellulosic hydrolysates, and the other sugars are the residual biomass from sugar-manufacturing or food-processing industries.

Hexose metabolism

Glucose and fructose

Glucose is a major carbon-based compound that is found in both starchy substrates and lignocellulosic biomass. When clostridia grow on this preferred carbon source, they can generally reach a production level of 20 g/L of total solvents by depleting approximate 60 g/L of glucose in a batch fermentation process; however, it is difficult to exceed this level at higher substrate concentrations due to the toxicity of ABE solvents to *Clostridium* strains. Although some mutant strains (e.g. *C. acetobutylicum* EA 2018 and *C. beijerinckii* BA 101) have demonstrated an increased butanol-producing ability compared to wild-type strains [13,16], more efforts are needed to develop real industrially applicable *Clostridium* strains with improved fermentation abilities.

The PTS plays an essential role during process of glucose uptake and phosphorylation in bacteria [9]. With regard to solventogenic clostridia, the availability of the *C. acetobutylicum* ATCC 824 genomic sequence initiated an intensive search for PTS in these anaerobes [11]. Tangney *et al.* first identified the genes responsible for PTS in *C. acetobutylicum*, which included *ptsH* (encoding a histidine-containing protein HPr) and *ptsI* (encoding enzyme I), and gene *hprK* (encoding Hpr kinase) responsible for the phosphorylation of the PTS component HPr in *C. acetobutylicum* [17]. The functional complementation of an *Escherichia coli* mutant defective in PTS with the system from *C. acetobutylicum* further confirmed the function of the *ptsH* gene, and an *in vitro* phosphorylation experiment also demonstrated that kinase activity was present in a cell extract [17]. A *glcG* gene that is suggested to encode enzyme II of the D-glucose PTS was also found in *C. acetobutylicum* ATCC 824 through sequence alignment [18]. Interestingly, the inactivation of *glcG* had no effect on glucose utilization, which may be attributed to residual glucose PTS activity and non-PTS glucose uptake in this organism; instead, inactivation of *glcG* significantly alleviated the glucose repression of xylose utilization [19]. These results demonstrate the potential importance of the *glcG* gene in the metabolic engineering of *C. acetobutylicum* to enhance sugar co-utilization.

PTS or non-PTS is involved in glucose uptake and phosphorylation in *C. beijerinckii* [20–22], but the PTS activity rapidly declines during fermentation. Consequently, glucose uptake and metabolism at the end of the fermentation period are largely dependent on a non-PTS system, together with glucokinase [23].

Fructose is the main carbon source present in some inexpensive feedstocks and industrial by-products, such as Jerusalem artichoke and molasses. Compared to *C. acetobutylicum*, *C. beijerinckii* appears to be capable of better utilizing fructose (unpublished data). Indeed, a comparative genomic analysis showed that *C. beijerinckii* contains 47 sets of PTS II genes (not all complete sets), and 9 complete sets belong to the fructose/mannose/sorbose family [22], which is much more than that of *C. acetobutylicum*. However, none of these PTS II genes have been experimentally confirmed to be capable of transporting fructose.

As the preferred carbon source in many bacteria, glucose normally represses the utilization of some secondary substrates. Such a carbon catabolite repression (CCR) is mediated by CcpA, an important pleiotropic regulator, in Gram-positive bacteria [24]. A comparative transcriptome analysis showed that, similar to most other carbon

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