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Short communication

Effect of cassava pulp supplement on 1,3-propanediol production by *Clostridium butyricum*

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ABSTRACT

To improve its 1,3-propanediol (1,3-PD) production, *Clostridium butyricum* was cultivated on glycerol medium supplemented with cassava pulp (CP). At small concentrations, the CP improved the 1,3-PD productivity of *C. butyricum* from (0.25 ± 0.01) g/L/h (glycerol alone) to (0.43 ± 0.02) g/L/h (glycerol + 2 g/L CP) after 24 h fermentation.

The three-carbon diol 1,3-propanediol (1, 3-PD) is an important organic substrate for biopolymers such as polytrimethylene terephthalate (Liu et al., 2010). Glycerol, which is a by-product of biodiesel production (da Silva et al., 2009; Dobson et al., 2012) is the main substrate of 1,3-PD production by fermentation with microorganisms such as *Clostridium butyricum* (Chatzifragkou et al., 2011). However, the yield and productivity of 1,3-PD on glycerol are low because the growth and energy production are hampered by the low assimilation rate (Abbad-Andaloussi et al., 1998; Saint-Amans et al., 2001). Supplementing the glycerol medium with glucose is expected to enhance the growth and increase 1,3-PD production, but leads to catabolite repression in *C. butyricum* (Abbad-Andaloussi et al., 1998; Saint-Amans et al., 2001). Although *C. butyricum* can produce solvents from polysaccharides such as starch (Whelan and Nasr, 1951), the effect of polysaccharides on 1,3-PD production by this organism has not been reported. In this note, we report that supplementing the glycerol medium with small amounts of cassava pulp (CP) rather than starchy polysaccharides can improve the 1,3-PD productivity of *C. butyricum*. CP is a promising starchy-lignocellulosic biomass for biochemical production (Khempaka et al., 2009; Vaithanomsat et al., 2013), because both of its major components, namely, starch (50% dry basis) and cellulose fiber (approximately 30% dry basis), can be hydrolyzed to fermentable sugars (Apiwatanapiwat et al., 2015). For the present study, CP was obtained from Sanguan Wongse Industrial Co., Ltd. (Nakhon Ratchasima, Thailand). To ensure strong amylolytic activity of our employed *C. butyricum* strain, we compared the amylolytic activities of *C. butyricum* strain NBRC3858 and our originally isolated *C. butyricum* strain (I5-42). To assay the amylolytic ability, the culture supernatant was clarified and the reducing sugar was released from the starch by the Somogyi-Nelson method (Vaithanomsat et al., 2013). On M1 medium (Chatzifragkou et al., 2011) containing 10 g/L soluble starch (Wako Pure Chemicals, Osaka, Japan) as the sole carbon source, *C. butyricum* I5-42 exhibited stronger ability to degrade extracellular starch than the type strain (see Table 1). When *C. butyricum* I5-42 was cultured on M1 medium containing 30 g/L glycerol (Wako Pure Chemicals) as the sole carbon source, the 1,3-PD production was 9.0 g/L in 48 h of fermentation (Fig. 1A). The 1,3-PD and glycerol consumption were measured by high-performance liquid chromatography (HPLC) in a Shodex RSpak DE-613 column (Showa Denko K.K., Tokyo, Japan), with dis-

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Table 1
Comparison of extracellular amylolytic ability of *C. butyricum* strains.

Strain	Amylolytic activity[b](units/ml culture broth)	Source
C. butyricum NBRC3858	0.4 ± 0.03	NITE[c]
C. butyricum I5–42[a]	1.32 ± 0.02	This study

[a] The strain was originally isolated from compost on Ishigaki Island, Japan, and was identified as *C. butyricum* by 16S rRNA sequence analysis.
[b] Amylase activity was carried out in the supernatant of starch-grown cultures, and analyzed in 100 mM acetate buffer (pH 6.0) using soluble starch for 30 min at 55 °C.
[c] Culture Collection Division, Biological Resource Center, National Institute of Technology and Evaluation, Japan.

tilled water flowing at 0.6 ml/min at 60 °C. When the M1 medium containing 30 g/L glycerol was supplemented with 2 g/L and 4 g/L CP (1 g/L and 2 g/L starch, respectively), the 1,3-PD concentrations were 9.5 g/L and 8.2 g/L, respectively, similar to that of glycerol alone (Fig. 1A). However, the CP-supplementation increased the rate of 1,3-PD production by *C. butyricum* (Fig. 1A). Specifically, in M1 medium containing 30 g/L of glycerol supplemented with 2 g/L and 4 g/L of CP, the 1,3-PD productivity (g/L/h) after 24 h of fermentation was enhanced from 0.25 ± 0.01 (in 30 g/L of glycerol alone) to 0.43 ± 0.02 and 0.34 ± 0.04, respectively. The starch concentration was determined by a total starch assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland). The starch content of the supplemented CP was completely consumed by *C. butyricum* during the fermentation. To confirm whether the starch in CP increased the 1,3-PD productivity, a fermentation test was carried out in M1 medium containing 30 g/L of glycerol supplemented with 1 g/L of soluble starch (Wako Pure Chemicals) instead of CP (Fig. 1A). The starch supplement also increased the 1,3-PD productivity by *C. butyricum*. Conversely, in M1 medium containing 30 g/L of glycerol supplemented with 1 g/L of glucose, the maximum 1,3-PD concentration was 5.7 g/L, approximately 60% lower than when supplemented with 2 g/L CP. To assess the effect of glucose released from the supplemented CP, the released glucose concentration was measured by HPLC with a refractive index (Shimadzu RID-10A) detector on a Bio-Rad Aminex HPX-87 P column (Bio-Rad Laboratories, Inc., Hercules, CA, USA) operated at 80 °C with distilled water at a flow rate of 0.4 ml/min. CP-released glucose was detected in the cultures from 4 h, and had accumulated by 12 h. However, the accumulated glucose was rapidly consumed by *C. butyricum* (Fig. 1A, B). Although glucose was produced at 0.6 g/L and 1.93 g/L in glycerol cultures supplemented with 2 g/L and 4 g/L of CP, respectively, the 1,3-PD productivity was enhanced from that in glycerol alone (Fig. 1A, B). CP was released a lot of glucose into the medium when glycerol cultures supplemented with 8 g/L of CP would provide a delayed glucose supply to the medium for uptake by the *C. butyricum* cells. Meanwhile, it appears that different metabolic response to the glucose released from CP and the present in the medium. In fact, although *C. butyricum* rapidly consumed glucose in M1 glycerol medium supplemented with 1 g/L of glucose, the presence of glucose originally delayed 1,3-PD production (Fig. 1A, B). These results suggest that glycerol catabolism by *C. butyricum* differs in the presence of glucose originally present in the medium, and glucose that is gradually released from polysaccharides such as starch.

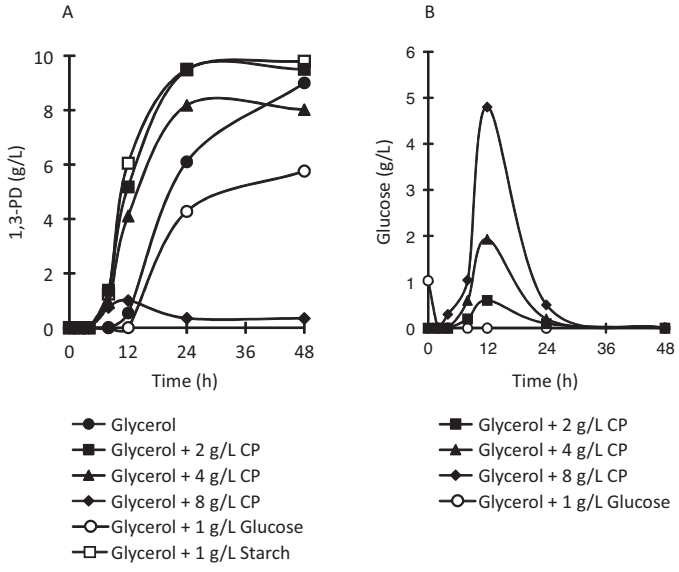

Fig. 1. Profiles of fermentative 1,3-PD production (A) and released glucose from CP (B) by *C. butyricum* I5-42 during batch fermentation on M1 medium (containing 30 g/L glycerol) supplemented with CP at various concentrations.

To understand the mechanism of the increased productivity, we measured the cell growth (as a proxy of the protein concentration) after 48 h of fermentation. To this end, the cells (not separated from CP and its fiber) were directly lysed by boiling in NaOH/SDS solution [0.2 N NaOH (Wako Pure Chemicals) containing 0.2% (w/v) SDS (sodium dodecyl sulfate; Wako Pure Chemicals)], and the protein concentration in the supernatant was estimated using a Pierce BCA assay kit (Thermo Fisher Scientific, Waltham, MA, USA). After 48 h, the *C. butyricum* cell proteins in the CP-supplemented glycerol reached 0.9 ± 0.07 mg/ml, more than double of the protein contents on glycerol alone (0.4 ± 0.02 mg/ml). Conversely, the 1,3-PD productivity was decreased in M1 medium containing 30 g/L of glycerol and supplemented with 8 g/L of CP. Similar trends have been reported for 1,3-PD production by *C. butyricum* in glucose-glycerol mixtures (Abbad-Andaloussi et al., 1998). Furthermore, glycerol medium induces the production of glycerol dehydrogenase (GDH), dihydroxyacetone (DHA) kinase, glycerol dehydratase and 1,3-PD dehydrogenase by *C. butyricum* (Saint-Amans et al., 2001). Glucose is known to repress the inducible synthesis of these enzymes, especially in fermentation (Abbad-Andaloussi et al., 1998; Saint-Amans et al., 2001). The cells consume glucose mainly as an energy source for acetate-butyrate production and NADH (Abbad-Andaloussi et al., 1998; Saint-Amans et al., 2001). Thus, excess CP supplementation might release sufficient glucose from starch degradation to repress these enzymes (Fig. 1A, B). In contrast, small amounts of CP will not interfere with the enzymes that convert glycerol to 1,3-PD (Fig. 1A, B). This phenomenon might depend on the available initial glucose concentration in the medium, and would directly affect the expression and activity of the above enzymes (Saint-Amans et al., 2001). These results indicate that supplementation with small quantities of CP not only improves the poor growth of *C. butyricum* on glycerol medium, but might also enhance 1,3-PD production by this organism.

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