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Short communication

Engineering of *Corynebacterium glutamicum* to utilize methyl acetate, a potential feedstock derived by carbonylation of methanol with CO

Seungjung Choo[a,b], Youngsoon Um[a,d], Sung Ok Han[b], Han Min Woo[a,c,d,*]

[a] *Clean Energy Research Center, Korea Institute of Science and Technology, Hwarangro 14-gil 5, Seongbuk-gu, Seoul 02792, Republic of Korea*
[b] *Department of Biotechnology, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea*
[c] *Green School (Graduate School of Energy and Environment), Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 02841, Republic of Korea*
[d] *Department of Clean Energy and Chemical Engineering, Korea University of Science and Technology, 217 Gajeong-ro, Yuseong-gu, Daejeon 34113, Republic of Korea*

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ABSTRACT

The possibilities to utilize one-carbon substrates (C_1) like CO, methane and methanol have been explored as a cheap alternative feedstock in the biotechnology. For the first time, methyl acetate (MeOAc), which can be formed from carbonylation of methanol with CO, was demonstrated to be an alternative carbon source for the cell growth of *Corynebacterium glutamicum* as a model microbial cell factory. To do so, a carboxyl esterase activity was necessary to hydrolyze MeOAc to methanol and acetate. Although the wild-type has an unknown esterase activity to MeOAc, the activity was not high enough to grow from 270 mM MeOAc as sole carbon source, reaching OD_{600} of 5.28 ± 0.2 in 32 h. Based on the literatures studied for the esterase, we chose three esterases (MekB of *Pseudomonas veronii* MEK700, AcmB of *Gordonia* sp. Strain TY-5, and Est of *Pyrobaculum calidifontis* VA1) and cloned into the wild-type. As a result, the recombinant *C. glutamicum* expressing the highly active MekB esterase (28.6 ± 0.77 U/mg protein) showed complete degradation of MeOAc and utilization of acetate, resulting in OD_{600} of 16.5 ± 0.02 at 24 h. In addition, the recombinant strain exhibited the rapid degradation of MeOAc to methanol and acetate in 2 h under anaerobic condition. Therefore, MeOAc can be used as another C_1-derived carbon source in the biotechnology.

Recent advances in metabolic engineering for microbial cell factories and integrated processes have progressed for production of value-added chemicals from various lignocellulosic biomass or marine biomass (Anbarasan et al., 2012; Peralta-Yahya et al., 2012). However, technological and economic barriers in biotechnological process still hinder the commercial scale production of various biofuels and biochemicals, compared to petroleum-based process. As a cost-advantage, the possibilities to utilize one-carbon substrates (C_1) like CO, methane and methanol have been explored as an alternative feedstock in the biotechnology (Dürre and Eikmanns, 2015). In addition, methanol and CO-derived chemicals such as methyl formate and methyl acetate (MeOAc) can be alternative feedstock (Morris, 2005). Especially, MeOAc is formed in situ during the carbonylation of methanol with CO insertion in presence of metal catalyst (i.e. cobalt-based BASF, rhodium-based Monsanto, iridium-based Cativa process) (Thomas and Suss-Fink, 2003). Using heterologous or iridium and ruthenium catalyst, MeOAc can also be synthesized from methanol with CO (Le Berre et al., 2000; Uhm et al., 1996). The availability of economically feasible MeOAc raises the question of whether MeOAc also serves as an alternative feedstock for microbial cell factories as renewable carbon and energy source.

Herein, we chose an industrial host, *Corynebacterium glutamicum* which is able to produce value-added chemicals such as succinate, isobutanol, cadaverine, and ethanol (Becker and Wittmann, 2012; Wendisch et al., 2006; Wieschalka et al., 2013). Because MeOAc is relatively hydrophobic and likely transported across the membrane barrier, firstly, *C. glutamicum* wild-type was cultivated in 50 mL CgXII medium containing 270 mM MeOAc (CAS No. 79-20-9) as sole carbon source at 30 °C on a rotary shaker at 200 rpm (Lee et al., 2014) in order to investigate whether the wild-type cell is able to utilize MeOAc for cell growth (Fig. 1). Three independent experiments were performed, accompanied by a MeOAc evaporation control. 83% of MeOAc in the cell-free medium was evaporated during the shacking, however the amount of evaporated MeOAc differed in presence of cells degrading MeOAc

* Corresponding author.
E-mail address: hmwoo@kist.re.kr (H.M. Woo).

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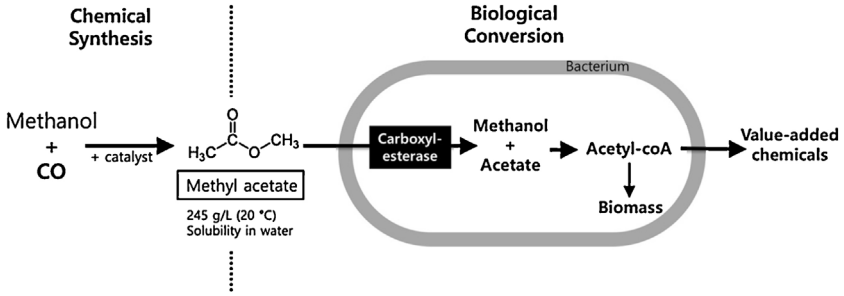

Fig. 1. Scheme of chemical synthesis and biological conversion of methyl acetate (MeOAc). Methyl acetate can be synthesized by carbonylation of methanol with CO in presence of catalysts. For biological conversion of MeOAc, bacteria that possess the activity of carboxyl esterase are capable of degrading MeOAc to methanol and acetate, of which substrates can be used as the sole carbon and energy sources. Further metabolic engineering of bacteria could be applied for production of value-added chemicals from MeOAc.

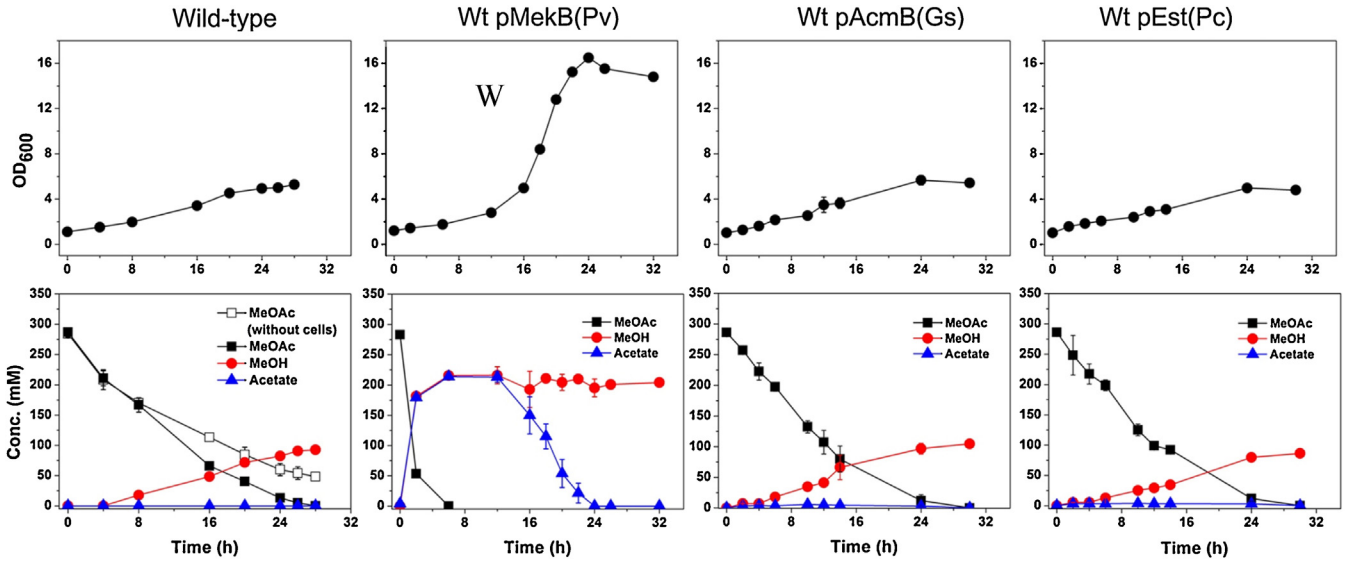

Fig. 2. Time course profiles of the cell growth and metabolites in *C. glutamicum* wild-type and the recombinants with methyl acetate as sole carbon source under aerobic condition. Recombinant strain Wt pMekB(Pv), Wt pAcmB(Gs), or Wt pEst(Pc) harbor a pBbEB1c plasmid carrying the codon-optimized *mekB*, *acmB*, or *est* gene, respectively. *C. glutamicum* cells were cultivated in 50 mL CgXII medium containing 270 mM methyl acetate (MeOAc) as sole carbon source at 30 °C on a rotary shaker at 200 rpm. All chemicals (methyl acetate; CAS No. 79-20-9) used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cell growth were measured at 600 nm (upper panel; black circle). In the lower panel, acetate (blue triangle), methanol (red circle), and MeOAc in the supernatant (black solid square) were measured. In addition, MeOAc in the cell-free medium (black open square) was also measured as a control. Metabolites were quantified by gas chromatography (Agilent Technologies, Model 6890) equipped with a HP-INNOWAX polyethylene glycol column (30 m × 0.25 mm × 0.25 m) and a flame ionization detector (FID) under the following conditions: oven temperature, from 50 °C to 240 °C at a rate of 10 °C/min; injector temperature, 250 °C; detector temperature, 250 °C; carrier gas (He); flow rate, 25 mL/min; and split ratio of 1:10. Mean values and standard deviations of triplicate cultures are shown (s.d. less than 1% not shown). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

Table 1
Specific enzyme activities of esterase (U/mg) in recombinant *C. glutamicum* strains.

	Strains			
	Wild-type	Wt pMekB(Pv)	Wt pAcmB(Gs)	Wt pEst(Pc)
Specific activity[a] (U/mg total protein)	0.41 ± 0.02	28.6 ± 0.77	0.43 ± 0.07	0.63 ± 0.10

[a] Enzyme activity was measured with 4-nitrophenyl acetate using the previous assay (Onaca et al., 2007). The reaction was started by adding 10 μL cell extract solution, and the kinetics of hydrolysis was determined by measuring absorption at 410 nm every 10 s for 2 min. Specific activity (U mg^{-1} total protein) was calculated and defined as the amount of enzyme releasing 1 μmol 4-nitrophenol min^{-1} (ε, 15.2 × 10^3 M^{-1} cm^{-1} l) per mg of total protein.

over the culture time (Fig. 2). As a result of the wild-type cultivation with MeOAc as sole carbon source, cell growth was observed (OD_{600} of 5.28 ± 0.2 in 32 h) and only methanol (92.7 mM ± 4.4) was detected in the medium. Although the wild-type has an endogenous methanol degradation pathway (Witthoff et al., 2013), the amount of methanol that converted to CO_2 could be negligible in the 28 h culture. Whereas, the equal-molar acetate (assuming 92.7 mM if there is an esterase activity in the wild-type), that were not detected, must be used for the cell growth because the growth corresponds to the double growth of the wild-type with 50 mM of potassium acetate as sole carbon source (Youn et al., 2009). Thus, we concluded that the wild-type has an unknown carboxyl esterase activity that breaks 1 mol of MeOAc to 1 mol of methanol and 1 mol of acetate. In spite of unknown activity, compared to the complete utilization of 300 mM acetate as sole carbon source by the wild-type (Woo et al., 2010), significantly low cell growth was observed (OD_{600} of 5.28 ± 0.2 in 32 h) with 270 mM MeOAc where theoretically 270 mM acetate can be used for the growth. Therefore, it is