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단 신

Formatotrophic Production of Poly-β-hydroxybutyric Acid (PHB) from *Methylobacterium* sp. using Formate as the Sole Carbon and Energy Source

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Abstract – Formate has been considered as an environmentally sustainable feedstock that can be used to accelerate the production of valuable chemicals. This study presents brief results of the formatotrophic production of poly-β-hydroxybutyric acid (PHB) by *Methylobacterium* sp. To evaluate the production of PHB, five species of *Methylobacteria* were tested using formate as the sole carbon and energy source. *Methylobacterium chloromethanicum* CM4 exhibited the highest productivity of PHB, which showed 1.72 g/L PHB production, 32.4% PHB content, and 0.027 g-PHB/g-formate PHB yield. These results could be used for the formatotrophic production of PHB with the concurrent reduction of CO_2 to formate.

Key words: Poly-β-hydroxybutyric acid (PHB), Formatotrophy, Formate, *Methylobacterium chloromethanicum* CM4, Formate dehydrogenase (FDH)

The demand for a significant reduction in carbon dioxide (CO_2) emission has intensified due to increasing attention to global warming and climate change. As an alternative strategy for the sequestration of CO_2, the chemical or electro-chemical reduction of CO_2 to useful organic compounds, such as formate, methane, carbon monoxide, and ethylene, has been investigated [1]. Among these products, formate has been researched as a fuel alternative to methanol or hydrogen in fuel cells [2,3]. In addition, the National Research Council of the National Academies of the USA has suggested formate, along with methane and CO_2, as an economically feasible and environmentally sustainable feedstock for accelerating the production of fuels and high-volume chemicals via bio-processing [4].

The electrochemical reduction of CO_2 can produce formic acid at low overpotential and with high faradic efficiency [5]. In addition, an exceptionally higher capability to synthesize formate by the electro-biocatalytic reduction of CO_2 has been reported using an oxygen-stable whole-cell biocatalyst [6]. Therefore, there is an urgent demand to find a new route for the production of more valuable compounds using formate as a carbon source.

Formate can be used by living cells as a carbon and energy source [7,8]. Formate is one of the best electron mediators that have good electron carrier criteria, such as stability, non-toxicity, permeability through the cell membrane, and low volatility [9]. Pink facultative methylotrophs, non-pigmented *pseudomonads*, facultative autotrophs or phototrophs, and *Hyphomicrobia* are able to grow on formate through the serine pathway or the ribulose monophosphate (RuMP) pathway [10]. However, these studies are focused on the genetic, metabolic, and physiologic point of view rather than on the perspective for the production of a valuable product. In this study, we report a brief study for the formatotrophic production of poly-β-hydroxybutyric acid (PHB) from *Methylobacterium* sp. using formate as the sole carbon and energy source.

The microbial genus of *Methylobacteria*, including *Methylobacterium extorquens* AM1 (ATCC 14718), *M. chloromethanicum* (KCTC 32005), *M. suomiense* (KCTC 12963), *M. platani* (KCTC 12901), and *M. tarhaniae* (KCTC 23615), was obtained from KCTC (Korean Collection for Type Cultures) and ATCC (American Type Culture Collection). The culture medium contained $(NH_4)_2SO_4$ (1.0 g/L), KH_2PO_4 (1.3 g/L), $Na_2HPO_4 \cdot 7H_2O$ (0.45 g/L), $CaCl_2 \cdot 2H_2O$ (3.3 mg/L), $FeSO_4 \cdot 7H_2O$ (1.3 mg/L), $MnSO_4 \cdot H_2O$ (100 μg/L), $ZnSO_4 \cdot 7H_2O$ (130 μg/L), $CuSO_4 \cdot 5H_2O$ (40 μg/L), $Na_2MoO_4 \cdot 2H_2O$ (40 μg/L), $CoCl_2 \cdot 6H_2O$ (40 μg/L), and H_3BO_3 (30 μg/L) [11]. For formatotrophic PHB production, sodium formate (25 mM) was added initially as the sole carbon and energy source. The cultures for the PHB production were performed using a 250-mL vessel that was equipped with a pH control system. To maintain a pH of 7.0 and a continuous supply of formate, a pH-static formic acid feeding system was used. Then, 10 N formic acid was fed as a pH titrant and carbon source using a pH controller and peristaltic pump. Filter-sterilized air was supplied at a flow rate of 1 vvm, and agitation was performed with a magnetic bar at a rate of 500 rpm. Temperature (30 °C) and stirring were controlled in a personal organic synthesizer ChemiStation (Eyela, Tokyo, Japan). Samples were collected periodically to determine cell growth, residual formate, and PHB production.

The dry cell weight (DCW) was measured after washing and drying the culture samples. The PHB concentration was determined using

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‡ This article is dedicated to Prof. Choon Han on the occasion of his retirement from Kwangwoon University.

a gas chromatographic method as previously described [12]. A gas chromatograph (Agilent Technology 6890 GC System) that was equipped with an HP-INNOWax column (30 m×250 μm×0.25 μm, Agilent Technologies, CA, USA) and flame ionized detector was used. The oven temperature was programmed to increase from 50 °C to 170 °C at a rate of 1 °C/min. The injector and detector temperatures were set to 250 °C. Helium was used as a carrier gas with a flow rate of 1.0 mL/min. The concentration of formate was determined by HPLC (Agilent 1200 series) with an Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad, Hercules, CA) and a refractive index detector (RID) at 35 °C. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min.

To evaluate the formatotrophic production of PHB, a total of five species of *Methylobacteria* were tested using formate as the sole carbon and energy source. As shown in Table 1, among the tested bacteria, *M. chloromethanicum* CM4 showed the highest productivity of PHB compared to the other strains. *M. chloromethanicum* CM4 produced 1.72 g/L PHB, and the PHB content was 32.4%. These values are much higher than those of *M. extorquens* AM1 (1.08 g/L and 22.46%, respectively). PHB yields relative to the total formate consumed (g-PHB/g-formate) were in the range of 0.003~0.027. *M. chloromethanicum* CM4 showed the highest PHB yield (0.027), followed by *M. extorquens* AM1 (0.018).

PHB accumulation is a growth-associated process, so that the biomass yield from the carbon and energy source is critical. The molar yield values (g-DCW/mol of substrate utilized) of several bacteria that utilize C_1-compounds via the RuMP pathway and the serine pathway have been reported [7]. When using the serine pathway, the yield values of methanol and formate were 0.31~0.41 g-DCW/g-methanol and 0.07~0.15 g-DCW/g-formate, respectively. It means that the yield values decreased as increase of oxidation level of C_1 substrate. In this study, the biomass yield value of *M. chloromethanicum* CM4 was 0.084 g-DCW/g-formate, which agrees with previous reports.

The methanol-based PHB yields from a high-cell-density culture of *M. extorquens* AM1 and the phosphate-limited fed-batch culture of *M. organophilum* were 0.20~0.22 g/g-methanol and 0.19 g/g-methanol, respectively [13,14]. These values are approximately seven times higher than those of *M. chloromethanicum* CM4 on formate in this study. The theoretical yields of PHB from methanol and formate were estimated to 0.54 (g-PHB/g-methanol) and 0.13 (g-PHB/g-formate), respectively [15]. It means that the result of this study (0.027 g-PHB/g-formate) on formate is about 20% of the theoretical value. As shown in Fig. 1, PHB production showed a growth-associated

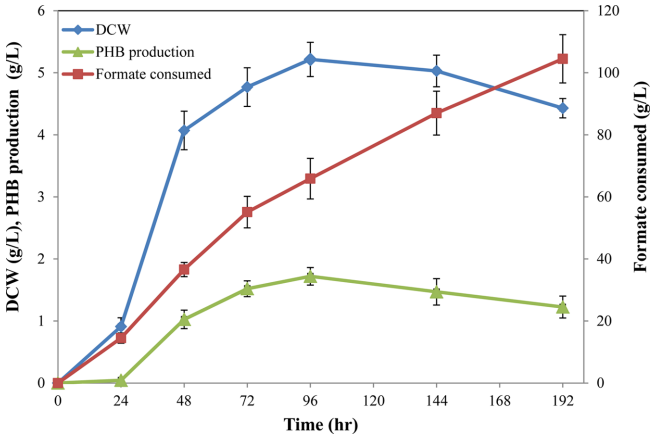

Fig. 1. Time course of PHB production by *M. chloromethanicum* CM4 using formate as the sole carbon and energy source.

pattern. Cell growth and PHB production peaked at 96 hr of culture and then decreased slowly. However, formate was continuously consumed until the end of culture. In addition, the formate consumption rate was maintained almost constant throughout the culture time, meaning that formate supply and consumption occurred continuously despite the cessation of growth. This result could be due to two reasons. First, the consumed formate was used to maintain cells rather than to support cell growth and PHB production. Second, formate could be oxidized by formate dehydrogenase (FDH).

In methylotrophic metabolism, formate serves as the main branch point between assimilatory and dissimilatory metabolism [16]. Formate can either be oxidized to CO_2 for energy generation or be converted to methylene-H_4F into biomass via the serine cycle. Formate oxidation to CO_2 is carried out by four known FDH enzymes in *M. extorquens* AM1 [17]. Therefore, some of the consumed formate after the stationary growth phase could be attributable to the oxidation of formate by FDHs. In fact, in the experiment of formate oxidation by resting cells of *M. chloromethanicum* CM4, 30 mM of formate was totally disappeared within 20 hr (data not shown).

Although *M. Chloromethanicum* CM4 exhibited the greatest ability to produce PHB, the yield value was not high compared to that of methanol. Nevertheless, the formatotrophic production of PHB has an advantage if it is connected with the process of the concurrent reduction of CO_2 to formate. In addition, this production is suited for the current demand of the use of formate as a feed stock instead of sugars for the production of valuable products through microbial fermentation.

Table 1. Formatotrophic production of PHB by *Methylobacterium* sp*

	PHB content (%)	PHB production (g/L)	PHB yield (g-PHB/g-formate)	Biomass yield (g-DCW/g-formate)
M. chloromethanicum CM4	32.40	1.72	0.027	0.084
M. extorquens AM1	22.46	1.08	0.018	0.080
M. platani	15.42	0.59	0.009	0.059
M. suomiense	3.59	0.19	0.003	0.116
M. tarhaniae	11.61	0.94	0.013	0.087

*The data were obtained after 96 hr of culture. All of the measurements were performed in triplicate, and the values were averaged

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