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Enantioselective Production of (*S*)-3-Hydroxybutyric Acid, (*S*)-1,3-Butanediol and (*R*)-1,3-Butanediol Using Methanol Yeast

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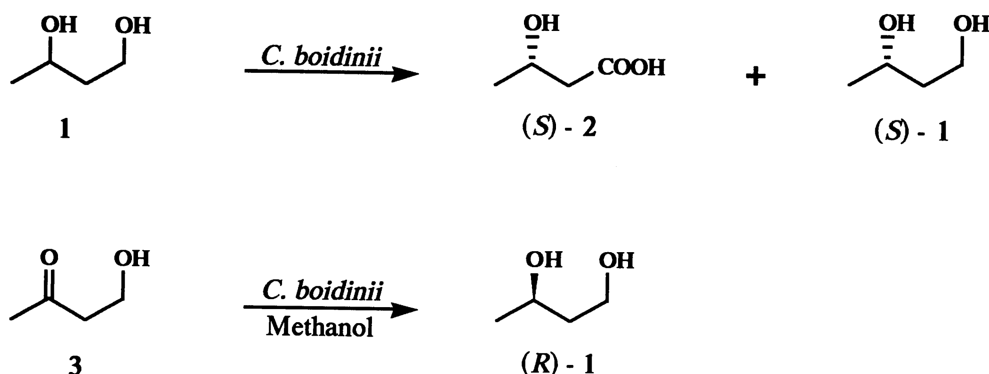
(*S*)-3-Hydroxybutyric acid and (*S*)-1,3-butanediol were obtained by the treatment of 1,3-butanediol with the resting cells of methanol yeast, *Candida boidinii* (IFO 10574). (*R*)-1,3-Butanediol was also obtained in high optical purity by the enantioselective reduction of 4-hydroxy-2-butanone in the presence of methanol using the same methanol yeast.

Optically active 1,3-polyol type segments are often found in naturally occurring bioactive substances, such as antibiotics, pheromons and medicines. [1,2] Optically active 1,3-diol and its derivative, 3-hydroxybutyric acid, will play an important role as environmentally acceptable compounds in the industrial field. On the other hand, 1,3-butanediol is one of the industrially available chemicals, and methanol produced from natural gas as well as carbon dioxide will be some of the most abundant and cheap carbon sources in the coming years. The utilization of methanol as a growing substrate for microbes[3,4] will make it feasible to introduce a microbial process for industrial applications. The microbial production of chiral 3-hydroxybutyric acid was recently reported,[2] but the microbial production of (*S*)-3-hydroxybutyric acid, (*S*)-1,3-butanediol and (*R*)-1,3-butanediol using methanol assimilating microbes has not been reported. This communication reports the microbial production of (*S*)-3-hydroxybutyric acid [(*S*)-**2**] and (*S*)-1,3-butanediol [(*S*)-**1**] as a residual substrate of 1,3-butanediol (**1**) using the methanol yeast, *Candida boidinii* KK912. (*R*)-1,3-Butanediol [(*R*)-**1**] was also prepared by the enantioselective reduction of 4-hydroxy-2-butanone (**3**) using the same methanol yeast, *C. boidinii* KK912.

Candida boidinii KK912 (IFO 10574) was first isolated by an enrichment culture technique from activated sludge as a methanol assimilating strain. *C. boidinii* KK912 was grown in an inorganic medium (200 mL, initial pH 5.0)[5] containing 2.0% methanol as the growing substrate in a shaking flask at 30 °C. After five days (OD_{660}



= 3.3), the cells were harvested by centrifugation, washed with distilled water to obtain wet cells (1.5 g wet cells corresponds to 0.3 g dry cells) for the reaction. The reaction mixture contained 45 mg of **1** and 300 mg of wet cells (corresponds to 60 mg of dry cells) in 10 mL of 0.05 mol•dm^{-3} phosphate buffer (pH 7). The reaction was performed in a shaking tube with reciprocal shaking at 30 °C in the dark. After the reaction, the cells were removed from the medium by centrifugation and the supernatant was filtered through a 0.2 μm membrane. The filtrate was then evaporated to dryness in vacuo to give a syrup. The crude product was purified by ion-exchange resin column chromatography (Dowex 50-W8 H^+-type and Dowex 2X8 HCO_3^- type) to give (*S*)-**2** and residual (*S*)-**1**. The isolated product was analyzed by IR, ^1H NMR and ^{13}C NMR spectroscopy.[6] These spectral data completely agreed with those of the authentic compound. Optical purity was estimated by calculating the enantiomeric excess (% e.e.) using the chiral HPLC column.[7] The yields of (*S*)-**2** and residual (*S*)-**1** were periodically analyzed by HPLC[8] as shown in Fig. 1. From these results, it was found that (*S*)-**2** and residual (*S*)-**1** were obtained in 13% and 32% yields, respectively, after 24 h incubation. The optical purities for (*S*)-**2** and residual (S)-**1** were 94% e.e. and 96% e.e., respectively, after 24 h incubation. No other product was detected in the reaction mixture during the incubation.

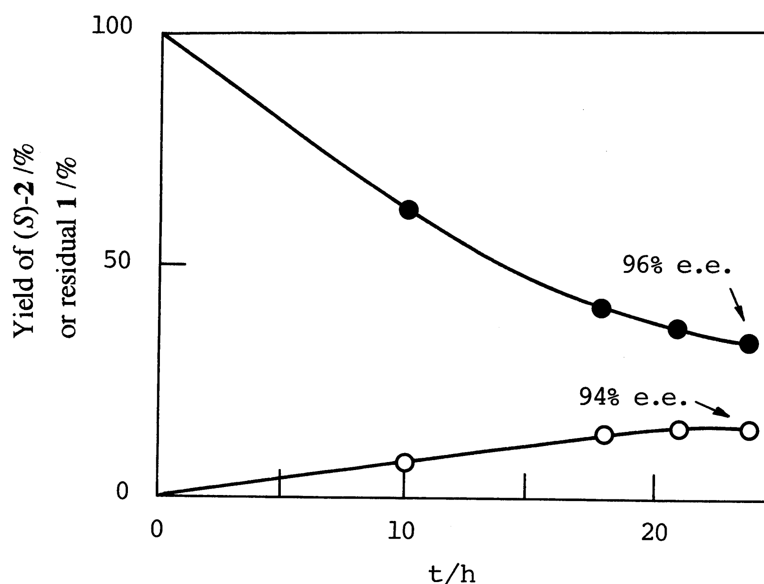


Fig. 1. Microbial transformation of **1** into (*S*)-**2** and (*S*)-**1** by *Candida boidinii* KK912 (IFO 10574).
○ : Yield of (*S*)-**2**, ● : residual **1**.

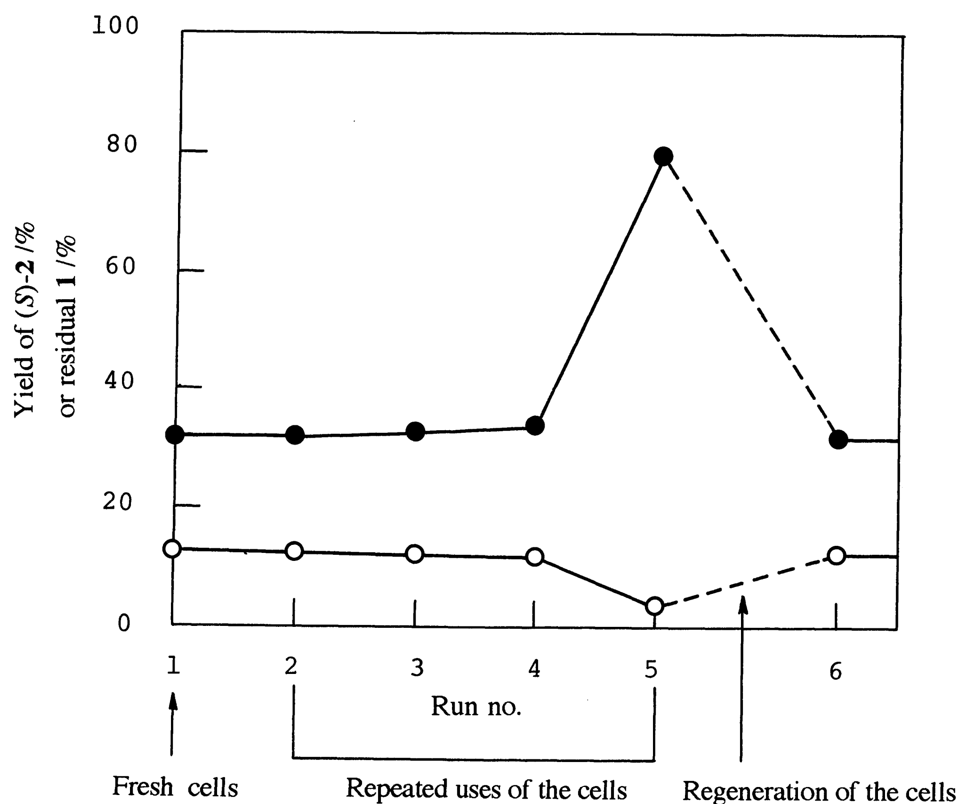


Fig. 2. Repeated uses of the microbial cells for the transformation of **1** into (*S*)-**2** and (*S*)-**1**. ○ : Yield of (*S*)-**2**, ● : residual **1**.

From the fact that both (*R*)-3-hydroxybutyric acid and (*R*)-1,3-butanediol were more rapidly assimilated than the corresponding (*S*)-enantiomers by *C. boidinii,* it is suggested that this enantioselective production was performed by kinetically controlled enantiomeric separations.

The microbial cells used for the reaction could be readily removed almost quantitatively from the incubation media by centrifugation. Thus obtained recovered cells could be used at least three times without being regenerated. The recovered cells worked actively in repeated uses as in the first use. Figure 2 shows a typical example of the results of the reaction of **1** with the recovered microbial cells for 24 h. The reaction was repeatedly carried out in the same manner as described for the fresh microbial cells. It was confirmed that similar reaction results were obtained using recovered cells. However, repeated use of more than four times caused a decrease in the activities of the microbial cells. So, after three times repeated use, the recovered cells were incubated with an inorganic medium containing 1.0% methanol for 24 h at 30 °C in a shaking flask for regeneration of the microbial cells. Thus regenerated microbial cells again worked actively against **1** as in the first use as shown in Fig. 2.

(*R*)-1,3-Butanediol [(*R*)-**1**] was obtained in high optical purity by the reduction of **3** with the resting cells of *C. boidinii* KK912 in the presence of methanol. Enantioselectively produced (*R*)-**1** was accumulated in the incubation media without assimilation when methanol was present in the incubation media. A typical reaction condition is as follows. The reaction mixture contained 100 mg of **3**, 5 g of wet cells and 500 mg of methanol in 50 mL of 0.05 mol•dm^{-3} phosphate buffer (pH 7). The reaction was performed in a shaking flask with reciprocal shaking at 30 °C in the dark. After the reaction, the cells were removed by centrifugation and the supernatant was filtered through a 0.2 μm membrane. The filtrate was then evaporated to dryness in vacuo to give a syrup. The crude product was purified by distillation under reduced pressure (110 °C/23 mmHg) to give (*R*)-**1**. The spectral data of IR, ^{1}H NMR, and ^{13}C NMR for the isolated product agreed completely with those of the authentic compound. The yield of (*R*)-**1** as determined by HPLC[9] was 60%, and the optical purity of (*R*)-**1** as determined by the HPLC method [7] was 99% e.e. after 6.5 h incubation. (R)-**1** was also obtained by the microbial reduction of **3** using D-glucose in place of methanol. However, both the yields and optical purity were reduced. That is, yield and enantiomeric excess of (*R*)-**1** were 40% and 77% e.e., respectively, after 6.5 h incubation.

Methanol yeast, *C. boidinii* KK912, was found to be a useful tool for the enantioselective preparation of 1,3-type diols and their derivatives.

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6) (*S*)-**2** (Na salt) : ^{13}C NMR (D_2O) : δ 22.9 (C-4), 65.3 (C-3), 43.9 (C-2), 176.8 (C-1).

7) Chiral HPLC column : Daicel Chemical Industries Ltd., chiral column, CHIRALCEL OB ; Eluent : hexane-isopropanol (19/1 v/v) ; UV detector : JASCO 875UV (220 nm).

8) HPLC column : TOSOH Co. Ltd., Cation-exchange chromatographic column, TSK-Gel SCX ; Eluent : 0.05 mol•dm^{-3} $HClO_4$; RI detector : Showa Denko Co. Ltd., Shodex RI SE-51.

9) For **3**, HPLC column : TOSOH Co. Ltd., Anion-exchange chromatographic column, TSK-Gel Sugar AXG ; Eluent : 0.5 mol•dm^{-3} borate buffer, pH 8.7, 65 °C ; UV detector : JASCO 875UV (210 nm). The system was calibrated with an authentic standard.

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