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Asymmetric Reduction of Benzil to (*S*)-Benzoin With Whole Cells of *Bacillus cereus*

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Abstract

Benzil **(1)** was selectively reduced to (*S*)-benzoin **(2)** in the presence of a wild-type *Bacillus cereus* Tim-r01. A 92% yield of **2** with 94% enantiomeric excess ratio was attained in phosphate-buffered saline (PBS) (pH 7.5) by using glucose as a nutrient at 37°C for 12 h. Compound **2** was not reduced further to hydrobenzoin **(3)** at all. The reduction activity differed greatly depending on the strain of *B. cereus*. Under these conditions the *B. cereus* strains IFO3001, IFO15305, IAM1110, IAM1229, IAM1656, and IAM1729 gave **2** in yields ranging from 23 to 46% and the configuration of **2** was (*S*)-form (7 to 86% ee).

Index Entries: Asymmetric reduction; benzil; benzoin; *Bacillus cereus*; diketone.

Introduction

Microorganisms have been increasingly applied to the transformation of organic compounds as environment-friendly catalysts since biochemical reactions take place in aqueous medium under ambient conditions and after the reaction the catalysts themselves are safely decomposed and regenerated in nature. Baker's yeast, which is a widely used biocatalyst, reduces various carbonyl compounds such as monoketones, diketones, and ketoesters to corresponding chiral alcohols with sucrose as a sole energy source *(1)*. However,

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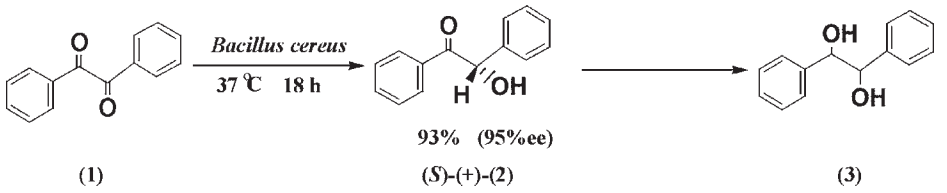

Scheme 1. Reduction of benzil by *B. cereus*.

in the reduction of benzil (**1**), baker's yeast gave only racemic benzoin *(2)*. Although chiral-ligand-coordinated metal complexes efficiently reduce ketones to chiral alcohols *(3)*, biocatalysts still have many advantages.

Konishi et al. *(4)* have recently reported the reduction of **1** to (*R*)-form of **2** in the presence of the bacterium *Xanthomonas oryzae* IAM 1657. Previously, they found that *Bacillus cereus* Tim-r01 isolated from soil in Gofuku, Toyama, Japan, gave (*S*)-form of **2** in good yield, and the reductase gene was isolated *(5,6)*. In the present study, we used whole cells as catalyst and selectively obtained (*S*)-form of **2** by controlling conditions such as the medium, temperature, strain of *B. cereus*, and atmosphere. Under our reaction conditions, **2** was not reduced further to hydrobenzoin (**3**) at all (Scheme 1).

Materials and Methods

B. cereus strains IFO 3001, 3836, 13494, and 15305 were obtained from the Institute for Fermentation Osaka (IFO), and strains IAM 1110, 1229, 1656, and 1729 were kindly provided by the Institute of Applied Microbiology (IAM).

Reduction was carried out by using typical species and strains of bacteria as follows. After *B. cereus* Tim-r01 had been grown in a sterilized minimum nutrient solution of 1% polypeptone, 1% meat (bonito) extracts, and 0.2% NaCl at 37°C for 12 h, the cells were harvested by centrifugation (2000 rpm), washed three times with phosphate-buffered saline (PBS), and then dispersed in a fresh nutrient solution or PBS (pH 7.5). Into an L-shaped test tube (20 mL) was added the culture solution (10 mL) containing the bacterial cells (0.2 wet g) and **1** (2 m*M*) dissolved in dimethylsulfoxide (DMSO) (100 µL). The addition of DMSO as the solvent of **1** was indispensable since the substrate coagulated and attached to the wall of the reaction vessel when the solvent was lacking.

The reduction was started by slow shaking (200 rpm) at 37°C. After the reaction, the products were extracted with ethylacetate (AcOEt) and analyzed using a high-pressure liquid chromatography (HPLC) apparatus (Gilson model 102, column YMC-pack, ODS-A; 4 mm id × 150 mm length). The enantiomeric excess ratio (ee%) of **2** was analyzed by HPLC using a chiral column (Chiralcel, 4.6 mm id × 150 mm length; Daicel, Japan) with acetonitrile:H_2O (4:6) as an eluent. The specific optical rotation of isolated products was confirmed by using a polarimeter at 987 nm (Model PM-101; Union Giken, Japan).

Table 1
Effects of Saccharides on Reduction of Benzil
by *B. cereus* Tim-r01 in PBS[a]

Saccharides	Medium	Yield (%)	ee (%)
—	—	24	84
Glucose	Saline	71	87
Glucose	PBS	92	94
Fructose	PBS	93	85
Xylose	PBS	19	73
Sucrose	PBS	31	81
Maltose	PBS	90	90
Lactose	PBS	37	82

[a]Reduction of benzil was done by using an L-shaped test tube (30 mL) containing PBS (10 mL), bacterial cells (0.2 wet g), benzil (2 m*M*), and 1% saccharides. The tube was shaken at 37°C for 12 h (200 rpm).

Results and Discussion

*Effects of Nutrients on Reduction of **1***

The effects of nutrients such as minimum nutrient and saccharides were examined. Without nutrients, the yield of **2** was very poor. In the presence of minimum nutrients, **1** was reduced smoothly at the initial stage of the reaction, but the yield of **2** decreased after having reached a maximum. Furthermore, various impurities from nutrients were extracted by AcOEt.

Therefore, several saccharides were used as nutrients. Table 1 provides the results of the reduction of **1** in the presence of mono- and disaccharides. In the case of the addition of glucose, the reduction of **1** continuously occurred to give **2** in good yield (92%) with 94% ee to (*S*)-form of **2** at 37°C for 12 h. One percent of glucose was sufficient for the reduction, but a concentration of <1% and >3% decreased the yield (data not shown). Interestingly, the hexoses glucose and fructose gave 92 and 93% yields, respectively. In addition, disaccharide of maltose gave a higher yield of (*S*)-form of **2**, but sucrose and lactose gave poor results. A pentose of xylose gave a poor yield (19%). These results suggest that the effectiveness of saccharides in the reduction greatly depended on the production of digestive metabolites in the cells as the energy source.

*Effects of Reaction Conditions on Yield of **2** in Presence of* B. cereus *Tim-r01*

The reduction of **1** was studied under various reaction conditions in the presence of glucose as a sole energy source; the results are given in Table 2. The only product observed was **2**. Temperature and atmosphere greatly affected the reduction of **1**. A reaction temperature of about 37°C resulted in good yields since temperatures higher than 50°C and lower than

Table 2
Effects of Reaction Conditions on Reduction of Benzil to Benzoin by *B. cereus* Tim-r01

Substrate (m*M*)	Time (h)	Temperature (°C)	Atmosphere	Sugar	Yield (%)	ee (%)
2	12	37	Air	—	24	84
2	12	37	Air	Glucose	92	94
2	18	37	Air	Glucose	93	95
2	12	37	O_2	Glucose	62	89
2	12	37	N_2	Glucose	22	80
4	12	37	Air	Glucose	34	92
2	12	25	Air	Glucose	18	92
2	12	50	Air	Glucose	2	73

[a]Reduction of benzil (2 or 4 m*M*) in PBS (10 mL) was done in the presence of bacterial cells (0.2 wet g) by shaking (200 rpm) for 12–18 h.

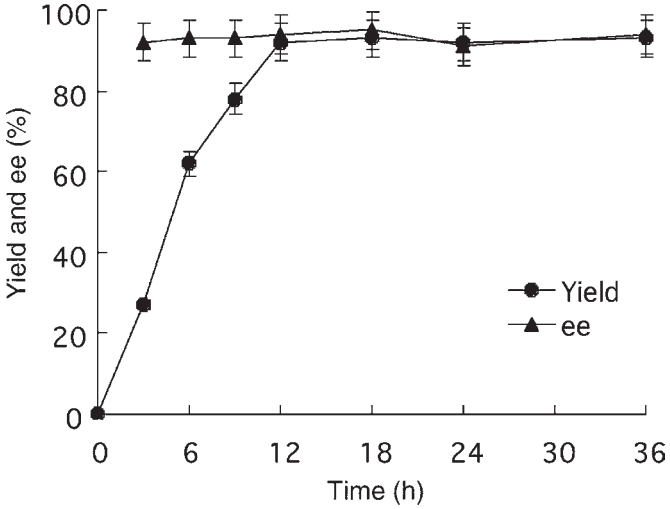

Fig. 1. Time courses of reduction of benzil to benzoin by *B. cereus* Tim-r01. Reduction was done in a 30-mL L-shaped test tube containing benzil (2 m*M*), bacterial cells (0.2 wet g), and 1% glucose in 10 mL of PBS at 37°C (200 rpm). The SD was obtained from three independent experiments.

25°C decreased the yields. A moderately oxidative atmosphere was needed to obtain a higher yield of **2**; an N_2 atmosphere gave a poor yield and an O_2 atmosphere hindered the reaction. An aluminum cap was then placed loosely on the reaction vessel to maintain the aeration by shaking. With an increase in the concentration of **1** from **2** to 4 m*M*, the yield of **2** decreased from 90 to 34% under the condition of a cell concentration of 0.2 wet g/100 mL. As a result, bacterial cells of 0.2 wet g reduced 2×10^{-2} mmol of **1** to give (*S*)-form of **2**. An excess amount of **1** inhibited the reduction. After the products had been extracted with AcOEt, **2** was purified on a silica-gel

Table 3
Reduction of Benzil to Benzoin
in Presence of Typical Species of Bacteria
and Several Strains of *B. cereus*[a]

Bacterium	Yield (%)	ee (%)
None	0	—
B. cereus		
Tim-r01	92	94
IFO3001	23	77
IFO3836	4	—
IFO13494	11	82
IFO15305	38	79
IAM1110	35	80
IAM1229	36	83
IAM1656	25	86
IAM1729	46	77
E. coli		
XL1-BLUE	4	—

[a]Reduction was done in the presence of bacterial cells (0.2 wet g) by the addition of benzil (2 m*M*) in a fresh culture at 37°C for 12 h.

column with MeOH-hexane as an eluent. Optical rotation and analysis by HPLC (chiral column) showed the configuration of **2** to be (*S*)-form *(5)*.

The time course of reduction of **1** to **2** in the presence of glucose in PBS at 37°C is shown in Fig. 1. Good reproductivity of the reduction of **1** in three independent experiments was observed. The yield of **2** linearly increased until 9 h and then became saturated and reached a 93% yield (95% after 18 h). An ee above 90% was maintained from the beginning of the reaction. Compound **2** was not reduced further to **3** when the reaction time was prolonged.

*Reduction of **1** With Various Strains of* B. cereus

In the reduction of **1**, living cells collected from the culture solution by centrifugation were used. The activities of several strains of *B. cereus* toward **1** in PBS are given in Table 3. Good reducing activities were attained by shaking at about 200 rpm since slow shaking retarded the reduction. In addition, an air supply was needed during the reaction. The yield of **2** by *B. cereus* Tim-r01 was the highest (92%), and the highest enantiomeric excess ratio (94%) was attained among the strains examined. The *B. cereus* strains IFO3001, IFO15305, IAM1110, IAM1229, IAM1656, and IAM1729 gave **2** in various yields ranging from 23 to 46%. Interestingly, the configurations of **2** obtained from *B. cereus* strains were (*S*)-form, ranging from 77 to 86% ee. The activities of *B. cereus* IFO3836 and IFO13494 were much weaker. These results suggested that the presence of reduction enzymes was not common to all of the strains of *B. cereus* examined.

When (*S*)-form of **2** was used for the substrate, reduction to **3** did not occur but oxidation to **1** occurred gradually. In the presence of a typical Gram-negative bacterium, *Escherichia coli*, no substantial amount of product was obtained. However, reductase gene–encoded *E. coli* reduced **1** to almost pure **2** with an excellent yield *(6)*. Therefore, it was considered that *E. coli* lacks the reductase gene for **1**. Among the strains of *B. cereus* examined, Tim-r01 specifically reduced **1** to (*S*)-form of **2**.

In baker's yeast, **1** was reduced to give (*S*)- and (*R*)-isomers by several reductases *(7)*. Therefore, the composition and activity of each enzyme determined the ee%. In addition, when molds were treated with acrylonitrile, the activity toward the (*S*)-form was suppressed *(8)*. As a result (*R*)-form was obtained selectively. In our reaction, it was thought that the activities toward (*R*)-form of **2** were very weak or that *B. cereus* Tim-r01 lacks reductase to (*R*)-form of **2**, resulting in the selective production of (*S*)-form of **2**.

The applicability of the cells to the reduction of other ketones such as benzyl phenyl ketone, *trans*-chalcone, 1-phenyl-1,3-butanedione, and dibenzoylmethane was examined. The yields of the reduction of these ketones were as low as 21–5%, indicating a high substrate specificity of *B. cereus* Tim-r01 in the reduction of ketone compounds.

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