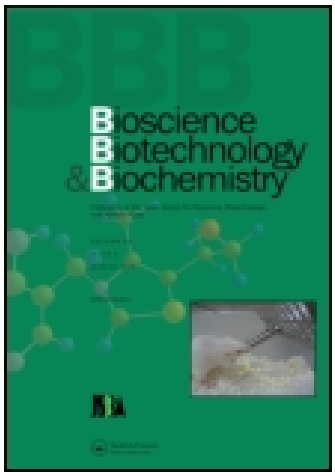

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Enantioselective Oxidation of Diols by Secondary Alcohol Dehydrogenase from Geotrichum sp. WF9101

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Note

Enantioselective Oxidation of Diols by Secondary Alcohol Dehydrogenase from *Geotrichum* sp. WF9101

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***Geotrichum* sp. WF9101 could degrade (*S*)-(+)-1,2-propanediol, (*S*)-(+)-1,3-butanediol, and (2*S*,4*S*)-(+)-2,4-pentanediol, but not the corresponding enantiomers. An NAD$^+$-linked secondary alcohol dehydrogenase purified from the strain showed the same enantioselective oxidations towards these diols. This enzyme is proposed to be useful for the preparation of (*R*)-(−)-diols from the racemates of these diols.**

Key words: NAD$^+$-linked secondary alcohol dehydrogenase; *Geotrichum* sp.; enantioselective oxidation of 1,3-butanediol

Enantiomerically pure diols are useful chiral auxiliaries for synthesis of agrichemicals and pharmaceutical chemicals. Recently, there has been growing interest in the chemical industry in the preparation of products from optically active diols using microbial[1–6] or enzymatic methods.[7–11]

In a previous study, we isolated a *Geotrichum* sp. (strain WF9101) that showed a high 2,4-pentanediol-degrading activity,[12] and succeeded in the purification of an NAD$^+$-linked secondary alcohol dehydrogenase (SADH) from the strain (T. Mori *et al.*, manuscript submitted to this journal). In this paper, we describe the enantioselective degradation of various diols by this strain and enantiospecificities of the SADH.

Geotrichum sp. strain WF9101, which was isolated and identified in our preceding study,[12] was used. The composition of the different media used for the enantioselective assimilation of various alcohols by the strain was 0.2% substrate, 0.5% NH_4NO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NaCl, and 0.02% yeast extract (pH 6.8). The cultivation of WF9101 was done in 50 ml of medium in a 100-ml Erlenmeyer flask on a rotary shaker at 120 rpm at 30°C. Optically active 1,2-propanediol, 1,3-butanediol, 2-butanol, and 2-pentanol were obtained from Tokyo Chemical Industries (Tokyo, Japan) and optically active 2-hexanol and 2,4-pentanediol were from Wako Pure Chemical Industries Ltd. (Osaka, Japan). The racemic alcohols were prepared by mixing both enantiomers. The assays for the optically active alcohols were done by a capillary gas-liquid chromatograph (Shimadzu GC-14A) coupled with a flame ionization detector. For separation of the enantiomers, a CDX-B column (J&W Scientific, U.S.A.) was used. The column temperatures were 120°C for 2-ols and 1,2-propanediol, 140°C for 1,3-butanediol, and 150°C for 2,4-pentanediol. Mass spectra for identification of the degradation product of 1,3-butanediol was made with a Shimadzu Q-2000 spectrometer coupled with a Shimadzu GC-14A gas chromatograph.

Table I shows degradation rates of the optically active diols and 2-ols by strain WF9101. When the strain was cultured for 3 days in the medium containing the enantiomer as a sole carbon source, it could degrade the (*S*)-(+)-forms of the diols, but not the (*R*)-(−)-forms. The degradation rates of (*S*)-(+)-1,2-propanediol, (*S*)-(+)-1,3-butanediol and (2*S*,4*S*)-(+)-2,4-pentanediol were 39.8%, 93.4%, and 83.0%, respectively. On the other hand, the strain could degrade both (*S*)-(+)- and (*R*)-(−)-forms of 2-ols. The degradation rates of 2-ols, except hexanol, were more than 80%. (*S*)-(+)- and (*R*)-(−)-2-hexanol were degraded by 58.3% and 48.2%, respectively. From these results, an enantioselective degradation of the (*S*)-(+)-form of diols from the racemates by strain WF9101 was proposed, although, as shown in Table II, the strain degraded about 10 to 30% of the initial (*R*)-(−)-form of

Table I. Assimilation of Enantiomers by *Geotrichum* sp. WF9101

Enantiomer	Degradation (%)
(*S*)-(+)-1,2-Propanediol	39.8
(*R*)-(−)-1,2-Propanediol	0
(*S*)-(+)-1,3-Butanediol	93.4
(*R*)-(−)-1,3-Butanediol	0
(2*S*,4*S*)-(+)-Pentanediol	83.0
(2*R*,4*R*)-(−)-Pentanediol	0
(*S*)-(+)-2-Butanol	80.2
(*R*)-(−)-2-Butanol	92.0
(*S*)-(+)-2-Pentanol	90.1
(*R*)-(−)-2-Pentanol	86.0
(*S*)-(+)-2-Hexanol	58.3
(*R*)-(−)-2-Hexanol	48.2

Geotrichum sp. WF9101 was incubated in the medium containing each enantiomer at a concentration of 0.2% at 30°C for 3 days.

Table II. The Changes of Enantiomeric Excess Percent of Racemic Diols in the Medium by the Cultivation of WF9101

Racemic diol	Enantiomer	Degradation (%)	Enantiomeric excess of optically active diol (%)
1,2-Propanediol	(*S*)-(+)-	57.3	38.4
	(*R*)-(−)-	31.5	61.6
1,3-Butanediol	(*S*)-(+)-	100	0
	(*R*)-(−)-	42.5	100
2,4-Pentanediol	(2*S*,4*S*)-(+)-	43.4	38.5
	(2*R*,4*R*)-(−)-	9.7	61.5

Geotrichum sp. WF9101 was incubated in the medium containing 0.2% racemic diol at 30°C for 3 days.

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Abbreviation: SADH, NAD$^+$-linked secondary alcohol dehydrogenase.

Table III. Stereospecificity of Secondary Alcohol Dehydrogenase towards Various Diols and 2-Ols

Enantiomer	Relative activity (%)	Enantiomer	Relative activity (%)
(*S*)-(+)-1,2-Propanediol	3.9	(*S*)-(+)-2-Butanol	128.5
(*R*)-(−)-1,2-Propanediol	0	(*R*)-(−)-2-Butanol	72.3
(*S*)-(+)-1,3-Butanediol	47.9	(*S*)-(+)-2-Pentanol	145.7
(*R*)-(−)-1,3-Butanediol	0	(*R*)-(−)-2-Pentanol	124.5
(2*S*,4*S*)-(+)-Pentanediol	100	(*S*)-(+)-2-Hexanol	187.5
(2*R*,4*R*)-(−)-Pentanediol	0	(*R*)-(−)-2-Hexanol	137.2

Percentages are relative to (2*S*,4*S*)-(+)-pentanediol.

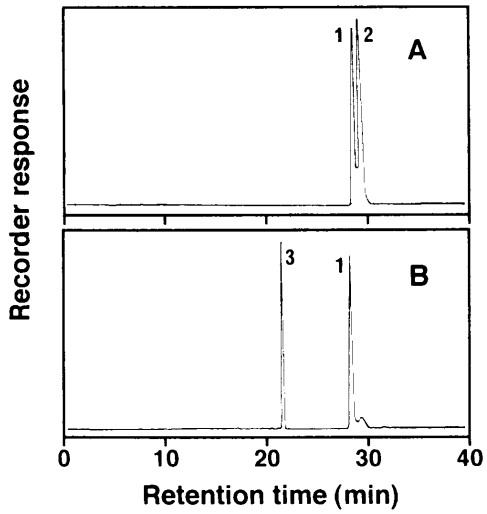

Fig. Gas-liquid Chromatograph of Reaction Products of Racemic 1,3-Butanediol by SADH.

A, before reaction; B, 60 min after reaction. Peak 1, (*R*)-(−)-1,3-butanediol; Peak 2, (*S*)-(+)-1,3-butanediol; Peak 3, 4-hydroxy-2-butanone.

the diols when it was cultured in medium containing the racemic mixture. The degradation rates of racemates of 1,3-butanediol, 2,4-pentanediol, and 1,2-propanediol were, respectively, 100%, 43.4% and 57.3% for the (*S*)-(+)-forms and 42.5, 9.7, and 31.5% for the (*R*)-(−)-forms.

Table III shows enantiospecificities of the purified SADH towards various diols and 2-ols. The reaction was done under the following conditions: the assay mixture (total volume of 200 μl), containing 2 μmol of NAD^+, 2 μmol of substrate, and 20 μg of enzyme (4 U/mg) in 50 mM Tris–HCl buffer, pH 8.0, was incubated at 30°C. The enzyme activity was measured spectrophotometrically by 2-ol or diol-dependent reduction of NAD^+ at 340 nm using a U-2000 spectrophotometer (Hitachi, Japan).

The enzyme showed activities towards (2*S*,4*S*)-(+)-2,4-pentanediol, (*S*)-(+)-1,3-butanediol, and (*S*)-(+)-1,2-propanediol, but not towards the (*R*)-(−)-enantiomers. The relative oxidation percentages of (*S*)-(+)-1,3-butanediol and (*S*)-(+)-1,2-propanediol (to 2,4-pentanediol) were 47.9 and 3.9, respectively. As for 2-ols, the SADH showed activities towards both the (*S*)-(+)- and (*R*)-(−)-forms. However, the relative oxidation percentages of the (*S*)-(+)-forms were higher than those of (*R*)-(−)-forms. These enantiospecificities corresponded to the enantioselective assimilation of this strain. NAD^+-linked secondary alcohol dehydrogenases, which were more active towards (*R*)-(−)-2-butanol than the (*S*)-(+)-form, have already been discovered from various microorganisms such as *Candida*, *Comamonas*, and *Pseudomonas*.[13-15] Contrary, the enzyme from strain WF9101 was more active towards the (*S*)-(+)-forms than the (*R*)-(−)-forms.

Based on the results obtained, the enantioselective oxidation of 1,3-butanediol by the SADH was analyzed. When the SADH (20 μg) was allowed to react with 0.2 μmol of racemic 1,3-butanediol in the presence of 2 μmol of NAD^+ for 30 min under the same conditions mentioned above, 97.7% of the initial (*S*)-(+)-enantiomer was oxidized to a corresponding ketone, and 100% of the initial (*R*)-(−)-enantiomer remained (Fig.). These results raised the possibility of purifying (*R*)-(−)-1,3-butanediol from the racemate with the SADH.

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