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Stereoinversion of 1-arylethanols by *Cyanidioschyzon merolae* NEIS-1332

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Abstract

The stereoinversion of *ortho*-, *meta*- and *para*-substituted fluoro, chloro, bromo and methyl 1-phenylethanols using red alga (*Cyanidioschyzon merolae*) was investigated. It was found that 1-(4′-chlorophenyl)ethanol (**1f**) gave the corresponding (*S*)-alcohols in high ee and high yield (95%, 91% ee). On the other hand, stereoinversion of 1-(3′-chlorophenyl)ethanol (**1e**) indicated moderate ee (**1e**, 54% ee). In the case of 1-(2′-chlorophenyl)ethanol (**1d**), the biotransformation did not proceed. Moreover, we discuss about stereoinversion of alkyl group for secondary alcohols (**3a**–**3d**) and *cis*-2-methylcyclohexanol.

Keywords: *Cyanidioschyzon merolae* NEIS-1332; Red algae; Stereoinversion; Acetophenone derivatives; 2-Methylcyclohexanone

1. Introduction

Enantiopure chiral secondary alcohols have been used in the pharmaceutical, food industry, flavour, fragrances and liquid crystal. In the synthesis of enantiopure chiral secondary alcohols, prochiral ketones or racemic alcohols are commonly used as starting material. For example, they are synthesized by the asymmetric reduction of corresponding prochiral ketone using chemical [1–3] or biocatalytic methods [4,5]. The biocatalytic reduction of ketones, the kinetic resolution of the alcohols via acyltransfer employing lipase [6] or via oxidation [7–10] is also widely applied. However, the yield is limited to 50% of each enantiomer. In order to overcome this problem, deracemisation techniques of the alcohols have been developed [11–13]. Deracemisation can be carried out by either stereoinversion or kinetic resolution. Stereoinversion of a secondary alcohol by biocatalyst consists of enantioselective oxidation of one enantiomer to the corresponding ketone and subsequent reduction to the other enantiomer.

Chiral diols [14–17], hydroxy acid derivatives [18–23], aliphatic [24–27] and aromatic alcohols [28–31] have been obtained in enantiopure by stereoinversion. Biocatalytic stereoinversion is divided into two systems. For example, a two-biocatalyst system [19,20,23,24] and a one-biocatalyst system [14–18,21,22,25–32] have been reported. Two biocatalysts, *Alcaligenes bronchiseptcus* and *Streptococus faecalis* have been used for stereoinversion of (±)-mandelic acid [20]. (*S*)-Mandelic acid was oxidized by *A. bronchiseptcus* to give benzoylformate. Next, benzoylformate was reduced by NADPH-dependent benzoylformate reductase of *S. faecalis* to give (*R*)-mandelic acid in 80% yield with >99% ee. About stereoinversion with one biocatalyst, it has been reported that (*R*)-3-pentin-2-ol was obtained enantioselectively from the corresponding racemic alcohols by *Nocardia fusca* [25–27]. Also, arylethanols by *Geotrichum candidum* [28] and *Sphingomonas pausimobilis* [29] have been reported. Moreover, Nakamura et al. reported the stereoinversion of aliphatic β-hydroxy esters using *G. candidum* IFO 5767 to produce the (*R*)-enantiomers in 97–99% ee and 26–48% isolated yield [33]. Plant cell cultures have also been used as biocatalysts

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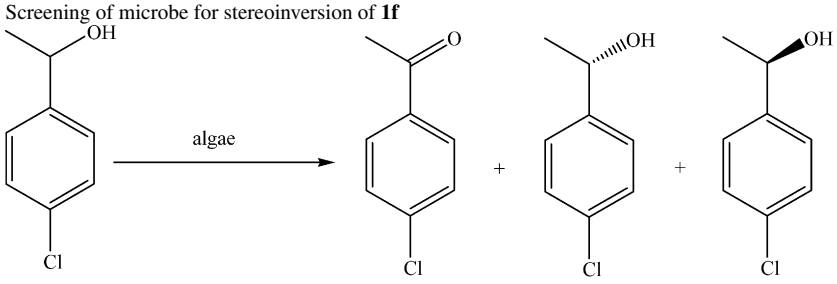
Table 1
Screening of microbe for stereoinversion of **1f**



Racemic **1f** **2f** (*S*)-**1f** (*R*)-**1f**

Algae	1-(4′ -Chlorophenyl)ethanol		
	Yield (%)	ee (%)	Config.
Cyanidioschyzon merolae NEIS-1332	89	91	*S*
Cyanidium caldarium IAM R-11	85	89	*S*
Synechococcus elongatus PCC 7942	70	41	*S*
Synechosystis sp. PCC 6803	72	42	*S*
Spirulina platensis NEIS-39	69	11	*S*

Reaction conditions: substrate (20 mg), algae (dry weight 1 g/L), and medium (100 mL) were employed for 7 days.

for the stereoinversion of racemic alcohols [22,29–32]. Several enantiopure chiral secondary alcohols have been prepared by stereoinversion. It is known that *C. merolae* is extremely characteristic algae inhabiting sulfate-hot springs (pH 2.5, 42 °C). More recently, we reported the reduction of acetophenone derivatives using *C. merolae* into stereoselective compounds [34]. From these results, it was found that fluoro, chloro and bromo acetophenone derivatives were reduced with good enantioselectivity. The reduction followed Prelog's rule, giving the (*S*)-alcohols in all cases. It is known that alga converts from CO_2 to O_2 by the direct use of light energy using photosynthetic microbe as biocatalysts. The growth rate of *C. merolae* is faster than other typical microbe. The problems using plant cultured-cells, as biocatalysts are it slowness and to require large amount of biocatalyst. For example, in the case of reduction of (+)- and (−)-camphorquinones by *N. tabacum* and *C. roseus*, 15 g plant cultured-cells are required to biotransform 20 mg of the substrates. However, 50 mg (dry weight) of *C. merolae* could be biotransformed stereoselectively.

Here, we report the stereoinversion of 1-arylethanols by *C. merolae*.

2. Experimental

The racemic alcohols were obtained by reduction of the corresponding ketones with sodium borohydride or authentic samples. *C. merolae* NEIS-1332 and *Spirulina platensis* NEIS-39 were obtained from the National Institute for Environmental Studies (NIES-Collection). *Cyanidium caldarium* IAM R-11 was obtained from the Institute of Applied Microbiology Culture Collection (IAMCC). *Synechococcus elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 were obtained from the Institut Pasteur. Gas chromatographic analysis was performed using chiral GC-column (Chirasil-DEX CB; 25 m) equipped on Shimadzu GC-17A. Gas chromatography/mass spectrometry analysis was Shimadzu GCMS-QP5050 (EI-MS 70 eV) using DB1 (0.25 mm × 30 m × 0.25 μm) capillary column GC; GC: GC-17A. The IR spectra were measured on a Jasco FT-IR 230. The NMR spectra were measured on a JEOL GSX 400 spectrometer. $CDCl_3$ with tetramethylsilane as the internal standard was used. Optical rotation was measured with a Jasco DIP-370.

The absolute configurations of the compounds were determined by comparing the specific rotation with the literature

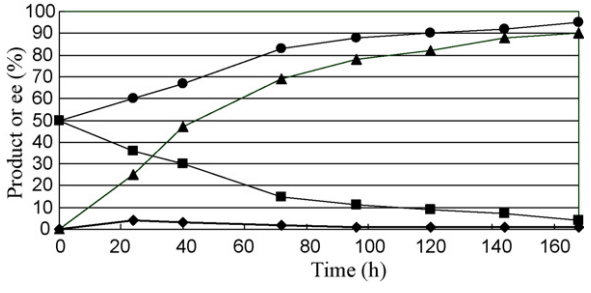


Fig. 1. Time course of stereoinversion of 1-(4′-chlorophenyl)ethanol by *C. merolae* NEIS-1332: (●) (1*S*)-(4′-chlorophenyl)ethanol; (■) (1*R*)-(4′-chlorophenyl)ethanol; (♦) 4-chloroacetophenone; (▲) ee.

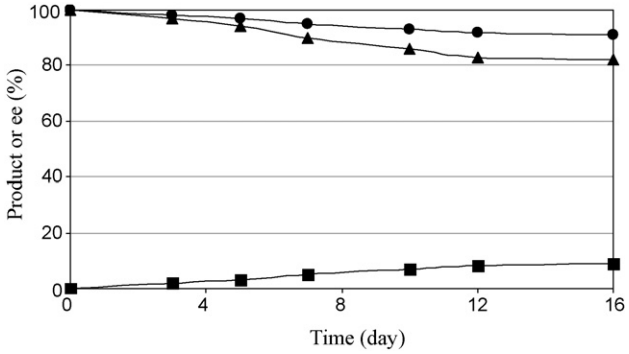


Fig. 2. Time course of stereoinversion of (*S*)-1-phenylethanol by *C. merolae* NEIS-1332: (●) (*S*)-1-phenylethanol; (■) (*R*)-1-phenylethanol; (▲) ee.

[34]: for 1-(3′-Fluorophenyl)ethanol (**1b**) $[\alpha]_D^{27}$ −29.0 (c = 0.7, $CHCl_3$), 75% ee; 1-(4′-Fluorophenyl)ethanol (**1c**) $[\alpha]_D^{27}$ −15.2 (c = 0.6, $CHCl_3$), 24% ee; 1-(3′-Chlorophenyl)ethanol (**1e**) $[\alpha]_D^{27}$ −20.7 (c = 1.5, $CHCl_3$), 54% ee; 1-(4′-Chlorophenyl)ethanol (**1f**) $[\alpha]_D^{27}$ −38.2 (c = 1.0, $CHCl_3$), 91% ee; 1-(3′-Bromophenyl)ethanol (**1h**) $[\alpha]_D^{27}$ −21.0 (c = 1.0, $CHCl_3$) 56% ee; 1-(4′-Bromophenyl)ethanol (**1i**) $[\alpha]_D^{27}$ −38.6 (c = 1.0, $CHCl_3$), 92% ee; 1-(3′-Methylphenyl)ethanol (**1k**) $[\alpha]_D^{27}$ −10.0 (c = 0.6, $CHCl_3$), 25% ee; 1-(4′-Methylphenyl)ethanol (**1l**) $[\alpha]_D^{27}$ −13.0 (c = 0.4, $CHCl_3$), 16% ee; 1-Phenylethanol (**3f**) $[\alpha]_D^{27}$ −32.8 (c = 0.4, $CHCl_3$), 75% ee; 1-Phenyl-1-propanol (**3b**) $[\alpha]_D^{27}$ −5.0 (c = 1.0, $CHCl_3$), 10% ee.

2.1. General procedure of stereoinversion conditions

1-Arylethanols (20 mg) were added to the culture of *C. merolae* (100 mL, 1 g/L as dry weight) in Allen's medium and shaken for the periods shown in Tables. The mixture was treated with a shaker (120 rpm) at 42 °C in the light (2000 lx). At the end of the reaction, alga was filtrated from the medium. The filtrate was extracted with ether (2× 20 mL), washed with water (2× 20 mL) and dried with Na_2SO_4. All the structures of products were identified by IR, ^{1}H NMR, optical rotation [34] and gas chromatographic analyses.

2.2. Preparation of microbial culture

Allen's medium was prepared by mixing $(NH_4)_2SO_4$ (2.64 g), KH_2PO_4 (0.544 g), $MgSO_4$ (0.492 g), $CaCl_2$ (0.148 g), and P4 metal (4 mL) in distilled H_2O (1 L). The medium was adjusted to pH 2.2–2.5 and sterilized.

P4 metal solution was $FeCl_3$ (196 mg), $MnCl_2$ (36 mg), $ZnSO_4$ (22.2 mg), $CoCl_2$ (4 mg), Na_2MoO_4 (2.5 mg) and Na_2EDTA (1000 mg) dissolved in distilled H_2O (1 L).

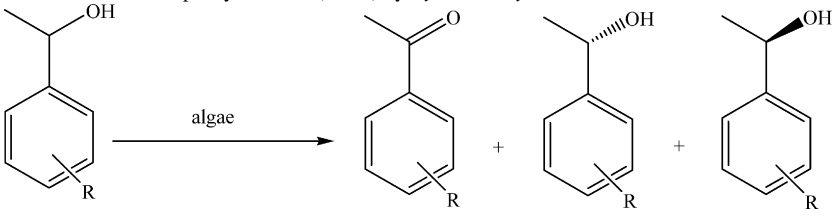
3. Results and discussion

3.1. Stereoinversion of 1-(4′-chlorophenyl)ethanol (1f) and 1-phenylethanol 1-d

First, we screened five algae for their abilities with the stereoinversion of 1-(4′-chlorophenyl)ethanol (**1f**). The results are summarized in Table 1. All algae gave high chemical yields. *Synechococcus elongatus* PCC 7942 afforded (*S*)-**1f** in 70% yield and 41% ee. On the other hand, the biotransformation using *S. platensis* afforded moderate yield in low enantioselectivity. Among the algae, *C. merolae* gave the best result (89% yield and 91% ee). Fig. 1 shows the time course of stereoinversion of **1f** by *C. merolae*. Initially, (*R*)-**1f** was oxidized to **2f**, which was subsequently reduced to (*S*)-**1f**. After 168 h, almost all of the (*R*)-**1f** was converted to (*S*)-**1f**, with the 91% ee.

However, reaction pathway of stereoinversion for the algae is not clear at present study. The mechanistic investigation of stereoinversion was carried out using a deuterated substrate, phenylethanol 1-*d* (**3a′**). Deuterium content in racemic **3a′**-1-*d* decreased to 11% and (*R*)-**3a** proceeds 9% after 7 days' incubation. In contrast, (*S*)-1-phenylethanol is converted to (*S*)-1-phenylethanol (91%) and (*R*)-1-phenylethanol (9%) after 16 days incubation (ee 82%) (Fig. 2). It was the same result for reduction of acetophenone reported previously (ee 80%) [34]. From these results, it seems that at least two active enzymes

Table 2
Stereoinversion of 1-phenylethanols (**1a**–**1l**) by *Cyanidioschyzon merolae* NEIS-1332

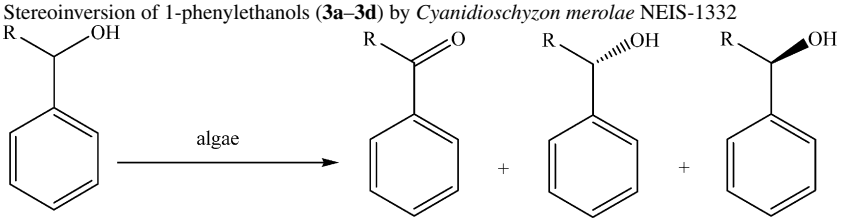


Racemic **1a-1l** **2a-2l** (*S*)-**1a-1l** (*R*)-**1a-1l**

Run	Substrate	R	Yield (%)	ee (%)	Config.
1	**1a**	*o*-F	75	10	*S*
2	**1b**	*m*-F	75	75	*S*
3	**1c**	*p*-F	73	24	*S*
4	**1d**	*o*-Cl	70	0	
5	**1e**	*m*-Cl	87	54	*S*
6	**1f**	*p*-Cl	89	91	*S*
7	**1g**	*o*-Br	94	0	
8	**1h**	*m*-Br	84	56	*S*
9	**1i**	*p*-Br	95	92	*S*
10	**1j**	*o*-CH_3	67	0	
11	**1k**	*m*-CH_3	74	25	*S*
12	**1l**	*p*-CH_3	60	16	*S*

Reaction conditions: substrate (20 mg), algae (dry weight 1 g/L), and medium (100 mL) were employed for 7 days.

Table 3
Stereoinversion of 1-phenylethanols (**3a**–**3d**) by *Cyanidioschyzon merolae* NEIS-1332



Racemic **3a-3d** **4a-4d** (*S*)-**3a-3d** (*R*)-**3a-3d**

Run	Substrate	R	Yield (%)	ee (%)	Config.
1	**3a**	CH_3	74	35	*S*
2	**3b**	CH_2CH_3	85	10	*S*
3	**3c**	$(CH_2)_3CH_3$	73	0	
4	**3d**	$(CH_2)_4CH_3$	70	0	

Reaction conditions: substrate (20 mg), algae (dry weight 1 g/L), and medium (100 mL) were employed for 7 days.

exist, one oxidizes (*R*)-1-phenylethanol into the ketone and the other reduces acetophenone into (*S*)-1-phenylethanol.

3.2. Stereoinversion of secondary alcohols

Stereoinversions using *C. merolae* were targeted for several aryl alcohols. The results are summarized in Table 2. *Para*-substituted 1-phenylethanols gave the corresponding (*S*)-alcohols in high ee and high yield. However, stereoinversion of *meta*-substituted 1-phenylethanols indicated moderate ee. Moreover, *ortho*-substituted chloro and bromo 1-phenylethanols did not proceed. But stereoinversion of *ortho*-substituted fluoro 1-phenylethanol proceeded. The result may be due to the steric hindrance by *ortho*-substituents. This behaviour is similar to the microbial stereoinversion in analogy with alcohols using *G. candidum* [28].

We investigated the effect of the size of alkyl group for secondary alcohols using *C. merolae*. The results are summarized in Table 3. From these results, it was found that as the length of the alkyl chain increases, the enantioselectivity for the corresponding (*S*)-alcohols decreases. Furthermore, 1-phenyl-1-butanol (**3d**) did not proceed (ee 0%). These results may be due to steric hindrance by length of the alkyl chain. Because stereoinversion of **3c** by *C. merolae* gives in 73% yield and 12% ee.

The configuration of the alcohol compared with the sign of the specific rotation with our literature data [34] allowed assignment of the (*S*)-configuration to the alcohol.

3.3. Biotransformation of 2-methylcyclohexanone and *cis*-2-methylcyclohexanol

Trans- and *cis*-2-methylcyclohexanol (**5a**) and (*R*, *S*)-2-methylcyclohexanone (**6a**) are used as model compounds of synthetic substrates for biotransformation. Bruni et al. reported that the (1*S*, 2*S*)-2-methylcyclohexanol (**5a**) (100%) was obtained from corresponding carbonyl compound using *Daucus carota* [35]. Moreover, Shimoda et al. reported that (1*S*, 2*S*)- and (1*S*, 2*R*)-2-methylcyclohexanol (**5a**) was obtained from 2-methyl-2-cyclohexen-1-one by *Synechococcus* sp. PCC 7942 [36]. More recently, we reported the reduction of (*R*, *S*)-2-methylcyclohexanone (**6a**) by various vegetables (carrot, potato, sweet potato, apple, Japanese radish, cucumber, burdock and onion) gave *trans*- and *cis*-2-methylcyclohexanol (**5a**) [37]. In order to discuss the flexibility for 6-ring, we report the biotransformation of (*R*, *S*)-2-methylcyclohexanone and *trans*- and *cis*-2-methylcyclohexanol by *C. merolae*.

We observed the stereoselectivity of (*R*, *S*)-2-methylcyclohexanone in the course of reaction using *C. merolae*. The results are shown in Fig. 3. As can be seen in Fig. 3, in the case of biotransformation of ketone **6a** using *C. merolae*, the *trans*-alcohol **5a** was preferentially formed. And then, a preference of the biotransformation of the mixtures was observed and the oxidation from *cis*-alcohol **5a** to ketone **6a** was promoted by *C. merolae*. On the end of the reaction, *trans*-alcohol **5a** was obtained preferentially. On the other hand, biotransformation of *cis*-2-methylcyclohexanol (**5a**) by *C. merolae* gave *trans*-alcohol **5a** in moderate yield after 16 days (*trans*-alcohol; 1*S*, 2*S* 62% ee. *cis*-alcohol 1*R*, 2*S* 77% ee). The results are shown in Fig. 4. In the case of biotransformation of *cis*-alcohol **5a** by *C. merolae*,

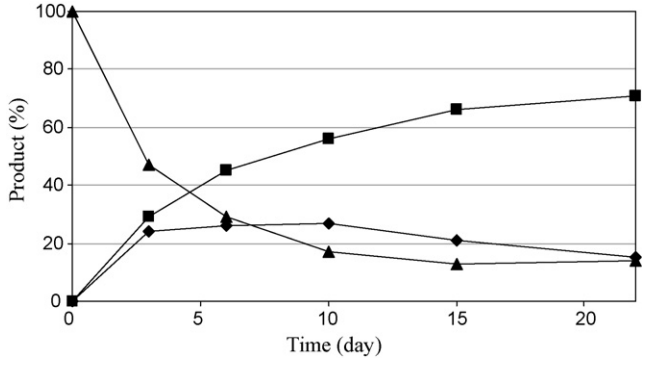


Fig. 3. Biotransformation of 2-methylcyclohexanone (**6a**) by *C. melorae* NEIS-1332: (♦) *cis*-2-methylcyclohexanol (**5a**); (■) *trans*-2-methylcyclohexanol (**5a**); (▲) 2-methylcyclohexanone (**6a**).

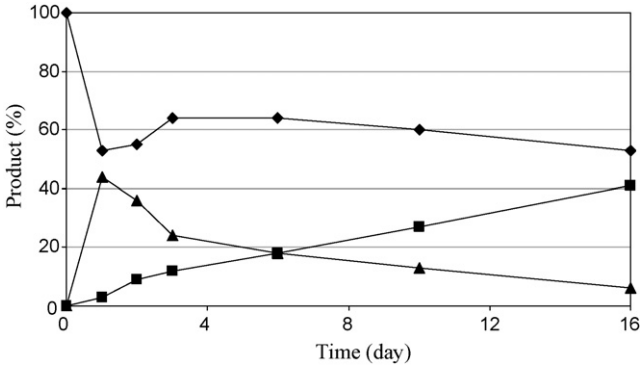


Fig. 4. Biotransformation of *cis*-2-methylcyclohexanol (**5a**) by *C. melorae* NEIS-1332: (♦) *cis*-2-methylcyclohexanol (**5a**); (■) *trans*-2-methylcyclohexanol (**5a**); (▲) 2-methylcyclohexanone (**6a**).

ketone **6a** was preferentially formed. And then, the reduction of ketone **6a** gave *trans*-alcohol **5a**. However, the biotransformation of *trans*-2-methylcyclohexanol (**5a**) did not proceed. It seems that in comparison with 1-arylethanols, biotransformation of 2-methylcyclohexanone and *cis*-2-methylcyclohexanol was too time-consuming due to the flexibility and the bulky for 6-ring.

4. Conclusion

The racemic alcohols were converted into the corresponding (*S*)-alcohols in good ee and chemical yield. However, *ortho*-substituted chloro and bromo 1-phenylethanols did not proceed. The result may be due to the steric hindrance by *ortho*-substituents. This is the first report that the stereoinversions of racemic alcohols using algae have been accomplished.

Acknowledgment

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