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ORIGINAL RESEARCH PAPER

# Photobiocatalyzed asymmetric reduction of ketones using *Chlorella* sp. MK201

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**Abstract** Aromatic ketones were reduced using suspension culture of *Chlorella* sp. MK201 under fluorescent light illumination producing the corresponding chiral alcohols in high yields with excellent enantiomeric excess (ee). For example, 2′,3′,4′,5′,6′-pentafluoroacetophenone at 0.25 mg/ml was converted to the corresponding (*S*)-alcohol in 80 % yield with >99 % ee by 1 mg dry wt of *Chlorella*/ml in 12 h illumination (2,000 lux).

**Keywords** Alcohol · Asymmetric reduction · *Chlorella* sp. MK201 · Ketones

## Introduction

*Chlorella* sp. MK201 that grew under high atmospheric $CO_2$ at a high temperature has been isolated in Japan (Muranaka and Murakami 2001). The *Chlorella* was registered as an official strain at the Research Institute of Innovative Technology for the Earth (RITE), and is considered useful for adsorbing and removing $CO_2$ in high $CO_2$ concentrations under high temperature such as exhaust gas from thermal power stations.

Microbes such as yeast and fungi are biocatalysts that reduce various ketones to the corresponding optically active alcohols (Matsuda et al. 2009). We reported that acetophenone derivatives and 3-acetylisoxazoles were reduced by photosynthetic microalgae such as *Synechococcus elongatus* PCC 7942 and *Synechocystis* sp. PCC 6803 (Nakamura et al. 2000, Nakamura and Yamanaka 2002a, b, Itoh et al. 2005), to the corresponding chiral alcohols in excellent ee. Chiral secondary alcohols are widely used as synthetic intermediates in organic chemistry, for instance, (*S*)-1-(2-chlorophenyl) ethanol or (*S*)-1-(2-trifluoromethylphenyl)ethanol are

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transformed into heteroaryl-linked 5-(1*H*-benzimidazol-1-yl)-2-thiophenecarboxamides as potent inhibitors of polo-like kinase 1 (PLK1) (Rheault et al. 2010).

Herein, we report an efficient asymmetric reduction of acetophenone derivatives using *Chlorella* sp. MK201 under fluorescent light illumination. We propose a synthetic method that affords useful chiral secondary alcohols by asymmetric reduction with a biocatalyst that can be prepared only for inorganic materials including $CO_2$.

## Materials and methods

### Chemicals

The substrates (ketones), ethyl acetate and other chemicals used here were obtained from Aldrich Chemical Co. Inc., Wako Pure Chemical Industries, Ltd., and Tokyo Chemical Industry Co. Inc.

### Cultivation

The cultivation of *Chlorella* sp. MK201 was performed in $MC^+$ liquid medium (3,750 mg $KNO_3$/l, 750 mg $KH_2PO_4$/l, 500 mg $MgSO_4 \cdot 7H_2O$/l, 20 mg $FeSO_4 \cdot 7H_2O$/l, 2.86 mg $H_3BO_3$/l, 2.5 mg $MnSO_4 \cdot 4H_2O$/l, 0.222 mg $ZnSO_4 \cdot 7H_2O$/l, 0.079 mg $CuSO_4 \cdot 5H_2O$/l and 0.021 mg $Na_2MoO_4$/l, see Muranaka and Murakami 2001) under continuous illumination provided by fluorescent lamps (MITSUBISHI/OSRAM FL40SW, 2000 lux) with air-bubbling at 25 °C.

The cultivation of *S. elongates* PCC 7942 and *Synechocystis* sp. PCC 6803 were performed in BG-11 liquid medium under continuous illumination provided by fluorescent lamps (MITSUBISHI/OSRAM FL40SW, 2,000 lux) with air-bubbling at 25 °C.

These cultures were being used as growing cells.

### Asymmetric reduction of acetophenone derivatives using *Chlorella* sp. MK201

The cultivated suspension culture of *Chlorella* sp. MK201 for nine days and $MC^+$ liquid medium were mixed to make the suspension culture of *Chlorella* ($OD_{730} = 1$). In this suspension, dry wt of *Chlorella* was obtained in 1 mg/ml. Acetophenone derivative in DMSO (50 μl) was added to the suspension culture (6 ml). The mixture was shaken at 120 rpm at 25 °C under fluorescent light illumination (2,000 lux). After the reaction, *n*-dodecane (10 μl) was added. The resulting mixture was extracted with ethyl acetate (5 ml). The extract was washed with saturated aqueous sodium chloride solution (2 ml). After drying with anhydrous sodium sulfate, the chemical yield was determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard (GC column: DB-1, 25 m). The enantiomeric purities were determined by GC analysis based on GC area ratio (GC column: CP-cyclodextrin-B-2,3,6-M-19 [CPCD], 25 m). The retention times of ketones, (*R*)-alcohols and (*S*)-alcohols in GC analysis were referred to the literature (Nakamura and Matsuda 1998).

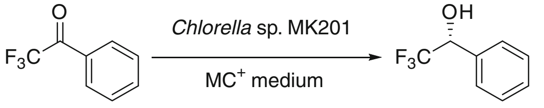
### Asymmetric reduction of acetophenone derivatives using *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803

The cultivated suspension culture of *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803 for nine days and BG-11 liquid medium were mixed to make the suspension culture of *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803 ($OD_{750} = 2$). In these suspensions, dry wt of *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803 were obtained in 0.6 mg/ml, respectively. After undergoing the same procedures in the *Chlorella* sp. MK201, the chemical yields and ee were determined by GC analysis.

## Results and discussion

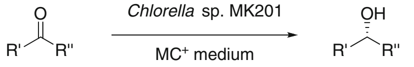
The growth and the reductive reactivity of *Chlorella* sp. MK201 were tested using 2,2,2-trifluoroacetophenone **1a**. When **1a** at 0.5 mg/ml was added to the suspension culture of *Chlorella* prepared from the cultivated *Chlorella* suspension for nine days and shaken for 18 h in the light, the corresponding alcohol (*R*)-**1b** was obtained in 84 % yield (97 % ee) (Fig. 1). The reproducibility of this reduction produced in 83.5 ± 2.5 % chemical yields and 96.5 ± 0.5 % ee. In the cultivation for 15 days, the reductive reactivity of *Chlorella* (reaction time: 24 h) was gradually lowered to 76 % yield (95 % ee) (Supplementary Table 1).

Based on these results, the optimum reaction conditions were investigated. The reaction of **1a** at 0.5 mg/ml using *Chlorella* for 18 h produced (*R*)-**1b**



**1a** (*R*)-**1b** (84 %, 97 % ee)

**Fig. 1** Asymmetric reduction of 2,2,2-trifluoroacetophenone **1a** by *Chlorella* sp. MK201: cultivation time (9 days); the starting concentration of the substrate (0.5 mg/ml); suspension culture (6 ml); dry wt of *Chlorella* (1 mg/ml); shaking 120 rpm at 25 °C under fluorescent light illumination (2,000 lux) for 18 h; chemical yield and ee were determined by GC analysis



**2a-14a** (*S*)-**2b-14b**

**2a**, **2b**: R' = Me, R'' = Ph
**3a**, **3b**: R' = Me, R'' = *p*-Me-Ph
**4a**, **4b**: R' = Me, R'' = *p*-F-Ph
**5a**, **5b**: R' = Me, R'' = *m*-F-Ph
**6a**, **6b**: R' = Me, R'' = *o*-F-Ph
**7a**, **7b**: R' = Me, R'' = *p*-Cl-Ph
**8a**, **8b**: R' = Me, R'' = *o*-Cl-Ph
**9a**, **9b**: R' = Me, R'' = *p*-Br-Ph
**10a**, **10b**: R' = Me, R'' = *p*-$CF_3$-Ph
**11a**, **11b**: R' = Me, R'' = *2',3',4',5',6'*-5F-Ph
**12a**, **12b**: R' = Me, R'' = *p*-MeO-Ph
**13a**, **13b**: R' = Et, R'' = Ph
**14a**, **14b**: R' = Me, R'' = $CH_2$-Ph

**Fig. 2** Asymmetric reduction of acetophenone derivatives using *Chlorella* sp. MK201: cultivation time (9 days); the starting concentration of the substrate (0.17–0.5 mg/ml); suspension culture (6 ml); dry wt of *Chlorella* (1 mg/ml); shaking 120 rpm at 25 °C under fluorescent light illumination (2,000 lux)

**Table 1** Asymmetric reduction of acetophenone derivatives using *Chlorella* sp. MK201

| Run[a] | Ketone (mg) | Time (h) | Yield ( %)[b] | | |
|---|---|---|---|---|---|
| | | | Ketone | Alcohol | Ee (Config.[c]) |
| 1[d] | **2a** (3) | 72 | 76 | 11 | 89 (*S*) |
| 2 | **2a** (1) | 72 | 72 | 19 | >99 (*S*) |
| 3 | **3a** (1) | 72 | 74 | 19 | >99 (*S*) |
| 4 | **4a** (1) | 72 | 45 | 40 | >99 (*S*) |
| 5 | **5a** (1) | 72 | 4 | 83 | >99 (*S*) |
| 6 | **6a** (1) | 48 | – | 99 | >99 (*S*) |
| 7 | **7a** (1) | 48 | – | 79 | 95 (*S*) |
| 8 | **8a** (1) | 48 | – | 69 | >99 (*S*) |
| 9 | **9a** (1) | 48 | – | 88 | >99 (*S*) |
| 10 | **10a** (1) | 48 | – | 84 | >99 (*S*) |
| 11[e] | **11a** (1.5) | 12 | – | 80 | >99 (*S*) |
| 12 | **12a** (1) | 72 | 84 | 5 | >99 (*S*) |
| 13 | **13a** (1) | 72 | 89 | 8 | >99 (*S*) |
| 14 | **14a** (1) | 48 | 68 | 16 | >99 (*S*) |

[a] Reaction conditions: cultivation time (9 days); the starting concentration of the substrate (0.17 mg/ml); suspension culture (6 ml); dry wt of *Chlorella* (1 mg/ml); shaking 120 rpm at 25 °C under fluorescent light illumination (2,000 lux)

[b] Determined by GC analysis

[c] Config.: Configuration

[d] The starting concentration of the substrate (0.5 mg/ml)

[e] The starting concentration of the substrate (0.25 mg/ml)

in 86 % yield (97 % ee). However, the chemical yield and enantiomeric purities were decreased by increasing the amount of **1** (1.5 mg/ml, reaction time: 48 h) into 31 % yield (82 % ee) (Supplementary Table 2).

The reaction using *Chlorella* was performed under dark conditions. The reaction of **1a** for 24 h in the dark decreased the chemical yield of (*R*)-**1b** into 37 % yield (93 % ee). When *Chlorella* pre-cultured under dark conditions for 24 h was used and the reaction was conducted under darkness, the chemical yield was decreased further into 22 % yield (87 % ee) (Supplementary Table 3). The results confirmed that the light conditions were necessary in the present asymmetric reduction system.

To apply the present proposed asymmetric reduction to other substrates, the reaction was performed with several acetophenone derivatives **2a–14a** (Fig. 2), and the results are summarized in Table 1 (Supplementary Table 4). All ketones were transformed into the corresponding alcohols **2b–14b** and produced (*S*)-alcohols in excellent ee. Among them, the reaction of **6a–11a** obtained the corresponding alcohols in high yield (Table 1, Runs 6–11). In the case of para-substituted acetophenones, *Chlorella* effectively reduced acetophenone derivatives including the electron-withdrawing group into the corresponding (*S*)-alcohol compared with the electron-donating group (Table 1, Runs 3, 4, 7, 9, 10 and 12). In fluoro-substituted acetophenones, ortho-substituted acetophenone was reduced into chiral alcohol in a short time (Table 1, Runs 4–6), and increasing the number of substituents accelerated the reduction (Table 1, Run 11).

More recently, Yang et al reported the comparison of a reduction with cyanobacteria and chlorella (Yang et al. 2012). Therefore, the reactions of **1a**, **6a**, **10a** and **11a** using *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803 were tested (Table 2). The results show that the reductive reactivity of *Chlorella* sp. MK201 in acetophenone derivatives was higher than *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803. Therefore, these results show that the biotransformation using *Chlorella* sp.

**Table 2** Asymmetric reduction of acetophenone derivatives using *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803

| Run[a] | Biocatalyst | Ketone | Time (h) | Yield ( %)[b] | | |
|---|---|---|---|---|---|---|
| | | | | Ketone | Alcohol | ee (Config.) |
| 1 | *S. elongatus* PCC 7942 | **1a** | 144 | 79 | 12 | >99 (*R*) |
| 2 | *S. elongatus* PCC 7942 | **6a** | 144 | 40 | 36 | >99 (*S*) |
| 3 | *S. elongatus* PCC 7942 | **10a** | 96 | 35 | 38 | >99 (*S*) |
| 4 | *S. elongatus* PCC 7942 | **11a** | 144 | – | 80 | >99 (*S*) |
| 5 | *Synechocystis* sp. PCC 6803 | **1a** | 96 | 90 | 4 | N.D. |
| 6 | *Synechocystis* sp. PCC 6803 | **6a** | 96 | 50 | 36 | >99 (*S*) |
| 7 | *Synechocystis* sp. PCC 6803 | **10a** | 48 | 36 | 40 | >99 (*S*) |
| 8 | *Synechocystis* sp. PCC 6803 | **11a** | 72 | – | 73 | >99 (*S*) |

[a] Reaction conditions: cultivation time (9 days); the starting concentration of the substrate (0.08 mg/ml); suspension culture (6 ml); dry wt of *S. elongates* PCC 7942 or *Synechocystis* sp. PCC 6803 (0.6 mg/ml); shaking 120 rpm at 25 °C under fluorescent light illumination (2,000 lux)

[b] Determined by GC analysis *N.D* Not detected

MK201 was widely and effectively used in the asymmetric reduction of acetophenone derivatives.

**In conclusion**, the present investigation revealed the potency of *Chlorella* in the asymmetric reduction of acetophenone derivatives to provide the corresponding alcohols. In particular, since *Chlorella* sp. MK201 grows under high atmospheric $CO_2$ at a high temperature, this reaction was demonstrated that an efficient material production by the indirect availability of $CO_2$.

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