ISSN : 0974 - 7427 Volume 8 Issue 4

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 8(4), 2014 [106-110]

Reduction of acetophenone derivatives by *Spirulina platensis* and *Nostoc minutum*

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ABSTRACT

The reduction of acetophenone derivatives using *Spirulina platensis* and *Nostoc minutum* was investigated. It was found that acetophenone derivatives **1a-1o** were reduced with good enantioselectivity. The reduction followed Prelog's rule, giving the (*S*)-alcohols in all cases.

KEYWORDS

Spirulina platensis NIES-39;
Nostoc minutum NIES-29;
Acetophenone derivatives;
Biotransformation.

INTRODUCTION

Optically active compounds are important building blocks for the synthesis of pharmaceuticals, pesticides, pheromones, flavors, fragrances and advanced materials. It is known that the synthesis of chiral alcohols can be achieved from the corresponding prochiral ketones by asymmetric reduction. Recently, many biocatalytic reductions of ketones have been reported[2,3,5]. Acetophenone derivatives are very interesting model compounds as foreign substrates for biotransformation, because either enantiomer may be formed, which may be determined easily. Reduction of the carbonyl group is performed using various biocatalysts such as cultured cells[1] and fresh-cut vegetables[9].

Nakamura et al.[4] reported that *Synechococcus elongatus* PCC 7942, a cyanobacterium as a biocatalyst reduce aryl methyl ketones into the corresponding (*S*)-alcohols with excellent enantioselectivities under illumination. Moreover, *S. elongatus* PCC 7942 reduced both the endocyclic C=C of *s-trans* enones and the exocyclic C=C of *s-cis* enones with high enantioselectivity to afford optically active (*S*)-α-substituted ketones[6]. Recently, we reported that the reduction of acetophenone derivatives and steroidal ketones using red algae (*Cyanidioschyzon merolae* 10D and *Cyanidium caldarium*) was investigated[7]. It is known that this method converts from CO_2 to O_2 by the direct use of light energy using photosynthetic microbe. Therefore, algae are an environment friendly catalyst.

Yazdi et al.[10] reported that hydrocortisone was converted in the culture of the cyanobacterium *Nostoc muscorum* PTCC 1636 into some androstane and pregnane derivatives. More recently, we reported that biotransformation of α-bromo and α, α'-dibromo alkanones were investigated with alga of *Spirulina platensis*[8]. Biotransformation of α-bromoketone with

S. platensis gave the corresponding α-hydroxyketone in good yields (80–95%). However, no literature report has been found in acetopheone derivatives biotransformation by *Spirulina platensis* and *Nostoc minutum.*

Here, we report a method for the asymmetric reduction with photosynthetic microorganism (*S. platensis* NIES-39 and *N.minutum* NIES-29)

MATERIAL AND METHODS

Analytical and algae

GC-MS: Shimadzu GCMS-QP5050 (EI-MS 70 eV) using DB1 (0.25 mm × 30m, 0.25μm) capillary column GC; GC: GC-17A. ^{1}H -NMR: Jeol GSX 400 spectrometer. CDCl3 with tetramethylsilane as the internal standared was used. *S. platensis* NIES-39 and *N. minutum* NIES-29 were obtained from the National Institute for Environmental Studies (NIES-Collection).

Cultivation

S. platensis was grown in SOT medium (pH 10.0) and *N. minutum* was grown in MDM medium (pH 8) under continuous illumination provided by fluorescent lamps (2000 lx) with air-bubbling at 25°C.

General reaction conditions

Substrate (20 mg) was added to suspended culture of *S. platensis* (0.8 g/L as dry weight) or *N. minutum* (0.7 g/L as dry weight) in medium (100 ml). The mixture was treated with a shaker (120 rpm) at 25 °C in the light (2000 lx). The end of the reaction, algae

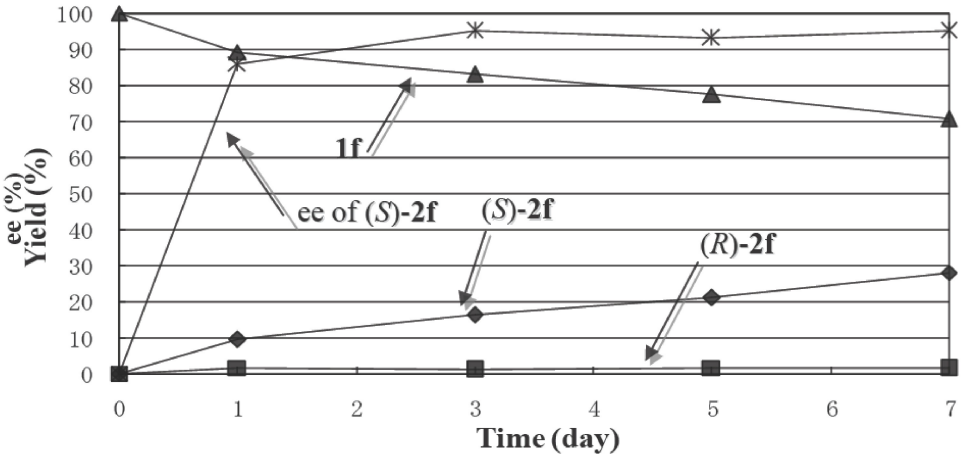

Figure 1 : Time course of reduction of *p*-chloroacetophenone using *N. minutum.* Reaction time: 1, 3, 5 and 7 days. Substrate and product: ▲ 4-chloroacetophenone, ♦ (*S*)-1-(4-chlorophenyl)ethanol, ■ (*R*)-1-(4-chlorophenyl)ethanol, × ee.

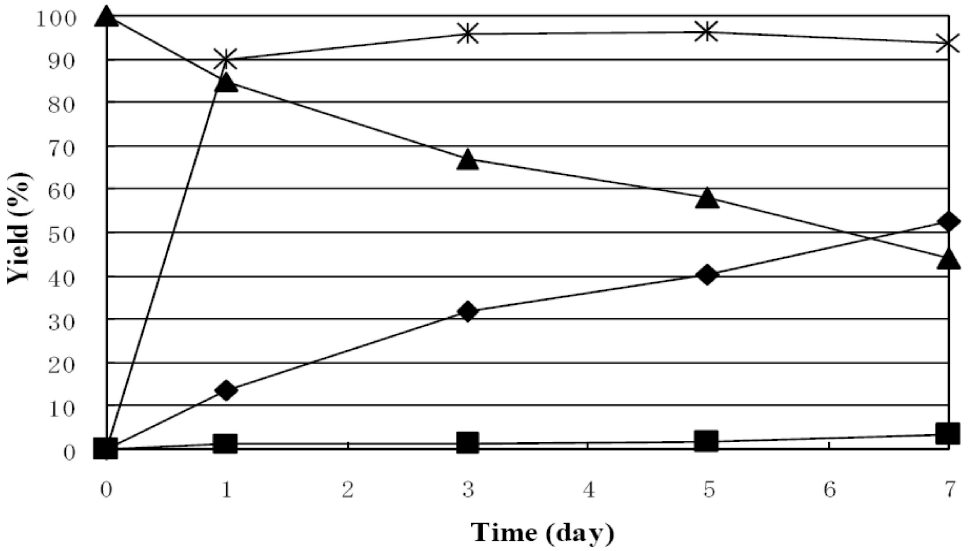

Figure 2 : Time course of reduction of *p*-chloroacetophenone using *S. platensis.* Reaction time: 1, 3, 5 and 7 days. Substrate and product: ▲ 4-chloroacetophenone, ♦ (*S*)-1-(4-chlorophenyl)ethanol, ■ (*R*)-1-(4-chlorophenyl)ethanol, × ee.

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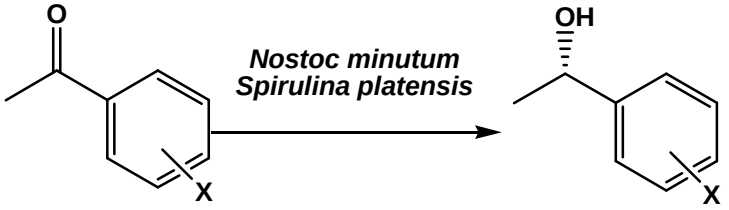
TABLE 1 : Reduction of acetophenone derivatives by *Spirulina platensis* and *Nostoc minutum*



1a-o 2a-o

Entry	Substrate	X	Product	*S. platensis*[a]			*N. mintum*[b]		
				Yield (%)	ee (%)	Config.	Yield (%)	ee (%)	Config.
1	**1a**	*o*-F	2a	58	>99	*S*	26	98	*S*
2	**1b**	*m*-F	2b	51	99	*S*	6	83	*S*
3	**1c**	*p*-F	2c	16	91	*S*	6	34	*S*
4	**1d**	*o*-Cl	2d	30	93	*S*	38	95	*S*
5	**1e**	*m*-Cl	2e	62	99	*S*	24	96	*S*
6	**1f**	*p*-Cl	2f	56	94	*S*	30	95	*S*
7	**1g**	*o*-Br	2g	32	>99	*S*	13	>99	*S*
8	**1h**	*m*-Br	2h	48	93	*S*	14	87	*S*
9	**1i**	*p*-Br	2i	48	97	*S*	41	95	*S*
10	**1j**	*o*-OCH_3	2j	32	89	*S*	6	74	*S*
11	**1k**	*m*-OCH_3	2k	33	90	*S*	10	78	*S*
12	**1l**	*p*-OCH_3	2l	4	89	*S*	1	89	*S*
13	**1m**	*o*-CH_3	2m	2	>99	*S*	8	>99	*S*
14	**1n**	*m*-CH_3	2n	15	72	*S*	1	80	*S*
15	**1o**	*p*-CH_3	2o	6	>99	*S*	4	94	*S*

Reaction conditions: a) Substrate (20 mg), *S. platensis* (dry weight 0.8g/L) and SOT medium (100 ml) were employed at 25 °C for 7 days (pH 10.0 and 1000Lx); b) Substrate (20 mg), *N. minutum* (dry weight 0.7g/L) and MDM medium (100 ml) were employed at 25 °C for 7 days (pH 8.0 and 1000Lx)

was filtered, and resulting mixture was extracted with Et_2O. All the products were determined by ^1H NMR, GC and GC-MS analyses.

Preparation of microbial culture

SOT medium was prepared by mixing $NaHCO_3$ (16.8 g), K_2HPO_4 (0.5 g), $NaNO_3$ (2.5 g), K_2SO_4 (1 g), NaCl (1 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $CaCl_2 \cdot 2H_2O$ (0.04 g), $FeSO_4 \cdot 7H_2O$ (0.01 g), Na_2EDTA (0.08 g) and A5 solution (1 ml) in distilled H_2O (1 L).

A5 solution was H_3BO_3 (286 mg), $MnSO_4 \cdot 7H_2O$ (250 mg), $ZnSO_4 \cdot 7H_2O$ (22.2 mg), $CuSO_4 \cdot 5H_2O$ (7.9 mg) and $Na_2MoO_4 \cdot 2H_2O$ (2.1 mg) dissolved in distilled H_2O (100 ml).

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MDM medium was prepared by mixing KNO_3 (1.0 g), $MgSO_4 \cdot 7H_2O$ (0.25 g), K_2HPO_4 (0.25 g), NaCl (0.1 g), $CaCl_2 \cdot 2H_2O$ (0.01 g), A5 solution (1 ml) and Fe solution (1 ml) in distilled H_2O (1 L). The medium was adjusted to pH 8.0.

Fe solution was $FeSO_4 \cdot 7H_2O$ (200 mg) and concH_2SO_4,(0.026 ml) dissolved in distilled H_2O (100 ml).

RESULTS AND DISCUSSION

Reduction of *p*-chloroacetophenone (1f)

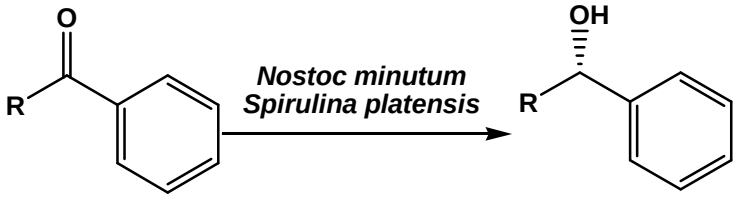
First, we screened two algae for their activities with the reduction of 4-chloroacetophenone (**1f**). Figure 1 and 2 show the time course of reduction of **1f** by *S. platensis* and *N. minutum*. These algae gave high enantioselectivity. *S. platensis* afforded (*S*)-**1f** in 56 % yield and 94 % ee for 7 days. On the other hand, the biotransformation using *N. minutum* afforded 30 % yield and 95 % ee for 7 days.

Reduction of acetophenone derivatives

Biotransformation using *S. platensis* and *N. minutum* were targeted for various acetophenone derivatives. The results summarized in TABLE 1. *Ortho*-, *meta*- and *para*-substituted fluoro, chloro, bromo, methyl and methoxy acetophenones **1a-o** were reduced to the corresponding (*S*)-alcohols in all cases. The alcohol products had the (*S*)-configuration, which is consistent with Prelog's rule. Reduction of fluoro, chloro, bromo, methyl, methoxy acetophenones provided good enantioselectivity (72-99 %) using *S. platensis*. On the other hand, biotransformation using *N. minutum* afforded good enantioselectivity (74-99 %) except *p*-fluoroacetophenone (**1c**, 34 %). These results indicate that the enantioselectivity of *S. platensis* and *N. minutum* are higher than other algae (*C. merolae* 10D and *C.caldarium*). However, reduction of acetophenone derivatives was observed in low to moderate yields (1-62 %).

The reduction of several aromatic ketones having alkyl chains of different length using *S. platensis* and *N. minutum* indicates in TABLE 2. It was found that compound **3a** was reduced after 7 days incubation in high enantioselectivities, while longer alkanones, 3b-3g, could not be reduced. This result is apparently due to

TABLE 2 : Reduction of acetophenone derivatives by *N. minutum* and *S. platensis*



3a-g 4a-g

Entry	Substrate	R	Product	*N. minutum*[a]			*S. platensis*[b]		
				Yield (%)	ee (%)	Config.	Yield (%)	ee (%)	Config.
1	**3a**	CH_3	4a	4	90	*S*	13	96	*S*
2	**3b**	CH_3CH_2	4b	-	-	-	-	-	-
3	**3c**	$(CH_3)_2CH$	4c	-	-	-	-	-	-
4	**3d**	$CH_3(CH_2)_3$	4d	-	-	-	-	-	-
5	**3e**	$CH_3(CH_2)_4$	4e	-	-	-	-	-	-
6	**3f**	$CH_3(CH_2)_5$	4f	-	-	-	-	-	-
7	**3g**	$CH_3(CH_2)_6$	4g	-	-	-	-	-	-

Reaction conditions: a) Substrate (20 mg), *N. minutum* (dry weight 0.7g/L) and MDM medium (100 ml) were employed at 25 °C (pH 8.0 and 1000Lx); b) Substrate (20 mg), *S. platensis* (dry weight 0.8g/L) and SOT medium (100 ml) were employed at 25 °C (pH 10.0 and 1000Lx)

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the size of the alkyl side chains and their lower solubility in the reaction medium.

CONCLUSION

Reduction of acetophenone derivatives using *S. platensis* and *N. minutum* gave the corresponding (*S*)-alcohols with good enantioselectivity (72-99 %) except *p*-fluoroacetophenone (**1c**, 34 %). These results indicate that the enantioselectivity of *S. platensis* and *N. minutum* are higher than other algae (*C. merolae* 10D and *C.caldarium*). The alcohol products had the (*S*)-configuration, which is consistent with Prelog's rule. We have established a convenient and environmentally benign enantioselectivie reduction system employing *S. platensis* and *N. minutum*.

ACKNOWLEDGEMENT

This work was partially supported by Frontier Project "Adaption and Evolution of Extremophile" and a Grant-in-Aid for Scientific Research (no 18550142).

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