

## Stereoselective Reduction of $\alpha$ -Keto Ester and $\alpha$ -Keto Amide with Marine Actinomycetes, *Salinispora* Strains, as Novel Biocatalysts

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**Abstract:** To clarify the potential ability of marine actinomycetes as biocatalysts, the stereoselective reduction of  $\alpha$ -keto esters and  $\alpha$ -keto amide using *Salinispora arenicola* and *Salinispora tropica* was tested. The reduction of ethyl pyruvate and ethyl 2-oxobutanoate by *S. tropica* gave corresponding alcohol with high conversion ratio and in high e.e. (96% e.e. (*S*) and 99% e.e. (*S*), respectively). In the presence of L-glutamate as an additive, the reduction of ethyl pyruvate by *S. tropica* afforded the corresponding (*S*)-ethyl lactate with >99% e.e. Furthermore, 2-chlorobenzoylformamide was reduced by *S. tropica* to the corresponding (*R*)-2-chloromandelamide with high conversion ratio and excellent enantioselectivity (>99% e.e.). Thus, it was found that marine actinomycetes, *Salinispora* strains, had high ability for the stereoselective reduction of carbonyl compounds as useful biocatalysts.

**Keywords:** carbonyl compound, stereoselective reduction, marine actinomycete, *Salinispora* strain

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## Introduction

Biotransformation of exogenous substrates have been widely studied and used in order to prepare chiral compounds.<sup>1–3</sup> Microbial reduction of carbonyl compounds is one of the convenient and environmental-friendly methods for obtaining optically pure alcohols. For example, bakers' yeast and fungi have often been used for the reduction to obtain optically active hydroxy esters.<sup>4–6</sup> To date, several studies concerning the reduction of keto esters and their analogues with other microorganisms such as actinomycete and algae have been reported.<sup>7–10</sup> However, little information is known about the reduction of keto esters using marine bacteria as biocatalysts.

Recently, marine actinomycetes were isolated, characterized, and named *Salinispora arenicola* and *Salinispora tropica*.<sup>11</sup> These *Salinispora* strains are attracting attention because useful bioactive compounds such as arenimycin (an antibiotic against methicillin-resistant *Staphylococcus aureus*) and salinisporamide A (a potent proteasome inhibitor) are produced by these marine actinomycetes.<sup>12,13</sup> Thus, marine actinomycete was a species of the microorganisms expected in medicine and pharmacy field. However, the potential ability and possibility of *Salinispora* strains as biocatalysts for asymmetric organic synthesis has not been investigated.

This study describes the stereoselective reduction of  $\alpha$ -keto esters and aromatic  $\alpha$ -keto amide by marine actinomycetes, *Salinispora* strains, as novel biocatalysts.

## Material and Methods

### Instruments and chemicals

Gas chromatography was done using GL Science GC-353 (DB-Wax, J&W Scientific, USA, 0.25mm×30m; TC-1, GL Science, Japan, 0.25mm×30m; CP-Chirasil-DEX CB, Chrompack, Netherlands, 0.25 mm × 25 m; Gamma DEX 225, Supelco, USA, 0.25 mm × 30 m) gas chromatographs. Ethyl pyruvate (**1a**), diatomaceous earth (granular), polypepton, sodium alginate (100–150 cP), monosodium L-glutamate,

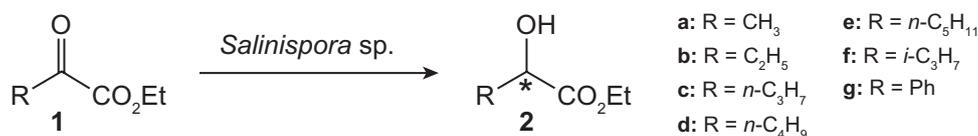
and Daigo's artificial seawater SP were purchased from Wako Pure Chemical Industries, Ltd., Japan. Ethyl 3-methyl-2-oxobutanoate (**1f**) was purchased from Sigma-Aldrich, USA. Ethyl benzoylformate (**1g**) was obtained from Tokyo Kasei Kogyo, Japan. Ethyl 2-oxobutanoate (**1b**), ethyl 2-oxopentanoate (**1c**), ethyl 2-oxohexanoate (**1d**), ethyl 2-oxoheptanoate (**1e**), and  $\alpha$ -hydroxy esters (**2a–g**) were prepared according to the procedures in the literature.<sup>14</sup> 2-Chlorobenzoylformamide (**3**) and 2-chloromandelamide (**4**) were synthesized according to the literature.<sup>15</sup> All other chemicals used in this study were of analytical grade and commercially available.

### Microorganism and cultivation

*Salinispora arenicola* NBRC105043 and *Salinispora tropica* NBRC105044 were purchased from the National Institute of Technology and Evaluation, Biological Resource Center, Japan (NBRC). These strains were maintained at 25 °C in the following synthetic medium solidified 1.5% agar. Medium (NBRC recommended medium 325) is comprised of 10 g polypepton, 2 g yeast extract, 0.5 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 27 g Daigo's artificial seawater SP, per one liter of distilled water (pH 7.3). The *Salinispora* strains were grown in each maintained medium (500 ml) for seven days at 25 °C with aerobic shaking in baffled 2-liter flasks in dark condition. The marine actinomycete cells were harvested by filtration on a filter paper *in vacuo* and washed with saline (0.85% NaCl aq.).

### Reduction of $\alpha$ -keto esters and $\alpha$ -keto amide with marine actinomycete whole cells

The saline-washed cell (0.5 g, corresponding 0.2 g of dry weight) was resuspended in a large test tube ( $\phi$  30 mm × 200 mm) containing 20 ml of saline, and then the substrate (0.15 mmol, corresponding substrate concentration is 7.5 mM) and additive (5.0 mmol) were added and incubated aerobically at 25 °C. A portion of the reaction mixture was filtered using a diatomaceous earth short column and



**Figure 1.** The reduction of  $\alpha$ -keto esters (**1a–g**) by *Salinispora* strain.

**Table 1.** The reduction of  $\alpha$ -keto esters (**1**) by *S. arenicola* or *S. tropica*.<sup>a</sup>

Product	<i>S. arenicola</i>			<i>S. tropica</i>					
	No additive			No additive			L-glutamate		
	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>
<b>2a</b>	>99	50	S	>99	96	S	>99	>99	S
<b>2b</b>	>99	77	S	>99	99	S	>99	84	S
<b>2c</b>	>99	73	S	>99	81	S	>99	55	S
<b>2d</b>	91	78	S	99	76	S	91	72	S
<b>2e</b>	41	49	S	99	73	S	41	69	S
<b>2f</b>	>99	27	R	>99	61	R	>99	28	S
<b>2g</b>	43	69	R	>99	40	S	43	30	R

**Notes:** <sup>a</sup>Substrate (0.15 mmol), additive (5.0 mmol), and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g), and the reaction mixture was incubated aerobically for 48 h at 25 °C; <sup>b</sup>Conversion was measured by GLC analysis; <sup>c</sup>Enantiomeric excesses (e.e.) and configuration were determined by GLC analysis with optically active capillary columns.

extracted with ether, and then concentrated under reduced pressure.

### Repetitive use of immobilized marine actinomycete cells

The Ca<sup>2+</sup>-alginate immobilized *S. tropica* cells (IMST) were prepared by according to the procedures in the literature.<sup>16</sup> Saline (20 mL), the additive (monosodium L-glutamate) and 2-chlorobenzoylformamide (**3**, 0.15 mmol) were added to the IMST (5 g, corresponding 0.5 g of wet cells) and the reaction mixture were incubated at 25 °C under aerobic shaking. The used IMST was recovered by filtration and washed with saline. The recovered IMST was used as a biocatalyst of the next batch reaction.

### Analysis

The conversions of products (**2a-g** and **4**) were measured using a GLC with a capillary DB-WAX (0.25 mm × 30 m, He, 100 kPa; 110 °C; **1a**, 3.78 min; **2a**, 4.75 min; **1b**, 4.73 min; **2b**, 5.92 min; **1f**, 4.54 min; **2f**, 6.41 min; 120 °C; **1c**, 4.84 min; **2c**, 6.45 min; 150 °C, **1d**, 3.83 min; **2d**, 4.68 min; **1e**, 4.78 min; **2e**, 6.07 min) or TC-1 column (0.25 mm × 30 m, He, 100 kPa, 175 °C; **3**, 6.85 min; **4**, 8.34 min). The enantiomeric excesses (e.e.) of the products were measured using a GLC equipped with an optically active capillary CP-Chirasil-DEX CB (**2a-g**) or Gamma DEX 225 column (**4**). The absolute configurations of  $\alpha$ -hydroxy esters (**2a-g** and **4**) were identified by comparing their retention times on GLC analyses with those of authentic samples.<sup>10,14</sup>

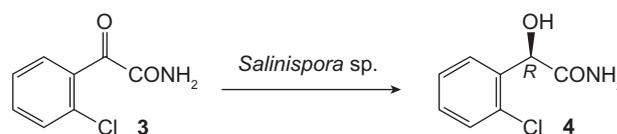
## Results and Discussion

### Reducing ability for $\alpha$ -keto esters

Two *Salinispora* strains were tested for reducing abilities toward  $\alpha$ -keto esters (see Fig. 1). The results of the reduction of  $\alpha$ -keto esters (**1a-g**) are summarized in Table 1. It was found that seven  $\alpha$ -keto esters were converted to the corresponding  $\alpha$ -hydroxy esters by *S. arenicola* and *S. tropica*.

*S. arenicola* reduced  $\alpha$ -keto esters have short alkyl chains (**1a-c** and **1f**) to the corresponding alcohols (**2a-c** and **2f**) with high conversion ratios in low e.e. In previous papers,<sup>9,16</sup> we found that the introduction of some hydride sources improve the stereoselectivity of the produced hydroxyl esters in the reduction of  $\alpha$ -keto esters by thermophilic actinomycete. Therefore, the reduction of  $\alpha$ -keto esters by marine actinomycetes in the presence of some additives was investigated. However, the low enantioselectivity did not improve by the addition of some additives such as glucose, glycerol, L-alanine, L-glutamate, L-aspartate, magnesium chloride, manganese chloride, and calcium chloride (data not shown).

The reduction of ethyl pyruvate (**1a**) and ethyl 2-oxobutanoate (**1b**) by *S. tropica* gave the corresponding alcohols (**2a** and **2b**) with high conversion ratios and in high enantiomeric excesses (96% e.e. and



**Figure 2.** The reduction of 2-chlorobenzoylformamide (**3**) by *Salinispora* strain.

**Table 2.** The reduction of 2-chlorobenzoylformamide (**3**) by *S. arenicola* or *S. tropica*.<sup>a</sup>

Reaction time (h)	<i>S. arenicola</i>			<i>S. tropica</i>					
	No additive			No additive			L-glutamate		
	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>
6	40	93	R	48	>99	R	45	>99	R
12	70	91	R	71	>99	R	90	>99	R
24	>99	90	R	89	>99	R	>99	>99	R
48	>99	92	R	>99	>99	R	>99	>99	R

**Notes:** <sup>a</sup>Substrate (0.15 mmol), additive (5.0 mmol), and 0.85% NaCl aq. (20 ml) were added to the wet cells (0.5 g), and the reaction mixture was incubated aerobically at 25 °C; <sup>b</sup>Conversion was measured by GLC analysis; <sup>c</sup>Enantiomeric excesses (e.e.) and configuration were determined by GLC analysis with an optically active capillary column.

99% e.e., respectively). Furthermore, in the presence of L-glutamate, the reduction of ethyl pyruvate (**1a**) by *S. tropica* afforded the corresponding (*S*)-ethyl lactate with >99% e.e.

### Reducing ability for aromatic $\alpha$ -keto amide

Two *Salinispora* strains were tested for reducing abilities toward aromatic  $\alpha$ -keto amide (see Fig. 2). The results of the reduction of 2-chlorobenzoylformamide (**3**) are summarized in Table 2. In the reduction of aromatic  $\alpha$ -keto amide by *S. arenicola*, the conversion ratio from 2-chlorobenzoylformamide (**3**) to 2-chloromandelamide (**4**) was reached >99% after 24 h; however, the enantioselectivity of the product (**4**) stayed about 90% e.e. (*R*). On the other hand, the reduction rate of **3** by *S. tropica* was slow and the maximum conversion ratio of **3** to **4** was reached 48 h later. However, the selectivity of the product indicated an excellent value (>99% e.e.) from the beginning of the reduction reaction. To improve the slowness of reaction rate

**Table 3.** The repetitive batch reduction of **3** to **4** by Ca<sup>2+</sup>-alginate immobilized cells of *S. tropica*.<sup>a</sup>

Batch	Conv. <sup>b</sup> (%)	e.e. <sup>c</sup> (%)	(R/S) <sup>c</sup>
1	>99	>99	R
2	>99	>99	R
3	>99	>99	R
4	99	>99	R
5	98	>99	R

**Notes:** <sup>a</sup>Substrate (0.15 mmol), monosodium L-glutamate (5.0 mmol), and 0.85% NaCl aq. (20 ml) were added to the immobilized cells (5 g), and the reaction mixture was incubated aerobically for 24 h at 25 °C; <sup>b</sup>Conversion was measured by GLC analysis; <sup>c</sup>Enantiomeric excesses (e.e.) and configuration were determined by GLC analysis with an optically active capillary column.

the reduction of **3** by *S. tropica* in the presence of L-glutamate was examined. As a result, it succeeded in shortening the time it took to reach to the maximum conversion rate with high enantioselectivity of the product maintained.

### Reduction of aromatic $\alpha$ -keto amide with immobilized *Salinispora* cells

Furthermore, the productivity of the reduction, the batch reduction of **3** by the IMST was investigated. In the IMST reduction from **3** to **4** using L-glutamate, the conversion ratio was on kept high value (>98%) at five batch reductions. Further, (*R*)-**4** was produced with high enantioselectivity in every batch reactions (Table 3). Thus, the repetitive production of enantio-pure (*R*)-2-chloromandelamide has been achieved using the IMST. It seems that the increase of reduced nicotinamide-adenine dinucleotide (NADH or NADPH) due to the oxidative degradation of L-glutamate in the cells of marine actinomycetes would accelerate the stereoselective reduction of aromatic  $\alpha$ -keto amide to the corresponding optically pure alcohols.

### Conclusion

We demonstrated the stereoselective reduction of  $\alpha$ -keto esters and aromatic  $\alpha$ -keto amide to the corresponding alcohols with marine actinomycetes. It was found that *Salinispora* strains were a useful tool for the preparation of chiral  $\alpha$ -hydroxy esters and (*R*)-2-chloromandelamide. To gain insight into the mechanistic interpretation of the marine actinomycete reduction, further detailed studies including purification of the reductase(s), which contribute to the reduction of  $\alpha$ -keto esters and aromatic  $\alpha$ -keto amide, are currently under investigation.



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## Disclosures

Author(s) have provided signed confirmations to the publisher of their compliance with all applicable legal and ethical obligations in respect to declaration of conflicts of interest, funding, authorship and contributorship, and compliance with ethical requirements in respect to treatment of human and animal test subjects. If this article contains identifiable human subject(s) author(s) were required to supply signed patient consent prior to publication. Author(s) have confirmed that the published article is unique and not under consideration nor published by any other publication and that they have consent to reproduce any copyrighted material. The peer reviewers declared no conflicts of interest.

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