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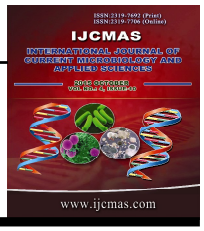

Original Research Article

Biocatalytic Preparation of Chiral Alcohols: Stereoselective Reduction of Carbonyl Compounds using Two Strains of the Streptomycetaceae family - *Streptacidiphilus* and *Kitasatospora*

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Keywords

Biocatalyst, Stereoselective reduction, Chiral alcohol, *Streptacidiphilus*, *Kitasatospora*

A B S T R A C T

To investigate the potential ability of members of the Streptomycetaceae family to act as biocatalysts, we screened 7 *Streptacidiphilus* and 16 *Kitasatospora* strains. Three recommended media (227, 266, and 1051 media) and a modified medium (P-MIM medium) were tested for use in the liquid culture of these actinomycetes. Two *Streptacidiphilus* strains (*Streptacidiphilus anmyonensis* NBRC103185 and *Streptacidiphilus rugosus* NBRC103186) and three *Kitasatospora* strains (*Kitasatospora azatica* NBRC13803, *Kitasatospora setae* NBRC14216, and *Kitasatospora phosalacinea* NBRC14372) showed good growth. Next, the stereoselective reduction of various carbonyl compounds using these five strains was investigated. It was found that these strains possess a reducing activity toward keto esters and an aromatic α-keto amide. Among them, the reduction of α-keto esters by *S. anmyonensis* NBRC103185 cultivated in the 1051 medium in the presence of L-alanine as an additive yielded the corresponding α-hydroxy esters with a high conversion ratio. Furthermore, the introduction of L-glutamate for *K. setae* NBRC14216-catalyzed reduction improved both the conversion ratio and the stereoselectivity of the produced alcohols. Thus, we found that *Streptacidiphilus* and *Kitasatospora* strains have great potential to be used as biocatalysts for the stereoselective reduction of carbonyl compounds.

Introduction

"Actinomycetes" is a common name used to refer to a group of specific microorganisms including bacteria and fungi, except bifidobacteria and mycobacteria in the order "*Actinomycetales*" (Gottlieb *et al.*, 1974). Actinomycetes are prokaryotic microorganisms that lack a nuclear membrane, and are cytologically and morphologically located between bacteria and fungi. However, in view of phylogenetic studies, actinomycetes have been classified into groups other than fungi or gram-positive and gram-negative bacteria (Embley and Stackebrandt, 1994). Since the discovery of streptomycin produced by *Streptomyces griseus* (Schatz *et al.*, 1944),

various actinomycetes have been isolated as antibiotic-producing bacteria, and extensive biochemical and genetic research has been conducted on these microorganisms. Furthermore, a new protease inhibitor "leupeptin" was discovered in the culture filtrate of *Streptomyces* species (Aoyagi *et al.*, 1969). Some studies have also shown that the actinomycetes can produce a variety of biologically active compounds such as antidiabetic (Kulkarni-Almeida *et al.*, 2011) and immunosuppressive substances (Al-Garni *et al.*, 2014; Bamzadeh *et al.*, 2014). Thus, actinomycetes are of importance in the medical and pharmaceutical fields.

As described above, there are several studies on the biochemical applications of secondary metabolites from actinomycetes. On the other hand, it was also found that some strains of the genus *Streptomyces* in the family Streptomycetaceae were useful biocatalysts for the asymmetric reductions of various carbonyl compounds (Ishihara *et al.*, 2013; 2008; 2004; 2003; 2000; 1997). While this genus has thus been extensively studied for the biocatalytic activities of its members, the potential biocatalytic activities of members of other genera in this family of microorganisms has not been investigated.

In this study, we investigated the stereoselective reduction of carbonyl compounds using the *Streptacidiphilus* and *Kitasatospora* strains from the Streptomycetaceae family as novel biocatalysts (Figure 1).

Materials and Methods

Instruments and chemicals

Gas chromatography (GC) was performed using the GL Science GC-353 gas chromatographs (GL Science Inc., Tokyo, Japan) equipped with capillary columns (DB-Wax, Agilent Technologies, Santa Clara, CA, USA, 0.25 μm, 0.25 mm x 30 m; TC-1, GL Science Inc., 0.25 μm, 0.25 mm x 30 m; CP-Chirasil-DEX CB, Varian Inc., Lake Forest, CA, USA, 0.25 μm, 0.25 mm x 25 m; Gamma DEX 225, Sigma-Aldrich Co., St. Louise, MO, USA, 0.25 μm, 0.25 mm x 30 m). Ethyl pyruvate (Figure 1, 1a), diatomaceous earth (granular) and polypepton were purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. Bacto™ peptone, Bacto™ yeast extract, and Difco™ soluble starch were purchased from Becton, Dickinson and Co. (Franklin Lakes, NJ, USA). Ethyl lactate (2a), ethyl 3-methyl-2-oxobutanoate (1f), ethyl 2-oxo-4-phenylbutanoate (1h), ethyl 2-hydroxy-4-phenylbutanoate (2h), ethyl 3-oxobutanoate (1i), ethyl 3-hydroxybutanoate (2i), and beef extract were purchased from Sigma-Aldrich. Ethyl benzoylformate (1g) and ethyl mandelate (2g) were obtained from Tokyo Chemical Industry, Co. Ltd. (Tokyo, Japan). Ethyl 2-oxobutanoate (1b), ethyl 2-oxopentanoate (1c), ethyl 2-oxohexanoate (1d), ethyl 2-oxoheptanoate (1e), 2-chlorobenzoylformamide (1h), 2-chloromandelamide (2h), and α-hydroxy esters (2b-f) were prepared according to the procedures described in previous literature (Nakamura *et al.*, 1998; Mitsuhashi and Yamamoto, 2005).

Microorganisms and culture

Streptacidiphilus albus NBRC100918, *Streptacidiphilus carbonis* NBRC100919, *Streptacidiphilus jiangxiensis* NBRC100920, *Streptacidiphilus neutrinimicus* NBRC100921, *Streptacidiphilus melanogenes* NBRC103184, *Streptacidiphilus anmyonensis* NBRC103185, *Streptacidiphilus rugosus* NBRC103186, *Kitasatospora azatica* NBRC13803, *Kitasatospora setae* NBRC14216,

Kitasatospora griseola NBRC14371, *Kitasatospora phosalacinea* NBRC14372, *Kitasatospora paracochleata* NBRC14769, *Kitasatospora mediocidica* NBRC14789, *Kitasatospora crystarginea* NBRC14836, *Kitasatospora kifunensis* NBRC15206, *Kitasatospora cineracea* NBRC16452, *Kitasatospora niigatensis* NBRC16453, *Kitasatospora putterlickiae* NBRC100917, *Kitasatospora arboriphila* NBRC101834, *Kitasatospora nipponensis* NBRC101836, *Kitasatospora paranensis* NBRC101837, *Kitasatospora terrestris* NBRC101838, and *Kitasatospora samplinensis* NBRC102069 were purchased from the National Institute of Technology and Evaluation, Biological Resource Center (NBRC, Japan). These strains were maintained at 28°C in NBRC-recommended medium (227, 228, 231, 245, 266, 268, 876, and 1051) solidified with 1.5%(w/v) agar. The 227 medium (International *Streptomyces* Project, ISP medium No. 2) comprised 4.0 g of Bacto™ yeast extract, 10.0 g of Bacto™ malt extract, and 4.0 g of D-glucose per liter of distilled water (pH 7.3). The 228 medium comprised 1.0 g of Bacto™ yeast extract, 1.0 g of beef extract, 2.0 g of NZ amine, type A, and 10.0 g of D-glucose per liter of distilled water (pH 7.3). The 231 medium comprised 1.0 g of Bacto™ yeast extract, 1.0 g of beef extract, 2.0 g of NZ amine, type A, and 10.0 g of maltose per liter of distilled water (pH 7.3).

The 245 medium (ISP medium No. 3) comprised 20.0 g of oatmeal, and 1.0 mL of trace salts solution per liter of distilled water (pH 7.2). Trace salts solution comprised 0.1 g of $FeSO_4 \cdot 7H_2O$, 0.1 g of $MnCl_2 \cdot 4H_2O$, 0.1 g of $ZnSO_4 \cdot 7H_2O$ per 100 mL of distilled water. The 266 medium comprised 2.0 g of Bacto™ yeast extract, and 10.0 g of Difco™ soluble starch per liter of distilled water (pH 7.3). The 268 medium (ISP medium No. 4) comprised 10.0 g of Difco™ soluble starch, 1.0 g of K_2HPO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 1.0 g of NaCl, 2.0 g of $(NH_4)_2SO_4$, 2.0 g of $CaCO_3$, 1.0 mL of trace salts solution. The 876 medium comprised 2.0 g of Bacto™ yeast extract, and 10.0 g of Difco™ soluble starch per liter of distilled water (pH 5.0). The 1051 medium comprised 4.0 g of Bacto™ yeast extract, 10.0 g of Bacto™ malt extract, and 4.0 g of D-glucose per liter of distilled water (pH 5.5). The P-MIM medium comprised 15.0 g of Bacto™ peptone, 2.0 g of Bacto™ yeast extract, 2.0 g meat extract, 2.0 g of glycerol, 2.0 g of KH_2PO_4, 2.0 g of K_2HPO_4, and $MgSO_4 \cdot 7H_2O$ per liter of distilled water (pH 7.2). The *Streptacidiphilus* strains were grown in the 227, 1051, and P-MIM media for 3 days at 25°C with aerobic shaking in baffled flasks in the dark, while the *Kitasatospora* strains were grown in the 227, 266, and P-MIM media for 3 days at 25°C with aerobic shaking in baffled flasks in the dark. The actinomycete cells were harvested by filtration on filter paper (Whatman, No. 4) *in vacuo* and washed with saline (0.85% NaCl aq.).

Reduction of α, β-keto esters and an aromatic α-keto amide using actinomycetes resting cells

Saline-washed wet actinomycete cells (0.5 g, dry weight approximately 0.15 g) were resuspended in a large test tube (ϕ 30 mm x 200 mm) containing 20 mL saline.

The substrate (0.15 mmol; 7.5 mM) was then added, and the reaction mixture was incubated aerobically (reciprocating shaking at 120 rpm) at 25°C. A portion (0.5 mL) of the mixture was filtered using a short diatomaceous earth column (ϕ 10 mm x 30 mm), extracted with diethyl ether (5.0 mL), and then concentrated under reduced pressure.

Analysis

The conversion of the alcohols produced (Figure 1, 2a-j) was measured using a GLC with a DB-WAX capillary column (100 kPa He at 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; and 2c, 6.45 min; 150°C: 1d, 3.83 min; 2d, 4.68 min; 1e, 4.78 min; 2e, 6.07 min; 180°C: 1g, 9.01 min; and 2g, 12.08 min) or a TC-1 capillary column (100 kPa He at 140°C: 1h, 10.02 min; 2h, 10.96 min, 130°C: 1i, 4.34 min; 2i, 5.16 min, 175°C: 1j, 6.85 min; and 2j, 8.34 min).

The enantiomeric excess (ee) of the product was measured using a GC instrument equipped with an optically active CP-Chirasil-DEX CB (2a-e, 2g-i) or Gamma DEX 225 capillary column (2f and 2j). The ee was calculated using the following formula: ee (%) = {(*R*-*S*)/(*R*+*S*)} x 100, where *R* and *S* are the respective peak areas in the GC analyses. The absolute configurations of the α-, β-hydroxy esters (2a-i) and aromatic α-hydroxy amide (2j) were identified by comparing their retention times determined by the GLC analyses with those of authentic samples (Nakamura *et al.*, 1998; Mitsuhashi and Yamamoto, 2005).

Results and Discussion

Screening of actinomycetes strains and culture media

To determine the suitable medium for liquid culture, the amount of wet cells obtained by cultivating 7 *Streptacidiphilus* and 16 *Kitasatospora* strains in several culture media was measured.

All *Streptacidiphilus* strains hardly grew in the P-MIM medium and even after few days to 1 week of culture, the resulting wet bacterial cell weight was 0.4 g or less (data not shown). However, only two strains, *S. anmyonensis* NBRC103185 and *S. rugosus* NBRC103186, in cultures with both the 227 and the 1051 media, yielded more than 1.2 g of wet cells/100 mL of the medium (Table 1). The 1051 medium was obtained by adjusting the pH of the 227 medium to 5.5; the components of the two media were identical. Although the recommended medium for these strains is the 1051 medium, the 227 medium also yielded satisfactory results for liquid culture. These results suggest that the type of carbon source is important, and that glucose is more suitable than glycerol for the culture of *Streptacidiphilus* strains. The growth of the *Kitasatospora* strains was not as good as that of the *Streptacidiphilus* strains. In particular, most of the *Kitasatospora* strains tested did not grow in the P-MIM medium (Table 2). However, three strains, *K. azatica* NBRC13803, *K. setae* NBRC14216, and *K. phosalacinea* NBRC14372 were able to grow in three kinds of liquid media (227, 266, and P-MIM medium).

Therefore, we investigated the possibility of two *Streptacidiphilus* (*S. anmyonensis* and *S. rugosus*) and three *Kitasatospora* strains (*K. azatica*, *K. setae*, and *K. phosalacinea*) acting as biocatalysts for the asymmetric reduction of carbonyl compounds.

Reduction of carbonyl compounds by *Streptacidiphilus* strains

Two *Streptacidiphilus* strains (NBRC103185 and 103486) cultivated in the 227 or 1051 medium were tested for their ability to reduce keto esters (1a-i) and an aromatic α-keto amide (1j) (Figure 1). The results of the microbial reductions are summarized in table 3.

Table.1 The cultivation of *Streptacidiphilus* strains in several culture media

NBRC No.	Scientific Name	227 medium[1] Wet cells (g)[2]	1051 medium[1] Wet cells (g)[2]	P-MIM[1] Wet cells (g)[2]
100918	*Streptacidiphilus albus*	0.13	0.55	<0.1
100919	*Streptacidiphilus carbonis*	<0.1	0.46	<0.1
100920	*Streptacidiphilus jiangxiensis*	0.27	0.13	<0.1
100921	*Streptacidiphilus neutrinimicus*	<0.1	<0.1	<0.1
103184	*Streptoacidiphilus melanogenes*	0.43	0.77	<0.1
103185	*Streptacidiphilus anmyonesis*	1.22	1.41	<0.1
103186	*Streptacidiphilus rugosus*	1.44	1.41	0.33

[1]Composition of each culture medium was described in materials and method section.
[2]The actinomycete were grown in the medium (100 mL) at 25°C for 72 hours with aerobic rotary shaking (100 min^{-1}) in baffled 500-mL flask in the dark condition.

Table.2 The cultivation of *Kitasatospor*a strains in several culture media

NBRC No.	Scientific Name	227 medium[1] Wet cells (g)[2]	266 medium[1] Wet cells (g)[2]	P-MIM[1] Wet cells (g)[2]
13803	*Kitasatospora azatica*	0.81	0.44	0.55
14216	*Kitasatospora setae*	0.69	0.47	0.58
14371	*Kitasatospora griseola*	0.73	<0.1	<0.1
14372	*Kitasatospora phosalacinea*	0.77	0.53	0.78
14769	*Kitasatospora paracochleata*	0.69	0.64	<0.1
14789	*Kitasatospora mediocidica*	0.21	<0.1	<0.1
14836	*Kitasatospora crystarginea*	0.49	<0.1	<0.1
15206	*Kitasatospora kifunensis*	0.45	0.44	<0.1
16452	*Kitasatospora cineracea*	0.60	0.45	<0.1
16453	*Kitasatospora niigatensis*	0.84	0.23	<0.1
100917	*Kitasatospora putterlickiae*	0.18	<0.1	<0.1
101834	*Kitasatospora arboriphila*	0.45	0.66	0.2
101836	*Kitasatospora nipponensis*	0.16	0.43	<0.1
101837	*Kitasatospora paranensis*	0.36	0.53	0.2
101838	*Kitasatospora terrestris*	0.65	0.28	<0.1
102069	*Kitasatospora samplinensis*	<0.1	<0.1	0.3

[1]Composition of each culture medium was described in materials and method section.
[2]The actinomycete were grown in the medium (100 mL) at 25°C for 72 hours with aerobic rotary shaking (100 min^{-1}) in baffled 500-mL flask in the dark condition.

Table.3 The reduction of various carbonyl compounds (**1a-j**) to corresponding alcohols (**2a-j**) with two *Streptacidiphilus* strains cultivated with two culture media

	Streptacidiphilus anmyonesis NBRC103185						*Streptacidiphilus rugosus* NBRC103186					
	227 medium			1051 medium			227 medium			1051 medium		
Product	conv.(%)	e.e. (%)	*R/S*	conv.(%)	e.e. (%)	*R/S*	conv. (%)	e.e.(%)	*R/S*	conv.(%)	e.e.(%)	*R/S*
2a	>99	40	*S*	>99	56	*S*	>99	63	*S*	>99	>99	*S*
2b	>99	29	*S*	>99	17	*S*	61	2.4	*S*	>99	8.0	*S*
2c	78	17	*R*	>99	6.2	*S*	86	32	*S*	96	19	*S*
2d	73	9.0	*S*	>99	2.4	*S*	52	40	*S*	>99	26	*S*
2e	41	7.4	*R*	84	5.2	*R*	23	39	*S*	86	15	*S*
2f	>99	50	*S*	>99	54	*S*	87	29	*S*	>99	37	*S*
2g	31	39	*S*	53	83	*R*	14	49	*S*	23	37	*R*
2h	83	18	*S*	66	18	*S*	28	38	*S*	39	26	*S*
2i	87	79	*S*	93	86	*S*	77	89	*S*	98	92	*S*
2j	60	>99	*R*	5.0	>99	*R*	38	>99	*R*	26	>99	*R*

Table.4 Effects of additives on the reduction of carbonyl compounds with *Streptacidiphilus anmyonensis* NBRC103185 cultivated in1051 medium

Product	Glycerol			Glucose			L-Alanine			L-Glutamate Na			Methyl vinyl ketone			Ethyl chloroacetate		
	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*
2a	>99	>99	*S*	>99	42	*S*	>99	50	*S*	>99	74	*S*	43	51	*S*	13	57	*S*
2b	98	91	*S*	48	34	*R*	>99	5.0	*S*	>99	27	*S*	37	15	*S*	64	40	*S*
2c	>99	88	*S*	72	1.0	*S*	>99	21	*R*	>99	40	*S*	24	27	*S*	19	54	*S*
2d	>99	78	*S*	46	20	*S*	>99	36	*S*	97	54	*S*	20	10	*R*	21	36	*S*
2e	97	74	*S*	>99	12	*S*	>99	63	*S*	90	35	*S*	11	52	*S*	14	22	*R*
2f	>99	>99	*S*	>99	>99	*S*	>99	74	*R*	>99	97	*S*	14	88	*S*	54	86	*S*
2g	>99	94	*S*	88	34	*S*	>99	72	*S*	81	44	*S*	12	70	*S*	20	27	*S*
2h	91	82	*S*	10	33	*S*	42	50	*R*	63	30	*R*	18	22	*R*	15	43	*R*
2i	88	96	*S*	14	89	*S*	86	79	*S*	75	88	*S*	22	43	*S*	31	51	*S*
2j	>99	>99	*R*	78	>99	*R*	>99	>99	*R*	86	>99	*R*	15	>99	*R*	19	>99	*R*

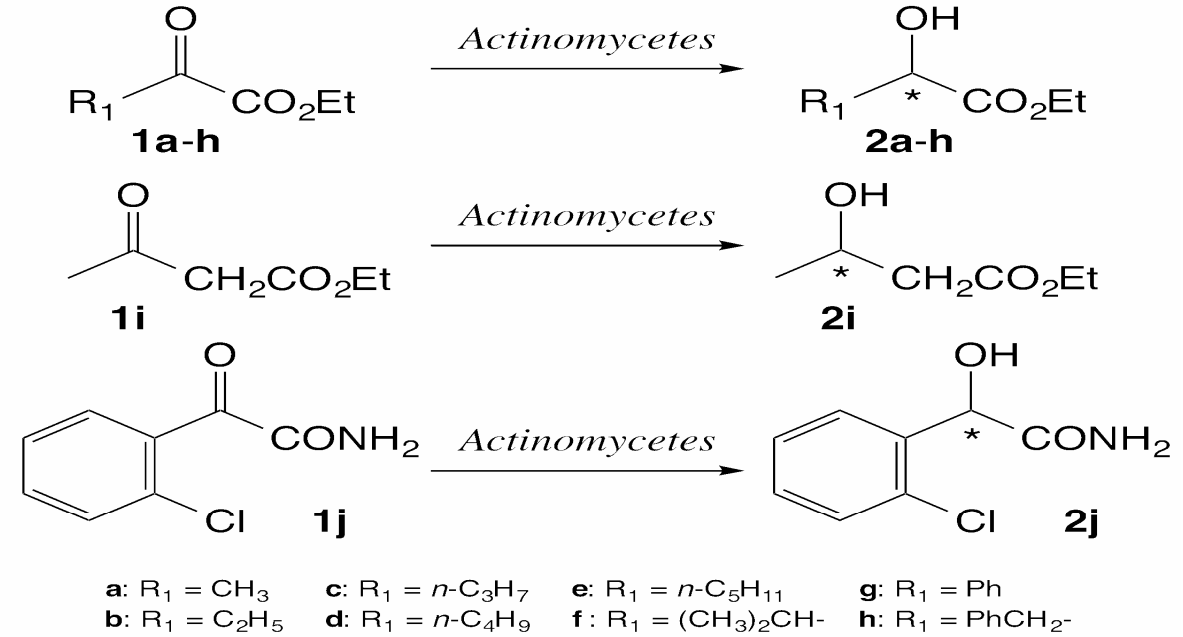
Table.5 The reduction of various carbonyl compounds (**1a-j**) to corresponding alcohols (**2a-j**) with three *Kitasatospora* strains cultivated with three media

Product	*Kitasatospora azatica* NBRC13803									*Kitasatospora setae* NBRC14216									*Kitasatospora phosalacinea* NBRC14372								
	227 medium			266 medium			P-MIM medium			227 medium			266 medium			P-MIM medium			227 medium			266 medium			P-MIM medium		
	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*
2a	4.5	>99	*S*	1.8	>99	*S*	>99	77	*S*	>99	94	*S*	69	91	*S*	>99	85	*S*	>99	78	*S*	>99	55	*S*	>99	73	*S*
2b	3.7	89	*S*	5.5	90	*S*	98	55	*S*	>99	44	*S*	>99	49	*S*	>99	64	*S*	>99	76	*S*	95	66	*S*	>99	54	*S*
2c	10	68	*S*	18	49	*S*	97	19	*S*	>99	87	*S*	89	31	*S*	>99	88	*S*	84	69	*S*	97	59	*S*	88	63	*S*
2d	8.8	49	*S*	21	67	*S*	29	38	*S*	>99	90	*S*	72	64	*S*	99	71	*S*	40	54	*S*	84	50	*S*	71	60	*S*
2e	6.9	39	*S*	19	47	*S*	5.3	46	*S*	97	93	*S*	75	53	*S*	81	79	*S*	57	61	*S*	68	29	*S*	68	39	*S*
2f	18	79	*S*	85	81	*S*	85	21	*R*	>99	36	*R*	>99	51	*R*	>99	45	*R*	>99	16	*R*	>99	31	*R*	>99	44	*R*
2g	5.1	16	*R*	10	39	*R*	11	19	*R*	96	87	*R*	82	32	*R*	80	89	*R*	66	88	*S*	49	70	*S*	60	78	*S*
2h	20	26	*R*	27	30	*R*	14	61	*R*	94	77	*R*	84	51	*R*	85	76	*R*	53	25	*R*	31	38	*R*	49	26	*R*
2i	99	89	*S*	94	47	*S*	98	33	*S*	96	40	*S*	98	60	*S*	97	29	*S*	99	5.4	*R*	>99	49	*S*	>99	13	*S*
2j	24	90	*R*	60	91	*R*	43	81	*R*	>99	>99	*R*	99	94	*R*	>99	>99	*R*	97	>99	*R*	>99	97	*R*	>99	99	*R*

Table.6 Effects of additives on the reduction of carbonyl compounds with *Kitasatospora setae* NBRC14216 cultivated in the 227 medium

Product	Glycerol			Glucose			L-Alanine			L-Glutamate Na			Methyl vinyl ketone			Ethyl chloroacetate		
	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*	conv. (%)	e.e. (%)	*R/S*
2a	98	>99	*S*	98	>99	*S*	>99	>99	*S*	>99	>99	*S*	25	71	*S*	43	74	*S*
2b	98	98	*S*	>99	97	*S*	>99	96	*S*	>99	97	*S*	18	55	*S*	37	63	*S*
2c	93	88	*S*	90	98	*S*	98	97	*S*	>99	98	*S*	24	48	*S*	52	44	*S*
2d	87	69	*S*	95	78	*S*	99	88	*S*	>99	>99	*S*	30	61	*S*	27	50	*S*
2e	90	83	*S*	98	83	*S*	99	87	*S*	99	93	*S*	11	38	*R*	19	37	*R*
2f	95	94	*R*	99	98	*R*	>99	84	*R*	>99	95	*R*	16	56	*S*	44	75	*S*
2g	97	81	*R*	92	89	*R*	97	90	*R*	98	92	*R*	15	40	*R*	10	17	*R*
2h	88	83	*R*	86	79	*R*	96	94	*R*	99	95	*R*	22	33	*R*	36	55	*R*
2i	98	94	*S*	93	95	*S*	98	>99	*S*	>99	>99	*S*	28	63	*S*	45	70	*S*
2j	>99	>99	*R*	>99	>99	*R*	>99	>99	*R*	>99	>99	*R*	18	>99	*R*	22	>99	*R*

Figure.1 The reduction of various carbonyl compounds (1a-j) to the corresponding alcohols (2a-j) by actinomycetes



In the present study found that the *Streptacidiphilus* strains tested in this study reduced 10 substrates (1a-i) to the corresponding alcohols (2a-i). The reduction using two *Streptacidiphilus* strains cultured in the 1051 medium was more than that in the 227 medium. Further, the conversion ratios for substrates with a short alkyl chain were higher than those for substrates with longer alkyl chains, while the stereoselectivity of the produced alcohols indicated low enantiomeric excesses except for the reduction of an aromatic α-keto amide (1j).

In the microbial reduction of carbonyl compounds using the common bakers' yeast or filamentous fungi, it is well known that the introduction of small organic molecules or metal ions increases the stereoselectivity of the alcohols produced (Kawai *et al.*, 1994; Kawai *et al.*, 1995; Nakamura *et al.*, 1996). In contrast, in the reduction using actinomycetes, several reports have shown that the addition of amino acids or sugars improves the conversion ratio and the stereoselectivity of the products (Ishihara *et al.*, 2013; 2011; 2010; 2000; 2003). Therefore, we investigated the effect of additives on the reduction of substrates by *S. anmyonensis* NBRC103185 cultivated in the 1051 medium (Table 4).

Among the various additives used (methyl vinyl ketone, ethyl chloroacetate, D-glucose, glycerol, L-alanine, and L-glutamate), methyl vinyl ketone and ethyl chloroacetate decreased the conversion ratio of the reduction. On the other hand, the introduction of sugars and amino acids improved the conversion ratio of the reduction. In particular, the reduction of α-keto esters (1a-g) and the α-keto amide (1j) in the presence of glycerol or L-alanine yielded the corresponding alcohols with excellent conversion ratios (>97%). It appears that the increase in reduced nicotinamide-adenine dinucleotide (NADH or NADPH) through the oxidative degradation of the additives accelerates the reduction of substrates to the corresponding alcohols. Furthermore, following the introduction of glycerol, the stereoselectivity of the produced alcohols in the reduction by NBRC103185 also improved.

Reduction of carbonyl compounds by *Kitasatospora* strains

Three *Kitasatospora* strains cultivated in three media were tested for their ability to reduce α-, β-keto esters, and the α-keto amide. As shown in Table 5, all the substrates were reduced to the corresponding alcohols by three actinomycete strains. In particular, *Kitasatospora* setae NBRC14216 cultured in the 227 medium reduced 10 substrates with high conversion ratios (>94%), but the stereoselectivity of the produced alcohols was not very high (36-99% ee). Therefore, the effect of additives on the reduction by *K. setae* NBRC14216 cultivated in the 227 medium was investigated (Table 6).

Similar to the results of our experiment focused on studying the effects of the additive on the reduction of carbonyl compounds by *S. anmyonensis*, this experiment involving reduction using *K. setae* BRC14216 cultured in the 227 medium showed that the introduction of small organic molecules decreased the conversion ratio. Further, both the conversion ratio and the stereoselectivity of the reduction improved after the introduction of amino acids. In particular, the addition of L-glutamate proceeded stereospecific reduction toward four kinds of substrates (1a, 1d, 1i and 1j).

In conclusion, Members of the Streptomycetaceae family, *Streptacidiphilus* and *Kitasatospora* strains converted various keto esters and an aromatic α-keto amide to the corresponding hydroxy esters and hydroxy amide. Based on the conversion ratios and the stereoselectivity of the products, we suggest *Streptacidiphilus anmyonensis* NBRC100742 and *Kitasatospora setae* NBRC14216 to be potential biocatalysts for the stereoselective reduction of keto esters and keto amide to yield the corresponding chiral alcohols.

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