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Microbial Preparation of Chiral Alcohols: Stereoselective Reduction of Carbonyl Compounds using Two Genera of the Streptosporangiaceae Family - *Streptosporangium* and *Nonomuraea*

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A B S T R A C T

To investigate the potential ability of two genera of the Streptosporangiaceae family to act as biocatalysts, we screened 13 *Streptosporangium* and 13 *Nonomuraea* strains. Five recommended media (227, 228, 231, 266 and 268 media) were tested for use in the liquid culture of these actinomycetes. Three *Streptosporangium* strains (*Streptosporangium* sp. NBRC16544, *Streptosporangium* sp. NBRC101289, and *Streptosporangium* sp. NBRC101798) showed good growth when cultured in 227 and 228 media, as did three *Nonomuraea* strains (*N. spiralis* NBRC14097, *N. helvata* NBRC14681, and *Nonomuraea* sp. NBRC16543) when cultured in 227 and 266 media. Therefore, the stereoselective reduction of various carbonyl compounds using these six strains was investigated. It was found that these strains possess reducing activity towardsα-keto esters (aliphatic and aromatic) and an aromatic α-keto amide. Based on the conversion ratios and the stereoselectivity of the produced alcohols, we suggest that *Streptosporangium* sp. NBRC101798 cultured in 228 medium and *N. helvata* NBRC14681 cultured in 266 medium are potential biocatalysts for the stereoselective reduction of α-keto esters and an aromatic α-keto amide to yield the corresponding chiral alcohols. In addition, our results also suggest that cryopreserved cells could be used as biocatalysts for the asymmetric reduction of carbonyl compounds.

Introduction

Actinobacteria (commonly called action-mycetes) are prokaryotic microorganisms that are cytologically and morphologically located between bacteria and fungi. However, in view of phylogenetic studies, actinomycetes have been classified as distinct from fungi and gram-positive or gram-negative bacteria (Embley and Stackebrandt, 1994). Actino-mycetes are ecosystem decomposers that are widely distributed in the soil, and they are well known to produce secondary metabolites (Solecka *et al*., 2012; Newman and Hill, 2006; Bérdy, 2005; Bull *et al*., 2000), notably antibiotics (Strohl, 2004), antitumor agents (Olano *et al*., 2009), immunosuppressive agents (Al-Garni *et al*., 2014; Bamzadeh *et al*., 2014; Mann, 2001), antidiabetic agents (Kulkarni-Almeida *et al*., 2011) and

industrially important enzymes (Pecznska-Czoch and Mordarski, 1988). Thus, actinomycetes are of important in the medical and pharmaceutical fields. As described above, there have been several studies on the biochemical applications of secondary metabolites from actinomycetes. In addition, it has been found that some strains of the genus *Streptomyces* in the family Streptomycetaceae are useful biocatalysts for the asymmetric reductions of various carbonyl compounds (Ishihara *et al*., 2015, 2013, 2008, 2004, 2003, 2000, 1997). While this family has thus been extensively studied for the biocatalytic activities of its members, the potential biocatalytic abilities of actinomycetes belonging to other families have not been investigated.

In this study, we investigated the stereoselective reduction of carbonyl compounds using two genera, *Streptosporangium* and *Nonomuraea* from the Streptosporangiaceae family, as novel biocatalysts (Figure 1). Furthermore, the possibility of microbial conversion using cryopreserved cells was also investigated for expanding the usage of these biocatalysts.

Materials and Methods

Instruments and chemicals

Gas chromatography (GLC) was performed using the GL Science GC-353 gas chromatographs (GL Science Inc., Tokyo, Japan) equipped with capillary columns (DB-Wax, 0.25 μm, 0.25 mm x 30 m, Agilent Technologies, Santa Clara, CA, USA; TC-1,0.25μm, 0.25 mm x 30 m GL Science Inc.; CP-Chirasil-DEX CB, 0.25 μm, 0.25 mm x 25 m, Varian Inc., Lake Forest, CA, USA; Gamma DEX 225, 0.25 μm, 0.25 mm x 30 m, Sigma-Aldrich Co., St. Louise, MO, USA). Ethyl pyruvate (Figure 1, **1a**), diatomaceous earth (granular), and NZ amine, type A were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Bacto™ malt extract, 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**), 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-chlorobenzoyl-formamide (**1i**), 2-chloromandelamide (**2i**), and α-hydroxy esters (**2b-f**) were prepared according to procedures described in the literature (Nakamura *et al*., 1998; Mitsuhashi and Yamamoto, 2005). All other chemicals used in this study were of analytical grade and commercially available.

Microorganisms and culture

Streptosporangium roseum NBRC3776, *Streptosporangium longisporum* NBRC13141, *Streptosporangium rubrum* NBRC13975, *Streptosporangium vulgare* NBRC13985, *Streptosporangium amethystogenes* subsp. *amethystogenes* NBRC13986, *Strepto-sporangium fragile* NBRC14311, *Strepto-sporangium vioaceochromogenes* NBRC15560, *Streptosporangium carneum* NBRC15562, *Streptosporangium* sp. NBRC16544, *Streptosporangium* sp. NBRC16545, *Strepto-sporangium* sp. NBRC101289, *Strepto-sporangium* sp. NBRC101793, *Strepto-sporangium* sp. NBRC101798, *Nonomuraea angiospora* NBRC13155, *Nonomuraea spiralis* NBRC14097, *Nonomuraea helvata* NBRC14681, *Nonomuraea pusilla* NBRC14684, *Nonomuraea africana* NBRC14757, *Nonomuraea* sp. NBRC14989, *Nonomuraea longicatena* NBRC16462, *Nonomuraea* sp. NBRC16541, *Nonomuraea*

sp. NBRC16543, *Nonomuraea* sp. NBRC101290, *Nonomuraea jiangxiensis* NBRC106679, *Nonomuraea maritime* NBRC106687, and *Nonomuraea candida* NBRC107354 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 media (227, 228, 231, 266, and 268) solidified with 1.5% (w/v) agar. The 227 medium (International *Streptomyces* Project, ISP medium No. 2) included 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 was composed of 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 was comprised of 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 266 medium included 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) was composed of 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 $CaCO_3$, and 1.0 mL of trace salts solution. The trace salts solution included 0.1 g of $FeSO_4 \cdot 7H_2O$, 0.1 g of $MnCl_2 \cdot 4H_2O$, and 0.1 g of $ZnSO_4 \cdot 7H_2O$ per 100 mL of distilled water. Thirteen *Streptosporangium* strains were grown in 227, 228, 231, and 266 media for 10 days at 25°C with aerobic shaking in baffled flasks in the dark, while 13 *Nonomuraea* strains were grown in 227, 266, and 288 media for 9 days at 25°C with aerobic shaking in baffled flasks in the dark. The actinomycetes were harvested by filtration on filter paper (Whatman, No. 4) *in vacuo* and washed with saline (0.85% NaCl aq.). In the case of the reduction using the resting cells, harvested cells were immediately used after washing with the saline. In the case of reduction using cryopreserved cells, after washing with saline, fresh wet cells were frozen and stored at -20°C for 3 weeks before use.

Reduction of α-keto esters and an aromatic α-Keto amide using actinomycetes

Saline-washed fresh wet cells (resting cells) or cryopreserved cells (0.5 g, dry weight approximately 0.15 g) were resuspended in a large test tube (ϕ30 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 using a reciprocating shaker 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-i**) was measured using 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; **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; **2g**, 12.08 min) or a TC-1 capillary column (100 kPa He at 140°C: 1h, 10.02 min; **2h**, 10.96 min; 175°C: **1i**, 6.85 min; **2i**, 8.34 min). The enantiomeric excess (e.e.) of the product was measured using a GC instrument equipped with an optically active CP-Chirasil-DEX CB (**2a-e**, **2g-h**) or Gamma DEX 225 capillary column (**2f** and **2i**). The e.e. was calculated using the following formula: e.e. (%) = [(*R*-*S*)/(*R*+*S*)] x 100, where *R* and *S* are the respective peak areas according to GLC analysis. The absolute configurations of the α-hydroxy esters (**2a-h**) and aromatic α-hydroxy amide (**2i**) were

identified by comparing their retention times as determined by GLC analysis 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 media for liquid culture, the biomasses of wet cells obtained by cultivating 13 *Streptosporangium* and 13 *Nonomuraea* strains in several culture media were measured. All *Streptosporangium* strains grew poorly in 231 and 266 media, and even after 10 days of culture, the resulting wet microbial cell weight was 0.9 g or less. However, three strains, *Streptosporangium* sp. NBRC16544, *Streptosporangium* sp. NBRC101289, and *Streptosporangium* sp. NBRC101798, yielded more than 1.5 g of wet cells/100 mL in culture with both 227 and 228 media (Table 1), even though the recommended medium for NBRC101289 and NBRC101798 is the 266 medium. These results suggest that the type of carbon source is important and that glucose is more suitable than starch for the culture of these *Streptosporangium* strains. Growth of the *Nonomuraea* strains was not as high as that of the *Streptosporangium* strains. In particular, no *Nonomuraea* strains tested grew in 268 medium (Table 2).

However, three strains, *N. spiralis* NBRC14097, *N. helvata* NBRC14681, and *Nonomuraea* sp. NBRC16543 produced up to 1.0 g wet cells/100 mL culture in 227 and 266 media. Therefore, the investigation was done for the potential of three *Streptosporangium* (NBRC16544, NBRC101289, and NBRC101798) and three *Nonomuraea* strains (NBRC14097, NBRC14681, and NBRC16453) strains to act as biocatalysts for the asymmetric reduction of carbonyl compounds.

Reduction of carbonyl compounds by *Streptosporangium* fresh wet cells

Three *Streptosporangium* strains (NBRC 16544, 101289, and 101798) cultivated in 227 and 228 media were tested for their ability to reduce α-keto esters (**1a-h**) and an aromatic α-keto amide (**1i**) (Figure 1). The results of the microbial reductions are summarized in table 3. It was found that the three *Streptosporangium* strains tested in this study reduced nine substrates (**1a-i**) to their corresponding alcohols (**2a-i**).

In the reduction of α-keto esters by the *Streptosporangium* sp. NBRC16544, the conversion ratios were higher when using the wet cells cultured in 228 medium than with those cultured in 227 medium. Moreover, a dramatic change in the enantioselectivity of ethyl pyruvate (**2a**) (from >99% e.e. *S* to >99% e.e. *R*) and a large improvement in the selectivity of ethyl 2-hydroxypentanoate (**2c**) (from 50% to 90% e.e.) were observed. In contrast, in the reduction by NBRC101289 wet cells cultured in 227 medium exhibited a higher conversion ratio than that by cells cultured in 228 medium; in particular, cells cultured in 228 medium exhibited high reducing activity for aliphatic α-keto esters with a short alkyl chain (**1a-d**). In the reduction of the substrates by NBRC101798, the wet cells cultured in both 227 and 228 media showed high reducing activity. Notably, the aliphatic α-keto esters with a short alkyl chain (**2a-c**) were reduced to their corresponding alcohols by the wet cells cultured in 228 medium with high stereoselectivity. The reduction of 2-chlorobenzoylformamide (**1i**), an aromatic α-keto amide, exhibited high stereoselectivity with any of the wet cells tested. In particular, it was found that the NBRC101798 wet cells cultured in 228 medium reduced **1i** to **2i** with a high conversion ratio and excellent stereoselectivity (>99% e.e.).

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

Scientific name	NBRC number	Recomm. medium[1]	Wet cell weights (g)[2]			
			227 medium[3]	228 medium[3]	231 medium[3]	266 medium[3]
Streptosporangium roseum	3776	231	0.2	1.4	0.7	<0.1
Streptosporangium longisporum	13141	227	0.2	0.2	<0.1	0.2
Streptosporangium rubrum	13975	231	<0.1	<0.1	<0.1	<0.1
Streptosporangium vulgare	13985	228	0.6	0.1	0.5	0.3
Streptosporangium amethystogenes subsp. *amethystogenes*	13986	227	0.3	<0.1	0.6	0.4
Streptosporangium fragile	14311	227	0.2	<0.1	0.3	0.2
Streptosporangium vioaceochromogenes	15560	266	0.2	1.4	0.1	0.2
Streptosporangium carneum	15562	268	0.5	1.2	0.2	0.7
Streptosporangium sp.	16544	227	2.3	1.6	0.3	0.9
Streptosporangium sp.	16545	268	0.7	0.1	0.4	0.2
Streptosporangium sp.	101289	266	8.4	1.7	0.9	0.1
Streptosporangium sp.	101793	231	0.3	0.9	0.2	0.1
Streptosporangium sp.	101798	266	6.4	1.8	0.6	0.3

[1]The culture medium number NBRC (NITE Biological Resource Center) culture collection recommends.
[2]The actinomycete were grown in the medium (100 mL) at 25°C for 10 days with aerobic reciprocating shaking (100 min^{-1}) in baffled 500-mL flask in the dark condition.
[3]Composition of each culture medium was described in materials and method section.

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

Scientific name	NBRC number	Recomm. medium[1]	Wet cell weights (g)[2]		
			227 medium[3]	266 medium[3]	268 medium[3]
Nonomuraea angiospora	13155	266	0.8	0.2	<0.1
Nonomuraea spiralis	14097	227	1.5	1.2	<0.1
Nonomuraea helvata	14681	227	1.4	1.3	<0.1
Nonomuraea pusilla	14684	227	0.7	0.6	<0.1
Nonomuraea africana	14757	227	0.3	0.4	<0.1
Nonomuraea sp.	14989	227	0.4	0.3	<0.1
Nonomuraea longicatena	16462	266	0.4	0.3	<0.1
Nonomuraea sp.	16541	268	0.8	0.6	<0.1
Nonomuraea sp.	16543	266	1.4	1.2	<0.1
Nonomuraea sp.	101290	266	0.8	0.4	<0.1
Nonomuraea jiangxiensis	106679	227	0.2	0.1	<0.1
Nonomuraea maritima	106687	227	0.2	0.2	<0.1
Nonomuraea candida	107354	227	0.8	0.5	<0.1

[1]The culture medium number NBRC (NITE Biological Resource Center) culture collection recommends.
[2]The actinomycete were grown in the medium (100 mL) at 25°C for 10 days with aerobic reciprocating shaking (100 min^{-1}) in baffled 500-mL flask in the dark condition.
[3]Composition of each culture medium was described in materials and method section.

Table.3 The reduction of carbonyl compounds (**1a-i**) to the corresponding alcohols (**2a-i**) by three *Streptosporangium* strains [1,2,3]

Products	*Streptosporangium* sp. NBRC16544						*Streptosporangium* sp. NBRC101289						*Streptosporangium* sp. NBRC101798					
	227 medium			228 medium			227 medium			228 medium			227 medium			228 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*
2a	>99	>99	*S*	>99	>99	*R*	>99	68	*R*	>99	59	*R*	>99	89	*S*	>99	>99	*R*
2b	56	94	*R*	>99	69	*R*	>99	56	*R*	92	68	*S*	>99	75	*S*	>99	>99	*R*
2c	>99	50	*S*	>99	>99	*S*	>99	80	*S*	89	92	*S*	>99	75	*S*	>99	>99	*S*
2d	>99	42	*S*	>99	40	*S*	>99	49	*S*	67	60	*S*	>99	79	*S*	>99	85	*S*
2e	38	39	*S*	62	51	*R*	45	41	*R*	82	39	*S*	49	72	*S*	>99	81	*S*
2f	76	35	*S*	>99	41	*S*	>99	58	*S*	>99	45	*S*	76	61	*S*	>99	88	*S*
2g	>99	14	*R*	>99	48	*R*	56	74	*S*	25	49	*R*	69	26	*R*	79	80	*R*
2h	41	51	*R*	79	29	*S*	81	29	*R*	31	55	*S*	48	37	*S*	76	57	*R*
2i	30	>99	*R*	60	>99	*R*	79	34	*R*	98	18	*S*	72	>99	*R*	97	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the wet cells (0.5 g) cultured in 227 or 228 media, and the reaction mixture was incubated aerobically (reciprocating shaking at120 min^{-1}) at 25 ℃ for 48 hrs. [2]Conversion was measured by a GLC analysis.
[3]Enantiomeric excess (e.e.) and absolute configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

Table.4 The reduction of carbonyl compounds (**1a-i**) to the corresponding alcohols (**2a-i**) by three *Nonomuraea* strains[1,2,3]

Products	*Nonomuraea spiralis* NBRC14097						*Nonomuraea helvata* NBRC14681						*Nonomuraea* sp. NBRC16543					
	227medium			266medium			227 medium			266medium			227 medium			266medium		
	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*	56	60	*R*	>99	92	*S*	>99	99	*S*	>99	90	*S*	>99	>99	*S*
2b	39	56	*S*	78	42	*S*	96	88	*S*	91	91	*S*	70	80	*S*	73	61	*S*
2c	19	94	*R*	74	84	*R*	79	64	*S*	90	>99	*R*	53	31	*R*	68	90	*R*
2d	33	>99	*R*	70	>99	*R*	80	44	*R*	93	80	*R*	65	63	*R*	54	94	*R*
2e	18	58	*R*	17	74	*R*	56	90	*S*	86	>99	*R*	21	44	*S*	33	46	*R*
2f	50	37	*R*	23	78	*R*	52	41	*R*	80	87	*R*	48	57	*R*	50	39	*R*
2g	49	44	*S*	80	66	*S*	79	74	*S*	92	81	*S*	<1	40	*S*	69	57	*S*
2h	38	67	*R*	21	81	*R*	18	33	*R*	81	88	*R*	11	64	*R*	44	71	*R*
2i	6	>99	*R*	30	>99	*R*	34	>99	*R*	95	>99	*R*	3	>99	*R*	11	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the wet cells (0.5 g) cultured in 227 or 266 media, and the reaction mixture was incubated aerobically (reciprocating shaking at120 min^{-1}) at 25 ℃ for 48 hrs.

Table.5 The reduction of carbonyl compounds (**1a-i**) to the corresponding alcohols (**2a-i**) with three *Streptosporangium* cryopreserved cells[1,2,3]

Products	*Streptosporangium* sp. NBRC16544 228 medium			*Streptosporangium* sp. NBRC101289 227 medium			*Streptosporangium* sp. NBRC101798 228 medium		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	>99	40	*R*	>99	68	*R*	>99	70	*R*
2b	38	52	*R*	19	56	*R*	>99	>99	*R*
2c	>99	>99	*S*	>99	80	*R*	71	77	*S*
2d	13	40	*S*	48	49	*S*	72	78	*S*
2e	12	51	*S*	23	41	*S*	24	64	*S*
2f	45	39	*S*	>99	65	*S*	41	56	*S*
2g	80	39	*R*	36	29	*S*	43	60	*S*
2h	19	63	*R*	26	53	*S*	35	84	*R*
2i	60	>99	*R*	50	45	*R*	48	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the cyropreserved cells (0.5 g) cultured in 227 or 228 media, and the reaction mixture was incubated aerobically (reciprocating shaking at120 min^{-1}) at 25 °C for 48 hrs. [2]Conversion was measured by a GLC analysis.
[3]Enantiomeric excess (e.e.) and absolute configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

Table.6 The reduction of carbonyl compounds (**1a-i**) to the corresponding alcohols (**2a-i**) with three *Nonomuraea* cryopreserved cells[1,2,3]

Products	*Nonomuraea spiralis* NBRC14097 266 medium			*Nonomuraea helvata* NBRC14681266 medium			*Nonomuraea* sp. NBRC16453266 medium		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	33	40	*S*	>99	>99	*S*	90	98	*S*
2b	48	52	*S*	55	86	*S*	80	73	*S*
2c	29	>99	*R*	60	>99	*R*	71	88	*R*
2d	31	40	*R*	41	88	*S*	72	79	*R*
2e	10	51	*R*	53	>99	*S*	24	55	*R*
2f	14	39	*R*	39	90	*S*	41	92	*S*
2g	39	39	*R*	41	77	*S*	43	55	*R*
2h	12	63	*R*	37	73	*R*	35	74	*R*
2i	25	>99	*R*	70	>99	*R*	9	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the cryopreserved cells (0.5 g) cultured in 266 medium, and the reaction mixture was incubated aerobically (reciprocating shaking at120 min^{-1}) at 25 °C for 48 hrs. [2]Conversion was measured by a GLC analysis. [3]Enantiomeric excess (e.e.) and absolute configuration (*R*/*S*) were determined by GLC analyses with optically active capillary columns.

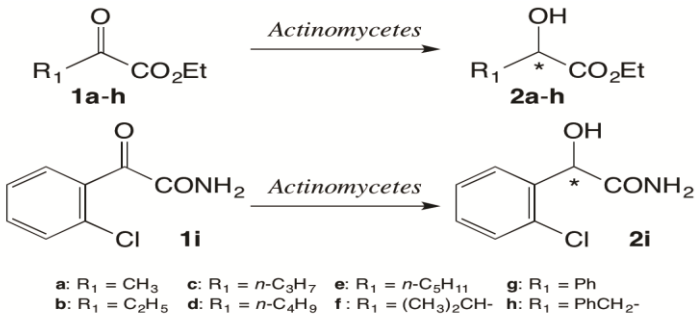

Figure 1. The reduction of various carbonyl compounds (**1a-i**) to the corresponding alcohols (**2a-i**) by actinomycetes.

These results indicate that the NBRC101798 strain cultured in 228 medium is a useful biocatalyst for the asymmetric reduction of carbonyl compounds such as α-keto esters and an aromatic α-keto amide.

Reduction of carbonyl compounds by *Nonomuraea* fresh wet cells

Three *Nonomuraea* strains cultivated in two media were tested for their ability to reduce α-keto esters and the aromatic α-keto amide as shown in table 4. The reduction of α-keto esters by NBRC14097 or NBRC16543 showed moderate conversion ratios except for in the reduction of ethyl pyruvate. In particular, eight α-keto esters were reduced by the wet cells of *N. helvata* NBRC14681 cultured in 266 medium with a high conversion ratio (>80%) and a high stereoselectivity (>80% e.e.). The aromatic α-keto amide **1i** was reduced enantiospecifically to the corresponding α-hydroxy amide **2i** by all of the strains tested, but the conversion ratios were generally low (3-34%). Only the NBRC14681 wet cells cultured in the 266 medium exhibited a high conversion ratio (95%). These results indicate that NBRC14681 cultured in 266 medium is also a useful biocatalyst for the asymmetric reduction of carbonyl compounds. Further improvements in the conversion ratio and stereoselectivity of the products by the introduction of additives to the reaction system should be explored.

Reduction of carbonyl compounds by cryopreserved cells of *Streptosporangium* and *Nonomuraea*

If microorganisms can be frozen and preserved, even microbes with poor growth can be used by accumulating wet cells, thereby expanding the utility of biocatalysts. Therefore, abilities of the cryopreserved cells of the three *Streptosporangium* strains and three *Nonomuraea* strains to reduce carbonyl compounds was investigated. When the cryopreserved cells of the three *Streptosporangium* strains were tested, a decrease in the conversion ratio was observed as compared with that of the reduction by fresh wet cells (Table 5). Among the strains, NBRC101798 cultured in 228 medium was considered useful as a biocatalyst because its conversion ratio was relatively high. As shown in table 6, similar to the cryopreserved cells of the *Streptosporangium* strains, reductions by the cryopreserved cells of the three *Nonomuraea* strains tended to exhibit lower conversion ratios than those by the corresponding wet cells. Only *N. helvata* NBRC14681 cultured in 266 medium was able to reduce the substrates with moderate conversion ratios. As described above, it was therefore determined that the *Streptosporangium* and *Nonomuraea* strains can be used as biocatalysts even when cryopreserved. In our previous research, microorganisms with poor growth in liquid medium have been excluded from screenings for useful microorganisms. However, as the

utility of cryopreserved cells has been demonstrated in this study, future reports should include the screening of such microbes.

In conclusion, members of two genera, *Streptosporangium* and *Nonomuraea*, from the Streptosporangiaceae family were shown to convert various α-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 *Streptosporangium* sp. NBRC101798 cultured in 228 medium and *N. helvata* NBRC14681 cultured in 266 medium for potential use as biocatalysts for the stereoselective reduction of α-keto esters and the α-keto amide to yield the corresponding chiral alcohols. In addition, our results suggest that cryopreserved cells could be used as biocatalysts for the asymmetric reduction ofcarbonyl compounds.

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