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Biocatalytic Reduction of Carbonyl Compounds by Actinobacteria from Two Genera of the Micromonosporaceae Family: *Actinoplanes* and *Dactylosporangium*

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A B S T R A C T

We screened 10 *Actinoplanes* and 14 *Dactylosporangium* strains to investigate the biocatalytic ability of two genera of the Micromonosporaceae family. Two *Actinoplanes* strains (*A. ferrugineus* NBRC15555 and *A. missouriensis* NBRC102363) exhibited good growth when cultured in 228 and 231 media, as did two *Dactylosporangium* strains (*Dactylosporangium* sp. NBRC101297 and *Dactylosporangium* sp. NBRC101730) when cultured in 227 and 266 media. The stereoselective reduction of various carbonyl compounds using these four strains was therefore investigated. The present study discovered that these strains can reduce aliphatic and aromatic α-keto esters and an aromatic α-keto amide. On the basis of the conversion ratio and stereoselectivity of the alcohols produced, *A. ferrugineus* NBRC15555 is a potential biocatalyst for the stereoselective reduction of α-keto esters and an aromatic α-keto amide to the corresponding chiral alcohols when cultured in the 227 medium. Our results also suggest that the reduction of ethyl 2-methylacetoacetate by *Dactylosporangium* sp. NBRC101730 cultured in 227 medium in the presence of D-glucose is useful for the production of chiral ethyl 3-hydroxy-2-methylbutanoate.

Introduction

Actinobacteria are among the most morphologically diverse prokaryotes, and are widely distributed in both terrestrial and aquatic ecosystems (Servin *et al.*, 2007; Embley and Stackebrandt, 1994). Micromonosporaceae, a family of bacteria of the class Actinobacteria, have been isolated from diverse habitats including soil, sediments, fresh and marine water, the rhizosphere, and plant tissues. Several species belonging to Micromonosporaceae produce useful enzymes (Peczyńska-Czoch and Mordarski, 1988) and degradea variety of polysaccharides (Yeager *et al.*, 2017) to

produce useful secondary metabolites (Al-Garni *et al.*, 2014; Solecka *et al.*, 2012; Bérdy, 2005; Shomura *et al.*, 1983). These bacteria therefore have important applications in industry, biotechnology, and agriculture (de Menezes *et al.*, 2008; Rose and Steinbüchel, 2005; Linos *et al.*, 2000). In addition, some strains of the genera *Micromonospora* and *Salinispora* in this family are useful biocatalysts for the asymmetric reduction of various carbonyl compounds such as α- and β-keto esters and aromatic α-keto amides (Ishihara *et al.*, 2013, 2011). Thus, although these genera have thus been extensively studied for their biocatalytic activities, the potential ability of other genera in this family to serve as biocatalysts has not been investigated.

In this study, we investigated the stereoselective reduction of carbonyl compounds by members of two genera, *Actinoplanes* (Stackebrandt and Kroppenstedt, 1987; Couch, 1950) and *Dactylosporangium* (Thiemann *et al.*, 1967), of the Micromonosporaceae family in order to identify potential novel biocatalysts (Figure 1).

Materials and Methods

Instruments and chemicals

Gas chromatography (GC) was performed using a GL Science GC-353 gas chromatograph (GL Science Inc., Japan) equipped with capillary columns (DB-WAX, 0.25 µm, 0.25 mm x 30 m, Agilent Technologies, 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., USA; Gamma DEX 225, 0.25 µm, 0.25 mm x 30 m, Sigma-Aldrich Inc., USA). Ethyl pyruvate (Figure 1, 1a), diatomaceous earth (granular), and NZ amine, type A were purchased from Wako Pure Chemical Industries Ltd. (Japan). Bacto™ malt extract, Bacto™ yeast extract, and Difco™ soluble starch were purchased from Becton Dickinson and Co. (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 Inc. Ethyl benzoylformate (1g), ethyl 2-methylacetoacetate (1j), and ethyl mandelate (2g) were obtained from Tokyo Chemical Industry, Co., Ltd. (Japan). Ethyl 2-oxobutanoate (1b), ethyl 2-oxopentanoate (1c), ethyl 2-oxohexanoate (1d), ethyl 2-oxoheptanoate (1e), 2-chlorobenzoylformamide (1i), 2-chloromandelamide (2i), β-hydroxy esters (2b-f), and ethyl 3-hydroxy-2-methylbutanoate (2j) were prepared as described previously (Mitsuhashi and Yamamoto, 2005; Kawai *et al.*, 1995; Nakamura *et al.*, 1988). All the other chemicals used in this study were of analytical grade and commercially available.

Microorganisms and Culture

Actinoplanes italicus NBRC13911,
Actinoplanes brasiliensis NBRC13938,
Actinoplanes garbadinensis NBRC13995,
Actinoplanes nipponensis NBRC14063,
Actinoplanes violaceus NBRC14458,
Actinoplanes ferrugineus NBRC15555,
Actinoplanes capillaceus NBRC16408,
Actinoplanes missouriensis NBRC102363,
Actinoplanes rishiriensis NBRC108556,
Actinoplanes siamensis NBRC109076,

Dactylosporangium salmoneum NBRC14103,
Dactylosporangium vinaceum NBRC14181,
Dactylosporangium matsuzakiense NBRC14259,
Dactylosporangium rosum NBRC14352,
Dactylosporangium fulvum NBRC14381,
Dactylosporangium sp. NBRC101297,
Dactylosporangum sp. NBRC101672,

Dactylosporangium sp. NBRC101673, *Dactylosporangium* sp. NBRC101730, *Dactylosporangium siamense* NBRC106093, *Dactylosporangium maewongense* NBRC106094, *Dactylosporangium darangshiense* NBRC109065, *Dactylosporangium tropicum* NBRC109071, and *Dactylosporangium luridum* NBRC109093

The above strains 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, and 266) solidified with 1.5% (w/v) agar. The 227 medium (International *Streptomyces* Project, ISP medium No. 2) contained 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 contained1.0 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 contained 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 contained 2.0 g of Bacto™ yeast extract, and 10.0 g of Difco™ soluble starch per liter of distilled water (pH 7.3). The 10*Actinoplanes* strains were grown in 227, 228, 231, and 266 media for 4 days at 25°C with aerobic shaking in baffled flasks in the dark, and the 14 *Dactylosporangium* strains were grown in 227 and 266 media for 8 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.). The harvested cells were immediately used for reduction after washing with the saline.

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

Saline-washed wet Actinomycete cells (0.5 g, dry weight approximately 0.15 g) were resuspended in a arge test tube (ϕ 30 mm x 200 mm) containing 20 mL of saline. The substrate (0.15 mmol; 7.5 mM) was then added, and the reaction mixture was incubated aerobically (with reciprocated shaking at 120 rpm) at 25°C. A portion (0.5 mL) of the mixture was applied to 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 production of alcohols (Figure 1, 2a-j)was measured using a GC 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;1j, 5.54 min; 2j-*anti*, 7.62 min;2j-*syn*, 8.13 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; 170°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, and 2j) or a 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 of the isomer in GC analyses. The absolute configurations of the α- and β-hydroxy esters (2a-h and 2j), and the α-hydroxy amide (2i) were identified by comparing their retention times as determined by the GC analyses with those of authentic samples (Mitsuhashi and Yamamoto, 2005; Kawai *et al.*, 1995; Nakamura *et al.*, 1988).

Results and Discussion

Screening of actinomycete strains and culture media

To determine the suitable media for liquid culture, 10 *Actinoplanes* and 14 *Dactylosporangium* strains were cultivated in several culture media, after which the wet weight of the cells was measured. All *Actinoplanes* strains grew poorly in the 266 medium, even after 8 days of culture, and the resulting wet cell weights were 0.1 g or less (see Table 1).

However, two strains, *Actinoplanes ferrugineus* NBRC15555 and *Actinoplanes missouriensis* NBRC102363, yielded more than 0.5 g of wet cells/100 mL of culture in both 228 and 231 media, even though the recommended medium for NBRC15555 strain is 266 medium (Table 2).

These results suggest that the amount of available carbon is more important than the type of carbon in liquid cultures of *Actinoplanes* strains.

Dactylosporangium strains exhibited good growth in liquid culture. The amount of wet cells obtained when culturing in 227 medium, which contains glucose as a carbon source, was larger than when using 266 medium, which contains soluble starch. Two strains in particular, *Dactylosporangium* sp. NBRC101297 and NBRC101730, produced up to 1.0 g wet cells/100 mL when cultured in both 227 and 266 media.

We therefore investigated the potential ability of two *Actinoplanes* strains (NBRC15555 and NBRC102363) and two *Dactylosporangium* strains (NBRC101297 and NBRC101730) to act as biocatalysts for the asymmetric reduction of carbonyl compounds.

Reduction of carbonyl compounds by *Actinoplanes* wet cells

Two actinomycete strains (*A. ferrugineus* NBRC15555 and *A. missouriensis* NBRC102363) that were cultivated in three media (227, 228, and 231) were tested for their ability to reduce several carbonyl compounds (Figure 1). The results of the microbial reductions are summarized in Table 3. Both strains could reduce aliphatic and aromatic α-keto esters (1a-h) and an aromatic α-keto amide (1i). However, there were differences in the reduction rate and stereo selectivity of the alcohols produced that were dependent on the culture medium. The reduction rate of substrates by NBRC15555 wet cells tended to be slightly higher when compared with NBRC1023063 wet cells. More specifically, the reduction by NBRC15555 wet cells in 227 medium produced reduction ratios of 70% or more for all nine substrates tested. We therefore tried to improve the conversion ratio and the stereo selectivity of the alcohols produced by the NBRC15555 strain by introducing additives into the reduction reaction catalyzed by NBRC15555 wet cells cultured in the 227 medium (see Table 4). Three additives were tested (glucose, sodium citrate, and soy oil), and considerable improvement was observed, especially with the addition of sodium citrate, with conversion ratios to >99% for all substrates. Furthermore, four substrates (1a, 1c-e) were stereo specifically reduced to an e.e. >99%, and the other substrates were converted to an e.e. of nearly 90%.The reduction of 2-chlorobenzoylformamide (1i), an aromatic α-keto amide, demonstrated high stereo selectivity with all the wet cells tested (Figure 1). In particular, both the NBRC15555 and NBRC102363 wet cells cultured in228 medium reduced 1i to (*R*)-2i with a high conversion ratio and excellent stereo selectivity (>99% e.e.). As shown in Table 5, the reduction of ethyl 2-

methylacetoacetate (1j), one of the *β*-keto esters, by the wet cells of NBRC15555 cultured in 231 medium resulted in a conversion ratio >99%; however, the stereo selectivity (*syn*/*anti*ratio and e.e.) was low and was not improved by the introduction of additives.

These results indicate that the NBRC15555 strain cultured in 227 medium is a useful biocatalyst for the asymmetric reduction of carbonyl compounds such as α-keto esters and aromatic α-keto amides.

Table.1 The cultivation of *Actinoplanes* strains in several culture medium

Scientific name	NBRC number	Recomm. medium[1]	Wet cell weight (g)[2]			
			227 medium[3]	228 medium[3]	231 medium[3]	266 medium[3]
Actinoplanes italicus	13991	227	0.1	0.3	0.1	<0.1
Actinoplanes brasiliensis	13938	227	0.2	0.1	0.4	<0.1
Actinoplanes garbadiensis	13995	227	0.3	0.1	0.3	<0.1
Actinoplanes nipponensis	14063	231	0.4	0.1	0.2	<0.1
Actinoplanes violaceus	14458	227	0.1	<0.1	<0.1	0.1
Actinoplanes ferrugineus	15555	266	0.3	0.5	0.6	<0.1
Actinoplanes capillaceus	16408	227	0.3	0.7	0.1	<0.1
Actinoplanes missouriensis	102363	231	0.3	0.6	0.9	<0.1
Actinoplanes rishiriensis	108556	228	0.2	0.1	0.1	<0.1
Actinoplanes siamensis	109076	227	0.1	0.6	0.2	<0.1

[1]The culture medium number NBRC (NITE Biological Resource Center) culture collection recommends
[2]The actinomycete were grown in the liquid medium (100 mL) at 25°C for 4 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 *Dactylosporangium* strains in several culture medium

Scientific name	NBRC number	Recomm. medium[1]	Wet cell weight (g)[2]	
			227 medium[3]	266 medium[3]
Dactylosporangium salmoneum	14103	227	0.7	0.4
Dactylosporangium vinaceus	14181	227	<0.1	0.2
Dactylosporangium matsuzakiense	14259	227	0.2	0.1
Dactylosporangium roseum	14352	227	1.1	0.4
Dactylosporangium fulvum	14381	227	0.4	<0.1
***Dactylosporangium* sp.**	101297	266	1.6	1.6
***Dactylosporangium* sp.**	101672	227	1.3	0.2
***Dactylosporangium* sp.**	101673	227	0.4	<0.1
***Dactylosporangium* sp.**	101730	266	1.0	1.0
Dactylosporangium siamense	106093	227	0.4	0.3
Dactylosporangium maewongense	106094	227	1.4	0.3
Dactylosporangium darangshiense	109065	227	0.3	0.1
Dactylosporangium tropicum	109071	227	0.3	0.3
Dactylosporangium luridum	109093	227	0.4	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 8 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 two *Actinoplanes* strains.[1,2,3]

Product	***Actinoplanes ferrugineus* NBRC15555**									***Actinoplanes missouriensis* NBRC102363**								
	227 medium			228 medium			231 medium			227 medium			228 medium			231 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*	92	73	*S*	90	72	*S*	37	52	*R*	>99	90	*S*	93	82	*S*
2b	82	66	*S*	80	46	*S*	83	39	*R*	20	89	*R*	67	9	*R*	32	63	*R*
2c	>99	>99	*S*	99	94	*S*	65	21	*R*	27	70	*R*	29	69	*R*	46	41	*R*
2d	89	54	*S*	82	99	*S*	51	13	*R*	18	79	*R*	70	11	*R*	41	31	*R*
2e	75	51	*R*	78	99	*S*	45	60	*R*	11	95	*R*	5	18	*R*	28	83	*R*
2f	72	30	*S*	49	19	*S*	75	83	*R*	16	73	*S*	39	27	*S*	74	33	*R*
2g	71	29	*S*	76	65	*R*	78	79	*S*	9	>99	*S*	39	27	*S*	14	>99	*R*
2h	70	67	*R*	28	65	*S*	28	92	*S*	4	>99	*S*	28	97	*S*	46	90	*S*
2i	92	>99	*R*	>99	>99	*R*	84	>99	*R*	85	>99	*R*	>99	>99	*R*	29	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the wet cells (0.5 g) cultured in liquid 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

Table.4 The reduction of 1 by *A. ferrugineus* NBRC15555 cultivated in 227 medium in the presence of additive.[1,2,3]

Product	Additive								
	D-Glucose			Sodium citrate			Soy oil		
	Con. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*	Conv, (%)	e.e. (%)	*R/S*
2a	>99	42	*S*	>99	>99	*S*	>99	43	*S*
2b	59	34	*R*	>99	92	*R*	>99	60	*S*
2c	62	19	*R*	>99	>99	*R*	>99	97	*S*
2d	74	13	*R*	>99	>99	*S*	95	40	*S*
2e	30	65	*R*	>99	>99	*S*	90	18	*S*
2f	>99	19	*S*	>99	99	*S*	>99	35	*S*
2g	42	>99	*R*	>99	90	*S*	49	43	*S*
2h	45	15	*R*	>99	88	*S*	90	43	*S*
2i	99	>99	*R*	>99	>99	*R*	98	>99	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) and additive (D-glucose and sodium citrate: 5 mmol, soy oil: 0.5 mL) were added to the wet cells (0.5 g) cultured in 227 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.

Table.5 The reduction of ethyl 2-methylacetoacetate (1j) to the corresponding alcohol (2j) by *Actinoplanes* strains.[1,2,3]

Strain	medium	Additive	Conv. (%)	*Syn* / *Anti*	e.e. (%)	
					Syn-(2*R*, 3*S*)	*Anti*-(2*S*, 3*S*)
A. ferrugineus	227	None	4	20 / 80	33	92
	228	None	16	44 / 56	38	45
	231	None	>99	59 / 41	34	17
A. missoriensis	227	None	7	38 / 62	30	78
	228	None	95	41 / 59	20	31
	231	None	6	35 / 65	42	84
A. ferrugineus	227	D-Glucose	10	33 / 67	40	80
	227	Sodium citrate	5	22 / 78	31	88
	227	Soy oil	12	39 / 61	44	71

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) and additive (D-glucose and sodium citrate: 5 mmol, soy oil: 0.5 mL) were added to the wet cells (0.5 g) cultured in liquid 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.

Table.6 The reduction of ethyl 2-methylacetate (1j) to the corresponding alcohol (2j) by two *Dactylosporangium* strains.[1,2,3]

Product	***Dactylosporangium* sp. NBRC101297**						***Dactylosporangium* sp. NBRC101730**					
	227 medium			266 medium			227 medium			266 medium		
	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*
2a	40	87	*S*	51	79	*S*	94	47	*S*	90	22	*S*
2b	29	53	*S*	95	76	*S*	48	18	*S*	43	24	*S*
2c	23	42	*S*	74	37	*S*	46	14	*S*	35	33	*S*
2d	14	23	*S*	8	>99	*S*	36	40	*S*	29	19	*S*
2e	5	14	*S*	3	1	*S*	14	36	*S*	7	>99	*S*
2f	32	66	*S*	60	14	*S*	69	>99	*R*	75	22	*R*
2g	11	12	*R*	10	25	*R*	26	64	*S*	19	47	*S*
2h	80	9	*S*	4	>99	*S*	16	39	*S*	7	28	*S*
2i	25	25	*R*	94	85	*R*	80	38	*R*	31	53	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) were added to the wet cells (0.5 g) cultured in liquid 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.

Table.7 The reduction of carbonyl compounds (1a-i) to the corresponding alcohols (2a-i) by *Dactylosporangium* sp. NBRC101297 strain in the presence of additive.[1,2,3]

Product	**227 medium**						**266 medium**					
	D-Glucose			L-Glutamate			D-Glucose			L-Glutamate		
	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*	Conv. (%)	e.e. (%)	*R/S*
2a	48	83	*S*	76	43	*S*	15	72	*S*	>99	>99	*S*
2b	53	73	*S*	68	78	*S*	29	64	*S*	82	69	*S*
2c	42	81	*S*	68	81	*S*	33	74	*S*	92	68	*S*
2d	23	>99	*S*	32	72	*S*	11	63	*S*	64	68	*S*
2e	14	>99	*S*	60	>99	*S*	25	>99	*S*	5	54	*S*
2f	66	2	*S*	80	28	*R*	52	21	*S*	90	30	*R*
2g	12	46	*R*	22	46	*R*	18	45	*S*	34	54	*R*
2h	9	48	*S*	35	58	*S*	14	46	*S*	34	45	*S*
2i	48	83	*R*	38	60	*R*	49	88	*R*	41	80	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) and additive (glucose and sodium hydrogen glutamate: 5 mmol) were added to the wet cells (0.5 g) cultured in liquid 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.

Table.8 The reduction of carbonyl compounds (1a-i) to the corresponding alcohols (2a-i) by *Dactylosporangium* sp. NBRC101730 strain in the presence of additive.[1,2,3]

Product	227 medium						266 medium					
	D-Glucose			L-Glutamate			D-Glucose			L-Glutamate		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	71	55	*S*	>99	49	*S*	71	36	*S*	79	54	*S*
2b	25	>99	*S*	62	58	*S*	59	45	*S*	25	55	*S*
2c	57	40	*S*	32	36	*S*	64	27	*S*	57	22	*S*
2d	45	55	*S*	39	39	*S*	58	22	*S*	39	33	*S*
2e	18	13	*S*	1	46	*S*	25	>99	*S*	36	>99	*S*
2f	71	20	*S*	>99	4	*S*	>99	18	*R*	71	75	*R*
2g	11	67	*R*	1	77	*S*	28	67	*R*	32	81	*R*
2h	5	10	*R*	8	44	*S*	31	42	*S*	13	48	*S*
2i	>99	91	*R*	95	27	*R*	47	90	*R*	41	80	*R*

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) and additive (glucose and sodium hydrogen glutamate: 5 mmol) were added to the wet cells (0.5 g) cultured in liquid 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.

Table.9 The reduction of ethyl 2-methylacetoacetate (1j) to the corresponding alcohol (2j) by two *Dactylosporangium* strains in the presence of additive.[1,2,3]

Strain	Medium	Additive	Conv. (%)	*Syn* / *Anti*	e.e. (%)	
					Syn-(2*R*, 3*S*)	*Anti*-(2*S*, 3*S*)
***Dactylosporangium* sp. NBRC101297**	227	None	18	10/90	>99	>99
		Glucose	9	10/90	>99	>99
		Glutamate	29	11 / 89	>99	>99
	266	None	86	8/92	>99	>99
		Glucose	20	11/89	>99	>99
		Glutamate	68	10 / 90	>99	>99
***Dactylosporangium* sp. NBRC101730**	227	None	38	7 / 93	>99	>99
		Glucose	98	<1 / >99	>99	-[4]
		Glutamate	46	<1 / >99	>99	-[4]
	266	None	26	6 / 94	>99	>99
		Glucose	63	<1 / >99	>99	-[4]
		Glutamate	50	<1 / >99	>99	-[4]

[1]Substrate (0.15 mmol), 0.85% NaCl aq. (20 mL) and additive (D-glucose and sodium hydrogen glutamate: 5 mmol, soy oil: 0.5 mL) were added to the wet cells (0.5 g) cultured in liquid 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.
[4]-: E.e. could not be measured because it could not detect the *Anti*-form

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

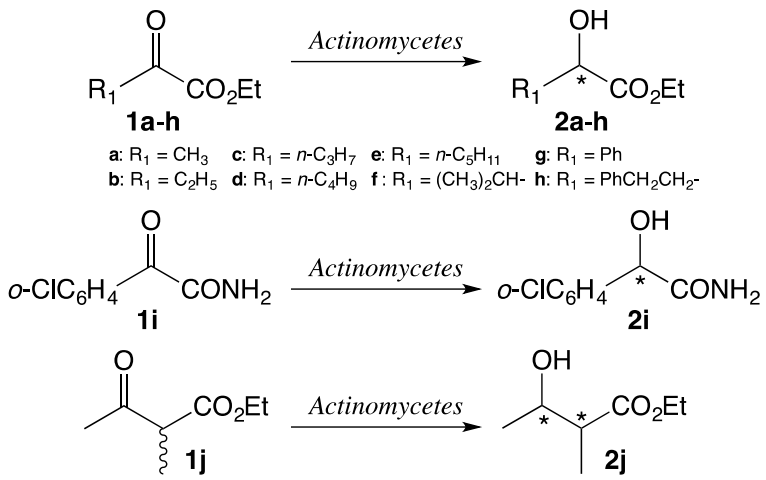
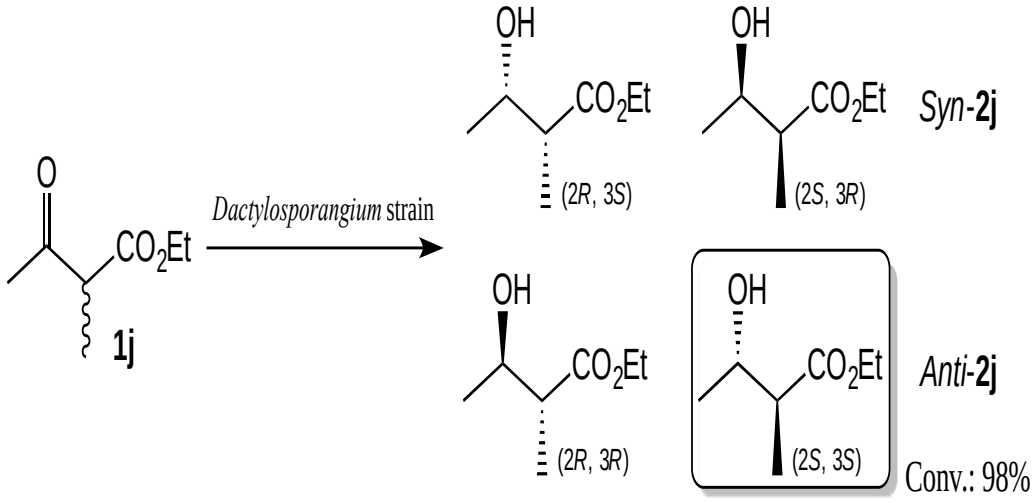


Fig.2 The reduction of 1j to (2*S*, 3*S*)-2j by *Dactylosporangium* sp. NBRC101730 strain in the presence of glucose



Reduction of carbonyl compounds by *Dactylosporangium* wet cells

Two *Dactylosporangium* strains cultivated in two media were tested for their ability to reduce several carbonyl compounds (Table 6). The *Dactylosporangium* strains could reduce aliphatic and aromatic α-keto esters and an aromatic α-keto amide, and no substantial difference was observed in the conversion ratio and stereoselectivity of the alcohols produced. The effects of additives on these microbial reduction reactions were also examined (Tables 7 and 8). The NBRC101297 strain cultured in 266 medium in the presence of sodium hydrogen L-glutamate reduced ethyl pyruvate (1a) to the corresponding (*S*)-alcohol (2a) with a high conversion ratio (>99%) and excellent e.e. (> 99%). However, the conversion rate and the

stereoselectivity of the reduction of other substrates by same strain were not greatly improved with additive (Table 7). Similarly, the reduction efficiency and stereoselectivity by the NBRC101730 strain were not improved with additives (Table 8).

In contrast, the effect of additives on the reduction of *β*-keto ester was remarkable, and ethyl 2-methylacetoacetate (1j) reduction by NBRC101730 cultured in 227 medium in the presence of glucose stereospecifically produced the corresponding alcohol (2*S*, 3*S*)-2j (Table 9). More specifically, in this reaction, the substrate was reduced to only one of the four theoretically possible isomers, in other words, this microbial reduction reaction was able to obtain a β-hydroxy ester having two chiral center carbons (Figure 2).

In conclusion, members of two genera (*Actinoplanes* and *Dactylosporangium*) from the Micromonosporaceae family were shown toconvert various α-keto esters and an aromatic α-keto amide to the corresponding hydroxy esters and hydroxy amide. On the basis of the conversion ratios and the stereoselectivity of the products, we suggest *Actinoplanes ferrugineus* NBRC15555 cultured in the 227 medium for potential use as a biocatalyst for the stereoselective reduction of α-keto esters and the α-keto amides to yield the corresponding chiral alcohols. Our results also suggest that the reduction of ethyl 2-methylacetoacetate by *Dactylosporangium* sp. NBRC101730 cultured in the 227 medium in the presence of D-glucose stereospecifically produces the corresponding chiral β-hydroxy ester.

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