Scientific Research Publishing

Advances in Enzyme Research, 2019, 7, 15-25
http://www.scirp.org/journal/aer
ISSN Online: 2328-4854
ISSN Print: 2328-4846

Microbial Production of Chiral Hydroxy Esters and Their Analogs: Biocatalytic Reduction of Carbonyl Compounds by Actinobacteria, *Agromyces* and *Gordonia* Strains

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How to cite this paper: Ishihara, K., Adach, N., Mishima, T., Kuboki, C., Shuto, A., Okamoto, K., Inoue, M., Hamada, H., Uesugi, D., Masuoka, N. and Nakajima, N. (2019) Microbial Production of Chiral Hydroxy Esters and Their Analogs: Biocatalytic Reduction of Carbonyl Compounds by Actinobacteria, *Agromyces* and *Gordonia* Strains. *Advances in Enzyme Research*, **7**, 15-25.
https://doi.org/10.4236/aer.2019.72002

Received: June 8, 2019
Accepted: June 27, 2019
Published: June 30, 2019

Abstract

We screened 15 *Agromyces* strains from the Microbacteriaceae family and 16 *Gordonia* strains from the Gordoniaceae family to investigate their biocatalytic ability to reduce carbonyl compounds. Two *Agromyces* strains (*A. soli* NBRC109063 and *A. humatus* NBRC109085) and two *Gordonia* strains (*G. hydrophobica* NBRC16057 and *G. malaquae* NBRC108250) grew well in 230 medium. The stereoselective reduction of various carbonyl compounds using these four strains was investigated. We discovered that these strains can reduce aliphatic and aromatic *α*-keto esters and an aromatic *α*-keto amide. On the basis of the conversion rate and stereoselectivity of the alcohols produced, *G. hydrophobica* NBRC16057 is a potential biocatalyst for the stereoselective reduction of *α*-keto esters and an aromatic *α*-keto amide to the corresponding chiral alcohols. Our results also suggest that the reduction of ethyl 2-methylacetoacetate by wet *G. hydrophobica* NBRC16057 cells in the presence of L-glutamate is useful for the production of chiral ethyl 3-hydroxy-2-methylbutanoate.

Keywords

Actinobacteria, Stereoselective Reduction, Carbonyl Reductase, Chiral Alcohol

1. Introduction

Biotransformation has been extensively investigated because it is useful for converting inexpensive and plentiful organic compounds into costly and valuable ones [1] [2] [3]. In particular, microbial reduction of carbonyl compounds is a convenient and environmentally-friendly method for obtaining optically pure alcohols. For example, bakers' yeast and fungi have often been used for the reduction of carbonyl compounds to obtain optically active hydroxy esters [4] [5] [6] [7]. To date, several studies concerning the reduction of keto esters and their analogs with other microorganisms such as actinomycetes have been reported. In particular, Streptomycetaceae (*Streptomyces*, *Kitasatospora*, and *Streptacidiphilus* genera) [8] [9] [10] [11] [12], Streptosporangiaceae (*Nonomuraea* and *Streptosporangium* genera) [13], and Micromonosporaceae (*Salinispora*, *Actinoplanes*, and *Dactylosporangium* genera) [14] [15] families have been studied for the biocatalytic activities of their members. Furthermore, several keto ester reductases have been purified from *S. thermocyaneoviolaceus*, *S. coelicolor* A3(2), and *S. avermitilis* whole cells, and the reaction mechanism of the reduction reaction has been elucidated based on the enzymatic properties of these reductases [16] [17] [18]. However, 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, *Agromyces* from the Microbacteriaceae family and *Gordonia* from the Gordoniaceae family, as novel biocatalysts (**Figure 1**).

2. Materials and Methods

2.1. 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 × 30 m, Agilent Technologies, USA; TC-1, 0.25 μm, 0.25 mm × 30 m, GL Science Inc.; CP-Chirasil-DEX CB, 0.25 μm, 0.25 mm ×

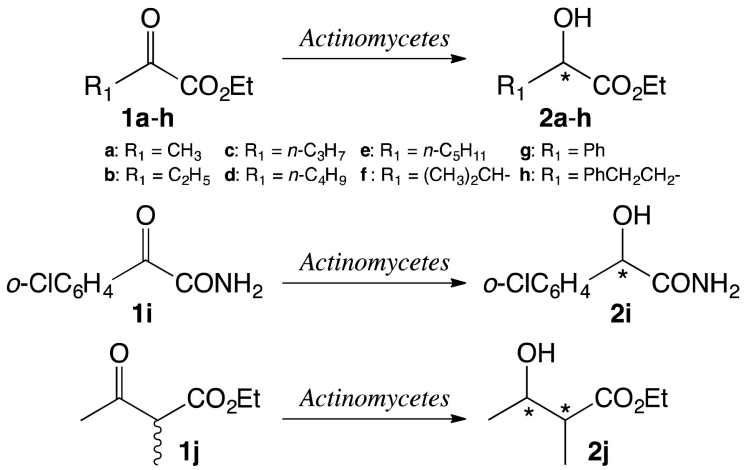

Figure 1. The reduction of carbonyl compounds by actinomycetes.

25 m, Varian Inc., USA; Gamma DEX 225, 0.25 μm, 0.25 mm × 30 m, Sigma-Aldrich Inc., USA). Ethyl pyruvate (Figure 1, **1a**), diatomaceous earth (granular), sodium hydrogen L-glutamate monohydrate, magnesium sulfate heptahydrate, glycerol, and hipolypepton were purchased from Wako Pure Chemical Industries Ltd. (Japan). Bacto™ malt extract, Bacto™ yeast extract, Bacto™ brain heart infusion, and Bacto™ tryptic soy broth 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**), *α*-hydroxy esters (**2b**-**f**), and ethyl 3-hydroxy-2-methylbutanoate (**2j**) were prepared as described previously [4] [7] [19]. All other chemicals used in this study were of analytical grade and commercially available.

2.2. Microorganisms and Culture

Agromyces ramosus NBRC13899, *Agromyces cerinus* subsp. *cerinus* NBRC15780, *Agromyces luteolus* NBRC16235, *Agromyces rhizospherae* NBRC16236, *Agromyces brachium* NBRC16238, *Agromyces albus* NBRC103057, *Agromyces iriomotensis* NBRC106452, *Agromyces subtropicus* NBRC106454, *Agromyces marinus* NBRC109019, *Agromyces soli* NBRC109063, *Agromyces flavus* NBRC109064, *Agromyces tropicus* NBRC109073, *Agromyces ulmi* NBRC109083, *Agromyces neolithicus* NBRC109084, *Agromyces humatus* NBRC109085, *Gordonia amarae* NBRC15530, *Gordonia hydrophobica* NBRC16057, *Gordonia rhizosphera* NBRC16068, *Gordonia polysprenivorans* NBRC16320, *Gordonia alkanivorans* NBRC16433, *Gordonia terrae* NBRC100016, *Gordonia namibiensis* NBRC108229, *Gordonia sihwensis* NBRC108236, *Gordonia westaflica* NBRC108237, *Gordonia paraffinivorans* NBRC108238, *Gordonia soli* NBRC108243, *Gordonia malaquae* NBRC108250, *Gordonia alkaliphila* NBRC109776, *Gordonia jinhuaensis* NBRC110001, *Gordonia defluvi* NBRC110695, and *Gordonia sinesedis* NBRC110696 were purchased from the National Institute of Technology and Evaluation, Biological Resource Center (NBRC; Japan). These strains were maintained at 25˚C in NBRC-recommended media (227, 230, 253, and 802) solidified with 1.5% (w/v) agar. The 227 medium 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 230 medium contained 30.0 g of Bacto™ tryptic soy broth per liter of distilled water (pH 7.3). The 253 medium contained 37.0 g of Bacto™ brain heart infusion per liter of distilled water (pH 7.3). The 802 medium contained 2.0 g of hipolypepton, 2.0 g of Bacto™ yeast extract, and 1.0 g of magnesium sulfate heptahydrate per liter of distilled water (pH 7.0). The 15 *Agromyces* strains were grown in 227, 230, 253, and 802 media for 8 days at 25˚C with aerobic shaking in

baffled flasks in the dark, and the 16 *Gordonia* strains were grown in 227, 230, 231, and 802 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) or glass fiber filters (ADVANTEC GA-55) *in vacuo* and washed with saline (0.85% NaCl aq.). The harvested cells were immediately used for reduction after washing with the saline.

2.3. Reduction of α- and β-Keto Esters, and an Aromatic α-Keto Amide Using Actinomycete Cells

Wet saline-washed wet actinomycete cells (0.5 g, dry weight approximately 0.15 g) were resuspended in a test tube (ϕ 30 mm × 200 mm) containing 20 mL of saline. The substrate (0.15 mmol; 7.5 mM) and additive (5.0 mmol; 250 mM) were 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 × 30 mm), extracted with diethyl ether (5.0 mL), and then concentrated under reduced pressure.

2.4. Analysis

The production of alcohols (Figure 1, **2a**-**j**) was measured using 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* from, 7.62 min; **2j**-*syn* form, 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. (%) = $\{(R - S)/(R + S)\} \times 100$, 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 [4] [7] [19].

3. Results and Discussion

3.1. Screening of Actinomycete Strains and Culture Media

To determine the most suitable medium for liquid culture, 15 *Agromyces* and 16 *Gordonia* strains were cultivated in several culture media, after which the wet weight of the cells was measured. All *Agromyces* strains grew poorly in 227, 253, and 802 media, even after 8 days of culture, and the resulting wet cell weights were 0.2 g or less (see Table 1). However, two strains, *A. soli* NBRC109063 and *A. humatus* NBRC109085, yielded more than 0.4 g of wet cells/100mL of culture in the 230 medium, even though the recommended medium for NBRC109063

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

NBRC No.	Scientific Name	Wet cells weight (g)			
		227 medium	230 medium	253 medium	802 medium
13899	*Agromyces ramosus*	<0.1	<0.1	<0.1	<0.1
15780	*Agromyces cerinus*	<0.1	<0.1	<0.1	<0.1
16235	*Agromyces luteolus*	<0.1	<0.1	0.2	0.2
16236	*Agromyces rhizosperae*	<0.1	<0.1	<0.1	0.2
16238	*Agromyces bracchium*	<0.1	<0.1	<0.1	<0.1
103057	*Agromyces albus*	0.2	0.1	0.2	0.2
106452	*Agromyces iriomotensis*	<0.1	<0.1	<0.1	<0.1
106454	*Agromyces subtropicus*	0.2	0.1	0.1	0.2
109019	*Agromyces marinus*	0.2	<0.1	0.2	0.2
109063	*Agromyces soli*	0.2	0.4	0.2	0.2
109064	*Agromyces flavus*	0.2	<0.1	0.2	0.2
109073	*Agromyces tropicus*	0.2	0.3	0.2	0.2
109083	*Agromyces ulmi*	0.1	<0.1	0.1	0.1
109084	*Agromyces neolithicus*	0.2	0.2	0.2	0.2
109085	*Agromyces humatus*	0.2	0.6	0.2	0.2

The actinomycetes were grown in liquid medium (100 mL) at 25˚C for 8 days with aerobic reciprocating shaking (100/min) in baffled 500-mL flask in the dark.

and NBRC109085 strain is 802 medium. These results suggest that the peptone contained in the culture medium is more likely to be assimilated from plants than from animals in liquid cultures of *Agromyces* strains. As in the case of the genus *Agromyces*, *Gordonia* strains also had poor growth in various liquid media. Only two strains, *G. hydrophobica* NBRC16057 and *G. malaquae* NBRC108250, yielded more than 0.4 g of wet cells/100mL of the liquid culture in the 230 medium (see Table 2).

Therefore, we investigated the potential ability of two *Agromyces* strains (NBRC109063 and NBRC109085) and two *Gordonia* strains (NBRC16057 and NBRC108250) to act as biocatalysts for the asymmetric reduction of carbonyl compounds.

3.2. Reduction of Carbonyl Compounds by *Agromyces* Wet Cells

Two actinomycete strains (*A. soli* NBRC109063 and *A. humatus* NBRC109085) cultivated in the 230 medium were tested for their ability to reduce several carbonyl compounds (**1a**-**j**) (Figure 1). The results of the microbial reductions are summarized in Table 3 and Table 4. Both strains could reduce aliphatic and aromatic *α*-keto esters (**1a**-**h**) and an aromatic *α*-keto amide (**1i**). Among the reduction of nine substrates tested, there were compounds in which the conversion rate was over 99% (**2c**, **2d**, and **2f**); however, the reduction of most substrates showed only low values to moderate conversion rates. Moreover, the

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

NBRC No.	Scientific Name	Wet cells weight (g)		
		227 medium	230 medium	802 medium
15530	*Gordonia amarae*	0.1	<0.1	<0.1
16057	*Gordonia hydrophobica*	0.1	0.6	0.2
16068	*Gordonia rhizosphera*	<0.1	<0.1	<0.1
16320	*Gordonia polysoprenivorans*	<0.1	<0.1	0.2
16433	*Gordonia alkanivorans*	0.2	<0.1	0.2
100016	*Gordonia terrae*	0.2	0.3	0.1
108229	*Gordonia namibiensis*	0.2	<0.1	0.2
108236	*Gordonia sihwensis*	0.2	0.2	0.2
108237	*Gordonia westaflica*	<0.1	<0.1	<0.1
108238	*Gordonia paraffinivorans*	0.2	<0.1	0.2
108243	*Gordonia soli*	0.1	<0.1	0.2
108250	*Gordonia malaquae*	<0.1	0.4	<0.1
109776	*Gordonia alkaliphila*	<0.1	<0.1	<0.1
110001	*Gordonia jinhuaensis*	0.1	0.1	<0.1
110695	*Gordonia defluvi*	<0.1	<0.1	<0.1
110696	*Gordonia sinesedis*	0.1	0.3	<0.1

The actinomycetes were grown in liquid medium (100 mL) at 25˚C for 8 days with aerobic reciprocating shaking (100/min) in baffled 500-mL flask in the dark.

Table 3. The reduction of *α*-keto esters and aromatic *α*-keto amide by *Agromyces soli* NBRC109063.

Product	No Additive			Glycerol			L-Glutamate		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	20	>99	*S*	24	81	*S*	89	46	*S*
2b	50	91	*R*	38	22	*S*	80	94	*R*
2c	>99	74	*R*	71	15	*S*	93	86	*R*
2d	62	86	*S*	60	48	*S*	>99	>99	*S*
2e	82	62	*R*	52	25	*S*	92	87	*R*
2f	72	25	*S*	43	78	*R*	80	55	*S*
2g	52	31	*R*	50	71	*S*	71	45	*R*
2h	96	95	*R*	69	90	*R*	92	96	*R*
2i	3	30	*R*	5	33	*R*	9	41	*R*

stereoselectivity of the product in the reduction of each substrate also showed low values except for the reduction of ethyl pyruvate (**1a**). We tried to improve the conversion rate and the stereoselectivity of the alcohols produced by two *Agromyces* strains by introducing additives (glycerol and sodium hydrogen

Table 4. The reduction of *α*-keto esters and aromatic *α*-keto amide by *Agromyces humatus* NBRC109085.

Product	No Additive			Glycerol			L-Glutamate		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	44	>99	*S*	71	>99	*S*	53	>99	*S*
2b	78	14	*S*	58	34	*S*	47	21	*R*
2c	>99	44	*S*	98	15	*S*	95	30	*R*
2d	>99	10	*S*	>99	38	*S*	>99	>99	*S*
2e	47	5	*S*	68	17	*S*	35	31	*R*
2f	>99	62	*R*	>99	77	*R*	>99	60	*R*
2g	71	68	*S*	66	46	*S*	75	65	*S*
2h	18	6	*R*	23	55	*R*	15	72	*R*
2i	49	91	*R*	61	89	*R*	3	21	*R*

L-glutamate). The introduction of glycerol contributed little to the improvement of conversion rates and stereoselectivity of produced alcohols. The addition of L-glutamate also showed no significant improvement in the reduction of substrates; the reduction of only one substrate (from **1d** to **2d**) improved the conversion rate of both NBRC109063 and NBRC109065 wet cells to >99% and the stereoselectivity to >99% e.e. (*S*). As shown in Table 5, reduction of ethyl 2-methylacetoacetate (**1j**), one of *β*-keto esters, by the wet cells of NBRC109063 strain showed high values of *syn*/*anti* ratios and enantioselectivity. However, the conversion rate from **1j** to **2j** was low and was not improved by the additives. Furthermore, reduction of **1j** by the wet NBRC109065 cells resulted in a low *syn*/*anti* ratio and low conversion rate and did not improve with the addition of additives.

3.3. Reduction of Carbonyl Compounds by *Gordonia* Wet Cells

Two *Gordonia* strains (*G. hydrophobica* NBRC16057 and *G. malaquae* NBRC108250) cultivated in 230 medium were tested for their ability to reduce the keto esters and keto amide (Table 6 and Table 7). The *Gordonia* strains could reduce aliphatic and aromatic *α*-keto esters and an aromatic *α*-keto amide. However, as with the reduction by two *Agromyces* strains, the substrate was reduced only at low to moderate conversion rates, and the stereoselectivity of the products was low except for the reduction of **1h** by the *G. hydrophobica* NBRC16057 strain. Therefore, the effects of additives on these microbial reduction reactions were also examined. In the reductions by two *Gordonia* strains, NBRC16057 and NBRC108250, the introduction of glycerol did not improve the conversion rate or stereoselectivity. On the other hand, the addition of L-glutamate improved the conversion rate and the stereoselectivity of the product. In particular, the reductions of **1a**, **1c**, **1d**, and **1g** by the NBRC16057 wet cells improved the conversion rates to >99%, and **1d** and **1e** were reduced to the

Table 5. The reduction of ethyl 2-methylacetoacetate by two *Agromyces* strains.

Strain/ NBRC No.	Additive	Conv. (%)	*Syn*/*Anti*	e.e. (%)	
				Syn-(2*R*, 3*S*)	*Anti*-(2*S*, 3*S*)
A. soli NBRC109063	None	12	7/93	>99	>99
	Glycerol	10	11/89	86	>99
	L-Glutamate	23	19/81	>99	>99
A. humatus NBRC109065	None	49	24/76	>99	>99
	Glycerol	31	19/81	84	>99
	L-Glutamate	48	18/88	88	>99

---: Not detected.

Table 6. The reduction of *α*-keto esters and aromatic *α*-keto amide by *G. hydrophobica* NBRC16057.

Product	No Additive			Glycerol			L-Glutamate		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	41	40	*S*	30	41	*S*	>99	46	*S*
2b	96	15	*S*	78	22	*S*	80	54	*S*
2c	>99	18	*S*	89	15	*S*	>99	86	*S*
2d	82	54	*S*	77	38	*S*	>99	>99	*S*
2e	65	28	*S*	38	17	*S*	92	>99	*S*
2f	59	35	*R*	44	24	*R*	60	44	*R*
2g	84	56	*S*	78	88	*S*	>99	45	*R*
2h	91	>99	*R*	30	>99	*R*	54	36	*S*
2i	16	25	*R*	10	20	*R*	3	21	*R*

Table 7. The reduction of *α*-keto esters and aromatic *α*-keto amide by G. hydrophobica NBRC108250.

Product	No Additive			Glycerol			L-Glutamate		
	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*	Conv. (%)	e.e. (%)	*R*/*S*
2a	38	24	*S*	27	36	*S*	>99	>99	*S*
2b	23	19	*S*	51	10	*S*	75	78	*S*
2c	3	30	*S*	10	55	*S*	91	89	*S*
2d	18	45	*S*	23	63	*S*	88	82	*S*
2e	55	32	*R*	32	21	*R*	83	79	*R*
2f	28	65	*R*	36	45	*R*	70	81	*R*
2g	44	20	*S*	29	38	*S*	90	45	*S*
2h	69	72	*S*	40	81	*S*	77	36	*S*
2i	7	66	*R*	12	79	*R*	54	80	*R*

(*S*)-hydroxy esters (>99% e.e.) stereospecifically.

As shown in Table 8, in the reduction of *β*-keto ester (**1j**) by *G. malaquae* NBRC108250 wet cells, the conversion rate and *syn*/*anti* ratio of the produced *β*-hydroxy ester (**2j**) were low and were not improved by the introduction of additives. In contrast, the reduction of **1j** by the wet cells of the NBRC16057 strain stereospecifically produced the corresponding alcohol *syn*-(2*R*, 3*S*)-**2j** in high conversion rate (81%). Furthermore, when L-glutamate was introduced as an additive to the reduction of **1j**, the stereoselectivity (*syn*/*anti* ratio and e.e.) of the product remained high and the conversion rate was improved to 98%. More specifically, in this reaction, the substrate was reduced to only one of the four theoretically possible stereoisomers. We reported in a previous paper that reduction by the wet cells of *Dactylosporangium* sp. NBRC101730 strain specifically reduces **1j** to the corresponding *β*-hydroxy ester having *anti*-(2*S*, 3*S*) form [15]. In other words, we succeeded in the specific preparation of *syn*-(2*R*, 3*S*) and *anti*-(2*S*, 3*S*) forms of **2j** by using two strains from different actinomycete genera, *G. hydrophobica* NBRC16057 strain and *Dactylosporangium* sp. NBRC101730 strain as shown in Figure 2.

In this study, the introduction of glycerol could not improve the conversion rate of the reduction, while the addition of L-glutamate improved the conversion rates. These results are likely due to the fact that the enzyme (keto ester reductase) involved in the reduction of the substrate depends on NADPH, and the coenzyme is excessively supplied during oxidative degradation of the added L-glutamate. In future work, we aim to purify and characterize the reductase from actinomycete whole cells and elucidate the reaction mechanism at the enzyme molecular level.

Table 8. The reduction of ethyl 2-methylacetoacetate by two *Gordonia* strains.

Strain/NBRC No.	Additive	Conv. (%)	*Syn*/*Anti*	e.e. (%)	
				Syn-(2*R*, 3*S*)	*Anti*-(2S, 3*S*)
G. hydrophobica NBRC16057	None	81	>99/<1	>99	---
	Glycerol	59	90/10	>99	>99
	L-Glutamate	98	>99/<1	>99	---
G. malaquae NBRC108250	None	33	68/32	28	>99
	Glycerol	18	55/45	44	>99
	L-Glutamate	52	72/28	39	>99

---: Not detected.

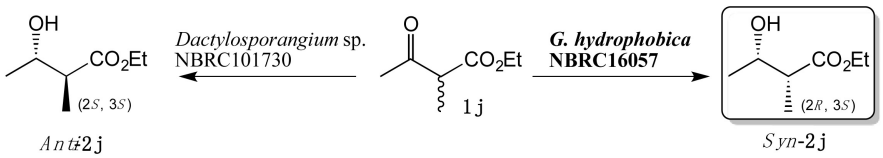

Figure 2. The stereospecific reduction of ethyl 2-methylacetoacetate by two actinomycetes. Left direction: Reference 15, Right direction: in this study.

4. Conclusion

We discovered reducing activities for nine keto esters and an aromatic *α*-keto amide in *Agromyces* strains from the Microbacteriaceae family and *Gordonia* strains from the Gordoniaceae family. We also found that the introduction of L-glutamate as an additive improved the conversion rate and stereoselectivity of the product in the reduction of some substrates. Furthermore, we have demonstrated that the *G. hydrophobic* NBRC16057 strain cultured in the 230 medium is an excellent and useful biocatalyst for the stereoselective reduction of ethyl 2-methylacetoacetate to the corresponding alcohol with *syn*-(2*R*, 3*S*) form.

Conflicts of Interest

The authors declare no conflicts of interest.

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