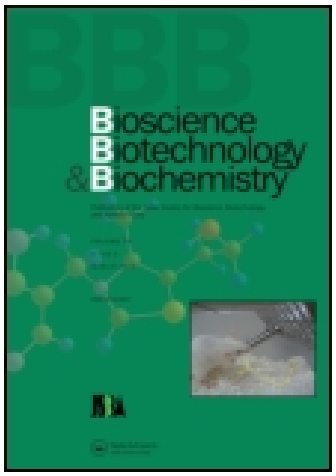

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Kohji Ishihara[a], Keisuke Iwai[a], Hitomi Yamaguchi[a], Nobuyoshi Nakajima[ab], Kaoru Nakamura[ac] & Toshihisa Ohshima[a]

[a] Department of Chemistry, Kyoto University of Education, Fushimi-ku, Fukakusa, Kyoto 612, Japan

[b] Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719-11, Japan

[c] Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

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Note

Stereoselective Reduction of α- and β-Keto Esters with Aerobic Thermophiles, *Bacillus* Strains

Kohji ISHIHARA,† Keisuke IWAI, Hitomi YAMAGUCHI, Nobuyoshi NAKAJIMA,* Kaoru NAKAMURA,** and Toshihisa OHSHIMA††

Department of Chemistry, Kyoto University of Education, Fushimi-ku, Fukakusa, Kyoto 612, Japan
**Department of Nutritional Science, Okayama Prefectural University, Soja, Okayama 719–11, Japan*
***Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan*
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The first example of stereoselective reduction with aerobic thermophiles is reported. Various α- and β-keto esters were reduced stereoselectively to the corresponding alcohols by the aerobic thermophiles, *Bacillus* strains. In particular, the reduction of ethyl 3-methyl-2-oxobutanoate with *B. stearothermophilus* DSM 297 gave the corresponding (*R*)-alcohol with high yield in excellent enantioselectively (>99% *e.e.*). The conversions of keto esters to the corresponding hydroxy esters with *Bacillus* strains were increased by introduction of glycerol in the reaction mixture as an additive.

Key words: stereoselective reduction; keto esters; aerobic thermophile; *Bacillus stearothermophilus*

Microbial conversion has been studied widely for preparation of various chiral compounds.[1] For example, bakers' yeast has been used often for the reduction of pro-chiral ketones to obtain optically active alcohols.[2] The alcohols are used as useful chiral building blocks for the synthesis of various biologically active compounds.[3] Until now, the reductions of various carbonyl compounds with an anaerobic thermophile, *Thermoanaerobactor brockii* DSM 1457 (*Thermoanaerobium brockii*) has been the only one reported.[4] The commercial TBADH (the alcohol dehydrogenase from *T. brockii*) has high thermostability and high stereospecificity toward various ketones.[5,6] Therefore, the enzyme is available for organic syntheses. However, a little information has been known about the reduction of carbonyl compounds using other thermophiles as biocatalysts. In this paper, we would like to report the stereoselective reduction of α- and β-keto esters with aerobic thermophilic bacteria.

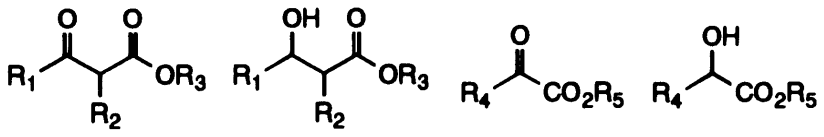
The bacteria (*Bacillus* strains) were incubated in the medium[7] (200 ml) for 48 h at 55°C aerobically with vigorous shaking in baffled 500-ml flasks. The wet cells were collected by centrifugation at 5000 × *g* for 15 min. Saline (20 ml) and the substrate (0.1 mmol) were added to the collected wet cells (0.2 g) and the reaction mixture were incubated at 37°C for 20 h, aerobically shaking. The conversion ratio (%) and enantioselectivity (*e.e.* %) of the product were measured by gas chromatography (HR-20M capillary column, 0.25 mm × 20 m, 100°C or 150°C, and Chiraldex G-TA optically capillary column, 0.25 mm × 30 m, 110°C) analysis.[8]

Twenty-six selected *Bacillus* strains were tested for the reducing activity of α- and β-keto esters. The results of the conversion of β-keto esters (**1a–d**) and α-keto ester (**3a–c**) to the corresponding alcohols with ten *Bacillus* strains are summarized in Table I.

It was found that various keto esters were converted to the corresponding alcohols with *Bacillus* strains. In the conventional method (without additive), the substrates (**1a–d**, **3a–c**) were reduced to the corresponding alcohol (**2a–d**, **4a–c**) with a low conversion ratio (as shown in parentheses in Table I), while these ratio were increased by introduction of glycerol in the reaction mixture as an additive. The addition of glycerol increased only the ratio in almost cases tested, however introduction of other additives (citric acid, malic acid, glucose, 2,3-butanediol, 2-propanol, methanol, ethanol, and 1-butanol) did not increased the ratio in the reduction (under 5 mM of substrate and 250 mM of additives). The addition of 50-fold the molar quantity of glycerol against the substrate gave the satisfactory results.

In the stereochemistry of products, the reduction of ethyl 2-methyl-3-oxobutanoate (**1d**) by three *Bacillus* strains (DSM 297, 730, and 2027) gave the corresponding β-hydroxy alcohol (**2d**) in high diastereoselectivity (*syn*/*anti* = 80–89/11–20), compared with a bakers' yeast reduction of the same substrate.[8] Further, the enantioselectivity of *syn*-(2*R*,3*S*)- and *anti*-(2*S*,3*S*)-**2d** reduced by *Bacillus* strains were >99% *e.e.* and >98% *e.e.*, respectively.

Many useful methods for the reduction with bakers' yeast have been reported.[9–14] Among them, we found that the addition of a third reagent to the reaction system changed the stereoselectivi-



(1) **(2)** **(3)** **(4)**

1a: R_1=$ClCH_2$-, R_2=H, R_3=Et
1b: R_1=Me, R_2=H, R_3=Me
1c: R_1=Me, R_2=H, R_3=Et
1d: R_1=Me, R_2=Me, R_3=Et

3a: R_4=Me, R_5=Et
3b: R_4=Me_2CH-, R_5=Et
3c: R_4=Ph, R_5=Et

Scheme

† To whom correspondence should be addressed.
†† Present address: *Department of Biological Science and Technology, Faculty of Engineering, Tokushima University, Minami-Jyosanjima, Tokushima 770, Japan.*

Table I. The Conversion (%) of Substrates to the Corresponding Alcohols by *Bacillus* Strains with Glycerol[a,b]

Bacillus strains	DSM No.	β-Keto esters				α-Keto esters		
		1a	**1b**	**1c**	**1d**	**3a**	**3b**	**3c**
B. stearothermophilus	297	23 (20)[c]	8 (8)	29 (13)	73 (25)	55 (47)	99 (71)	89 (5)
B. stearothermophilus	457	34 (6)	32 (8)	7 (13)	32 (8)	64 (57)	34 (85)	75 (34)
B. smithii	460	22 (0)	13 (1)	11 (1)	1 (2)	8 (0)	99 (23)	85 (37)
B. sphaericus	461	9 (0)	14 (9)	8 (28)	78 (6)	89 (64)	93 (74)	69 (40)
Bacillus sp.	465	19 (13)	21 (5)	9 (6)	2 (2)	99 (79)	60 (36)	87 (28)
Bacillus sp.	466	42 (11)	19 (2)	39 (2)	6 (15)	70 (41)	71 (65)	77 (16)
B. stearothermophilus	494	13 (7)	5 (8)	5 (14)	50 (5)	91 (66)	67 (63)	74 (35)
B. thermocatenulatus	730	38 (11)	20 (6)	55 (3)	67 (4)	60 (37)	48 (31)	85 (60)
B. stearothermophilus	1550	7 (0)	17 (4)	80 (14)	57 (4)	41 (20)	98 (68)	90 (58)
B. stearothermophilus	2027	14 (4)	11 (6)	40 (39)	34 (6)	38 (29)	42 (29)	71 (42)

[a] The conversion ratios of the substrates to the corresponding alcohols were measured by GLC with a capillary column HR-20M (0.25 mm × 25 m).
[b] The substrates (5 mM), glycerol (250 mM), and saline (20 ml) were added to the wet cells (0.2 g) and the reaction mixture were incubated at 37°C for 20 h.
[c] The conversion ratios without glycerol are shown in parentheses.

Table II. Reductions of β-Keto Esters (**1d**) and α-Keto Esters (**3b**) by *Bacillus* Strains[a]

Ethyl 2-methyl-3-oxobutanoate (**1d**)						Ethyl 3-methyl-2-oxobutanoate (**3b**)			
Bacillus strains	DSM No.	Yield[b] (%)	*syn/anti*[c]	*e.e.* (%)[d]		*Bacillus* strains	DSM No.	Yield[b] (%)	*e.e.*[d] (%)
				syn-(2*R*,3*S*)	*anti*-(2*S*,3*S*)				
B. stearothermophilus	297	56	80/20	>99	99	*B. stearothermophilus*	297	82	>99 (*R*)
B. sphaericus	461	65	74/26	>99	96	*B. smithii*	460	85	7 (*S*)
B. thermocatenulatus	730	51	83/17	99	98	*B. sphaericus*	46	71	75 (*R*)
B. stearothermophilus	1550	36	51/49	>99	90	*Bacillus* sp.	466	60	79 (*R*)
B. stearothermophilus	2027	12	89/11	98	98	*B. stearothermophilus*	1550	79	72 (*R*)

[a] Saline (20 ml), the substrates (5 mM), and glycerol (250 mM) were added to the wet cells (0.2 g) and the reaction mixtures were incubated at 37°C for 20 h.
[b] Isolated yields.
[c] The ratios of *syn/anti* were measured by GLC with a capillary column HR-20M (0.25 mm × 25 m).
[d] The *e.e.* (%) and configuration were measured with GLC with an optically active capillary column Chiraldex G-TA (0.25 mm × 20 m).

ty of the reduction and allowed a product of the desired configuration to be obtained in high enantiomeric excess.[9-11] For example, the introduction of α,β-unsaturated carbonyl compound or metal salts shifted the stereoselectivity of the yeast reduction.[10] The ratio (80:20) of *syn/anti* of product (**2d**) in the reduction by *B. stearothermophilus* DSM 297 could be shifted to the extent of 98:2 by addition of $MgCl_2$, but the chemical yield was decreased to 12%. The ratio was not changed by introduction of other additives such as allyl alcohol, ethyl chloroacetate, or methyl vinyl ketone (data not shown).

Further, the reduction of ethyl 3-methyl-2-oxobutanoate (**3b**) by four *Bacillus* strains (DSM 297, 461, 466, and 1550) also gave the corresponding α-hydroxy ester (**4b**) in high enantioselectivity as shown in Table II.

In particular, the reduction of **3b** with *B. stearothermophilus* DSM 297 afforded (*R*)-**4b** in high chemical yield (82%) with excellent stereoselectivity (>99% *e.e.*). These results indicated that the reduction of keto esters with *Bacillus* strains would be available for a tool for organic asymmetric syntheses.

This work is the first example of the stereoselective reduction of carbonyl compounds by aerobic thermophiles.

The mechanism for the improvement of conversion ratio by addition of glycerol is not clear. It seems that the increasement of reduced nicotinamide-adenine dinuclotide (NADH or NADPH) due to the oxidative degradation of glycerol in the cells of these aerobic thermophiles would accelerate the stereoselective reduction of α and β-keto esters to the corresponding optically pure alcohols. Further detailed studies including purification of the enzymes which contribute to the reduction system are now in progress in our laboratory.

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