JOC*Article*

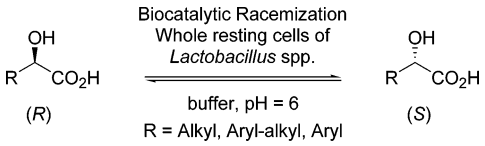
Biocatalytic Racemization of Aliphatic, Arylaliphatic, and Aromatic α-Hydroxycarboxylic Acids

Silvia M. Glueck,[†,§] Monika Pirker,[†] Bettina M. Nestl,[†,§] Barbara T. Ueberbacher,[†] Barbara Larissegger-Schnell,[†] Katrin Csar,[†] Bernhard Hauer,[‡] Rainer Stuermer,[‡] Wolfgang Kroutil,[†] and Kurt Faber*[,†]

Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz, Heinrichstrasse 28, A-8010 Graz, Austria, and BASF-AG, GVF/E-B9, D-67056 Ludwigshafen, Germany

kurt.faber@uni-graz.at

Received January 25, 2005



Biocatalytic racemization of a range of aliphatic, (aryl)aliphatic, and aromatic α-hydroxycarboxylic acids was accomplished by using whole resting cells of a range of *Lactobacillus* spp. The mild (physiological) reaction conditions ensured an essentially "clean" isomerization in the absence of side reactions, such as elimination or decomposition. Whereas straight-chain aliphatic 2-hydroxycarboxylic acids were racemized with excellent rates (up to 85% relative to lactate), steric hindrance was observed for branched-chain analogues. Good rates were observed for aryl−alkyl derivatives, such as 3-phenyllactic acid (up to 59%) and 4-phenyl-2-hydroxybutanoic acid (up to 47%). In addition, also mandelate and its *o*-chloro analogue were accepted at a fair rate (45%). This biocatalytic racemization represents an important tool for the deracemization of a number of pharmaceutically important building blocks.

Introduction

Racemization is an irreversible entropy-driven isomerization reaction leading to a loss of enantiomeric purity,[1] discovered by Pasteur in 1853. Since it usually goes in hand with a loss of the "chiral value" of materials, it has been generally considered as an unwanted side reaction rather than a synthetically useful transformation. As a consequence, the controlled racemization of organic compounds has been scarcely studied.[1,2] It was only recently that the need for "clean" racemization protocols had been recognized in particular due to the increasing demand for so-called deracemization processes, which allow the complete transformation of a racemate into a single stereoisomeric product in 100% theoretical yield.[3,4] Over the past few years, the latter have gained considerable interest in the field of asymmetric transformations, mainly due to the increasing pressure to enhance the economic balance of chemical processes, predominantly on an industrial scale.

Detailed analysis of the (chemical) racemization protocols published so far[2] reveals that the general reaction conditions employed for racemization favor strongly acidic or basic media, which are incompatible with the presence of a stereoselective (catalytic) in situ transformation. As a consequence, traditional (chemical) protocols for racemization are thus of limited use for dynamic kinetic resolutions.[5−8] To circumvent this limitation, enzymatic racemization—taking place under ambient reactions conditions—holds great potential.[9]

During our studies directed toward the deracemization of α-hydroxycarboxylic acids by coupling lipase-catalyzed acyl-transfer to racemization of the nonreacted substrate enantiomer using mandelate racemase [EC 5.1.2.2][10] we

[†] University of Graz.
[‡] BASF-AG.
[§] Research Centre Applied Biocatalysis, Graz, Austria.

(1) Eliel, E. L.; Wilen, S. H.; Doyle, M. P. *Basic Organic Stereochemistry*; Wiley: New York, 2001; pp 277−278.
(2) Ebbers, E. J.; Ariaans, G. J. A.; Houbiers, J. P. M.; Bruggink, A.; Zwanenburg, B. *Tetrahedron* **1997**, *53*, 9417.
(3) Faber, K. *Chem. Eur. J.* **2001**, *7*, 5004.
(4) Pamies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247.
(5) Pellissier, H. *Tetrahedron* **2003**, *59*, 8291.
(6) Pamies, O.; Bäckvall, J.-E. *Chem. Rev.* **2003**, *103*, 3247.
(7) Kim, M.-J.; Ahn, Y.; Park, J. *Curr. Opinion Biotechnol.* **2002**, *13*, 578.
(8) El Gihani, M. T.; Williams, J. M. J. *Curr. Opinion Chem. Biol.* **1999**, *3*, 11.
(9) Schnell, B.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* **2003**, *345*, 653.
(10) Strauss, U. T.; Faber, K. *Tetrahedron*: *Asymmetry* **1999**, *10*, 4079.

10.1021/jo050156n CCC: $30.25
Published on Web 04/21/2005

encountered a stringent substrate limitation for the latter enzyme: Although mandelate racemase was very tolerant toward various β,γ-unsaturated α-hydroxycarboxylic acids,[11] saturated (aliphatic) substrate analogues were not accepted at all. The latter fact can be explained by the lack of resonance stabilization of the corresponding enolate intermediate within the active site of the enzyme.[12,13] Furthermore, severe steric hindrance was observed for ortho-substituted mandelate derivatives.[11] To extend the applicability of our deracemization protocol toward saturated aliphatic and arylaliphatic α-hydroxycarboxylic acids, which are lacking the minimum structural requirements of mandelate racemase, i.e., at least one C=C bond in the β,γ-position, a matching isomerase/racemase enzyme was required.

Due to the fact that the vast majority of biochemical processes are stereospecific, Nature has faced little need for racemization and, as a consequence, "racemases" are a small group of enzymes, which have been biochemically classified as subgroup [EC 5.1.X.X] among the diverse and heterogeneous group of isomerases.[14−16] Despite their rare occurrence in Nature, their importance in synthetic organic chemistry lies in the fact that they often can catalyze "chemically impossible" isomerization reactions. Careful analysis of the biochemical literature on racemases acting on α-hydroxycarboxylic acids revealed the existence of a promising candidate: Lactate racemase [EC 5.1.2.1].[17]

The biochemical data on lactate racemase available to date are somewhat scattered and divers. The respective enzymatic activity was reported (or assumed) in various microbial strains in context with their ability to produce (or degrade) D-lactate through an L-specific lactate pathway. The biodegradation/formation of D-lactate from the L-isomer via lactate racemase was identified in particular among (anaerobic) rumen bacteria, such as *Megasphaera elsdenii*[18,19] and *Selenomonas ruminantium*.[20] Similar degradation pathways were found in *Staphylococcus aureus*,[21] *Lactobacillus sakei*,[22,23] and halophilic Archaea, such as *Haloferax volcanii* and various *Haloarcula* spp.[24]

Less is known about the enzyme properties and its mode of action: Although lactate racemase from *Clostridium butylicum*,[25] *Lactobacillus sake*,[26] and *L. curvatus*[27] was purified and biochemically characterized to a certain extent, detailed proof for the assumption on its mechanism of action through an internal hydride shift involving an α-carbonyl enzyme-bound intermediate is still missing. Most important, the biocatalytic racemization of saturated α-hydroxycarboxylic acids other than lactate has not been studied to date.[28]

Results and Discussion

In search of a suitable racemase activity applicable to a broad spectrum of substrates encompassing aliphatic and arylaliphatic α-hydroxycarboxylic acids, which could not be isomerized with use of mandelate racemase, a screening was initiated based on the data for lactate racemase discussed above. Thus, rehydrated (resting) cells of a representative set of *Lactobacilli* (20 strains), *Lactococci* (9 strains), and halophilic organisms, such as *Halococcus* (7 strains) and halobacteria (3 strains, *Halobacterium*, *Haloferax*, and *Haloarcula*), were screened for their ability to racemize α-hydroxycarboxylic acids in aqueous buffer at pH 6. To cover a reasonably wide substrate spectrum, straight-chain, branched, and cyclic aliphatic α-hydroxycarboxylic acids [(*S*)-**1**,**2**, (*R*)-**3**, and (*S*)-**4**−**6**] were chosen. Special emphasis was put on aryl−alkyl derivatives, such as phenyl-lactates and 4-phenyl-2-hydroxybutanoates [(*S*)-**7**−**12** and (*R*)-**7**,**8**], since compounds of this type are frequently used as chiral building blocks for the synthesis of pharmacologically active target molecules. For instance, (*S*)-3-cyclohexyl lactate (**6**) was found to be an essential component of sialyl Lewis^X-analogues, which are currently tested as inhibitors of E-selectin for the treatment of inflammatory disorders.[29] (*R*)-4-Phenyl-2-hydroxybutanoate (**8**), which has been prepared by kinetic resolution,[30] asymmetric bioreduction,[31,32] microbial stereoinversion,[33] and asymmetric synthesis[34] is an important intermediate for the synthesis of a wide range of ACE inhibitors.

3-Phenyl lactate (**7**) and derivatives thereof are frequently used components of pharmaceuticals and natural antibiotic agents.[35,36] Among them, the *p*-hydroxy ana-

(11) Felfer, U.; Strauss, U. T.; Kroutil, W.; Fabian, W. M. F.; Faber, K. *J. Mol. Catal. B*: *Enzym.* **2001**, *15*, 213.
(12) Powers, V. M.; Koo, C. W.; Kenyon, G. L.; Gerlt, J. A.; Kozarich, J. W. *Biochemistry* **1991**, *30*, 9255. Prat-Resina, X.; Garcia-Viloca, M.; Gonzalez-Lafont, A.; Lluch, J. M. *ChemPhysChem* **2002**, *4*, 5365.
(13) For a review on the substrate tolerance of mandelate racemase and a general substrate model see: Felfer, U.; Goriup, M.; Koegl, M.; Wagner, U.; Faber, K.; Kroutil, W. *Adv. Synth. Catal.* Submitted for publication.
(14) Adams, E. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1976**, *44*, 69.
(15) Glaser, L. *The Enzymes* **1972**, *6*, 355.
(16) Tanner, M. E. *Acc. Chem. Res.* **2002**, *35*, 237.
(17) Synonyms for lactate racemase are lacticoracemase, hydroxy acid racemase, and lactic acid racemase. No sequence or X-ray structure for lactate racemase or hydroxy acid racemase is available to date. www.Brenda.Uni-Koeln.de.
(18) Hino, T.; Kuroda, S. *Appl. Environ. Microbiol.* **1993**, *59*, 255.
(19) Hino, T.; Shimada, K.; Maruyama, T. *Appl. Environ. Microbiol.* **1994**, *60*, 1827.
(20) Melville, S. B.; Michel, T. A.; Macy, J. M. *FEMS Microbiol. Lett.* **1987**, *40*, 289.
(21) Stockland, A. E.; San Clemente, C. L. *J. Bacteriol.* **1969**, *100*, 347.
(22) Malleret, C.; Lauret, R.; Ehrlich, S. D.; Morel-Deville, F.; Zagorec, M. *Microbiology* **1998**, *144*, 3327.
(23) No lactate racemase activity was found in *Lactobacillus casei* NRRL-B445 and *L. delbrueckii* ATCC 11842, see: Hjörleifsdottir, S.; Seevaratnam, S.; Holst, O.; Mattiasson, B. *Curr. Microbiol.* **1990**, *20*, 287. Ragout, A.; Psece de Riuz Holgado, A.; Oliver, G.; Sineriz, F. *Biochimie* **1989**, *71*, 639.
(24) Oren, A.; Gurevich, P.; Silverman, A. *Can. J. Microbiol.* **1995**, *41*, 302.
(25) Cantwell, A.; Dennis, D. *Biochemistry* **1974**, *13*, 287. Pepple, J. S.; Dennis, D. *Biochim. Biophys. Acta* **1976**, *429*, 1036.
(26) Hiyama, T.; Fukui, S.; Kitahara, K. *J. Biochem.* **1968**, *64*, 99.
(27) Stetter, K. O.; Kandler, O. *Arch. Microbiol.* **1973**, *94*, 221.
(28) For a preliminary communication see: Glueck, S. M.; Larissegger-Schnell, B.; Csar, K.; Kroutil, W.; Faber, K. *Chem. Commun.* **2005**, 1904.
(29) Storz, T.; Dittmar, P.; Fauquez, P. F.; Marschal, P.; Lottenbach, W. U.; Steiner, H. *Org. Proc. Res. Dev.* **2003**, *7*, 559.
(30) Liese, A.; Kragl, U.; Kierkels, H.; Schulze, B. *Enzyme Microb. Technol.* **2002**, *30*, 673.
(31) Schmidt, E.; Blaser, H.-U.; Fauquex, P. F.; Sedelmeier, G.; Spindler, F. In *Microbial Reagents in Organic Synthesis*; Servi, S., Ed.; Kluwer: Dordrecht, The Netherlands 1992; pp 377−388.
(32) Oda, S.; Inada, Y.; Kobayashi, A.; Ohta, H. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1762.
(33) Chadha, A.; Baskar, B. *Tetrahedron*: *Asymmetry* **2002**, *13*, 1461.
(34) Blaser, H.-U.; Burkhardt, S.; Kirner, H. J.; Mössner, T.; Studer, M. *Synthesis* **2003**, 1679.
(35) For antifungal activity see: Ström, K.; Sjögren, J.; Broberg, A.; Schnürer, J. *Appl. Environ. Microbiol.* **2002**, *68*, 4322.
(36) For anti-*Listeria* activity see: Dieuleveux, V.; van der Pyl, D.; Chataud, J.; Gueguen, M. *Appl. Environ. Microbiol.* **1998**, *64*, 800.

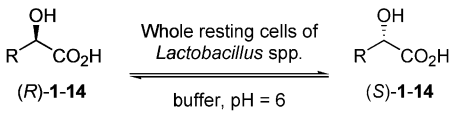
TABLE 1. Screening of *Lactobacillus* spp. for the Racemization of Substrates 1−14[a]

		strain							
substrate	R	*Lactobacillus halotolerans* DSM 20190	*Lactobacillus brevis* DSM 20054	*Lactobacillus acetotolerans* DSM 20749	*Lactobacillus paracasei* DSM 20008	*Lactobacillus paracasei* DSM 20207	*Lactobacillus delbrueckii* DSM 20074	*Lactobacillus paracasei* DSM 2649	*Lactobacillus sakei* DSM 20017
(*S*)-**1**	CH_3−	++	++	++	++	++	++	−	+
(*S*)-**2**	CH_3-CH_2−	+	−	−	+	++	−	+	−
(*R*)-**3**	CH_3-$(CH_2)_3$−	+	n.d.	n.d.	++	++	++	++	−
(*S*)-**4**	$(CH_3)_2CH$−	−	−	−	−	±	−	−	−
(*S*)-**5**	$(CH_3)_2CH$-CH_2−	±	−	−	±	±	±	±	±
(*S*)-**6**	*c*-C_6H_{11}-CH_2−	−	−	−	+	+	−	±	+
(*S*)-**7**	C_6H_5-CH_2−	−	−	+	++	++	++	+	±
(*S*)-**8**	C_6H_5-$(CH_2)_2$−	+	±	+	++	++	++	++	±
(*S*)-**9**	*p*-F-C_6H_4-CH_2−	−	−	−	++	++	++	++	n.d.
(*S*)-**10**	*p*-Cl-C_6H_4-CH_2−	n.d.	n.d.	n.d.	+	++	+	++	n.d.
(*S*)-**11**	*p*-Br-C_6H_4-CH_2−	n.d.	n.d.	n.d.	+	++	±	++	n.d.
(*S*)-**12**	*p*-OH-C_6H_4-CH_2−	±	−	−	+	++	++	−	−
(*S*)-**13**	C_6H_5−	−	−	−	±	+	−	−	−
(*R*)-**14**	*o*-Cl-C_6H_4−	−	−	−	±	+	+	±	±

[a] n.d. = not determined; (−) = no racemization; (±) = slow racemization (conversion ≤ 20%); (+) = moderate racemization (conversion 20−70%); (++) = fast racemization (conversion ≥ 70%); 100% conversion corresponds to complete racemization under standard conditions (i.e., ee = 0%, see the Experimental Section).

logue (**12**)[37] is an integral part of bioactive peptides, such as Aeruginosins[38] and Microcin[39] isolated from freshwater cyanobacteria, which were shown to be potent protease inhibitors. (*R*)-*p*-Fluoro-3-phenyl lactate (**9**) is a key building block for the synthesis of AG7088 (Ruprintrivir), a potent rhinovirus protease inhibitor currently being tested in clinical trials to treat the common cold.[40,41] The latter compounds are particularly difficult to racemize with conventional methods due to their ease of elimination forming cinnamic acid. To circumvent steric restrictions of mandelate racemase with respect to ortho-substituted mandelate derivatives, (*S*)-mandelate (**13**) and its (*R*)-*o*-chloro derivative (**14**) were chosen. The latter is a key intermediate for the synthesis of anticoagulant agents in cardiovascular therapy (Clopidogrel/Plavix).[42,43] Whereas no racemization activity could be detected among *Lactococci* and halophilic organisms (for a complete list of strains see the Experimental Section), the *Lactobacilli* proved to provide a rich source of racemase activity. Among 20 strains tested, eight "hits" were identified. The screening results depicted in Table 1 allow the active strains to be grouped into the following categories according to their substrate-activity pattern. (i) Lactate racemizers: *Lactobacillus* spp. DSM 20190, DSM 20054, and DSM 20749 showed high activity for the "natural" substrate−lactate (**1**) with little preference for 'nonnatural' substrates **2**−**12**. (ii) Lactate and α-hydroxy acid racemizers: On the contrary, *Lactobacillus* spp. DSM 20008, DSM 20207, and DSM 20074 had a desired broad substrate tolerance for all substrates, including lactate. (iii) α-Hydroxy acid racemizer: Most remarkably, *Lactobacillus* sp. DSM 2649 showed broad activity for nonnatural substrates, in particular 3-phenyl lactates (**7**−**11**), but was unable to racemize lactate.

SCHEME 1. Biocatalytic Racemization of Aliphatic, Arylaliphatic, and Aromatic α-Hydroxycarboxylic Acids (1−14)



(*R*)-**1-14** (*S*)-**1-14**

Substrate	R	Substrate	R
(*S*)-**1**	Me-	(*R*)-**8**, (*S*)-**8**	C_6H_5-$(CH_2)_2$-
(*S*)-**2**	Et-	(*S*)-**9**	*p*-F-C_6H_4-CH_2-
(*R*)-**3**	*n*-Bu-	(*S*)-**10**	*p*-Cl-C_6H_4-CH_2-
(*S*)-**4**	$(CH_3)_2CH$-	(*S*)-**11**	*p*-Br-C_6H_4-CH_2-
(*S*)-**5**	$(CH_3)_2CH$-CH_2-	(*S*)-**12**	*p*-HO-C_6H_4-CH_2-
(*S*)-**6**	*c*-C_6H_{11}-CH_2-	(*S*)-**13**	C_6H_5-
(*R*)-**7**, (*S*)-**7**	C_6H_5-CH_2-	(*R*)-**14**	*o*-Cl-C_6H_4-

No clear preference was detectable for *Lactobacillus sakei* DSM 20017. In contrast to straight-chain substrates, sterically demanding branched-chain α-hydroxy acids (**4**−**6**) showed reduced racemization rates with all strains.

To obtain quantitative data on racemization rates, the course of the racemization of substrates **1**−**14** was monitored over time, using *Lactobacillus paracasei* DSM 20207, which clearly emerged as the "champion" from the screening (Table 2). Initial rates were determined from the constant slope of progress curves at a conversion below 5%; relative rates were calculated by setting the racemization rate of the natural substrate (*S*)-**1** as standard (100%).

(37) Lawrence, N. J.; Brown, S. *Tetrahedron* **2002**, *58*, 613.
(38) Valls, N.; Lopez-Canet, M.; Vallribere, M.; Bonjoch, J. *Chem. Eur. J.* **2001**, *7*, 3446. Valls, N.; Vallribera, M.; Carmeli, S.; Bonjoch, J. *Org. Lett.* **2003**, *5*, 447. Ishida, K.; Okita, Y.; Matsuda, H.; Okino, T.; Murakami, M. *Tetrahedron* **1999**, *55*, 10971.
(39) Valls, N.; Vallribera, M.; Lopez-Canet, M.; Bonjoch, J. *J. Org. Chem.* **2002**, *67*, 4945.
(40) Tao, J.; McGee, K. *Org. Proc. Res. Dev.* **2002**, *6*, 520.
(41) Dragovich, P. S.; Prins, T. J.; Zhou, R.; Brown, E. L.; Maldonado, F. C.; Fuhrman, S. A.; Zalman, L. S.; Tuntland, T.; Lee, C. A.; Patick, A. K.; Matthews, D. A.; Hendrickson, T. F.; Kosa, M. B.; Liu, B.; Batugo, M. R.; Gleeson, J.-P. R.; Sakata, S. K.; Chen, L.; Guzman, M. C.; Meador, J. W.; Ferre, R. A.; Worland, S. T. *J. Med. Chem.* **2002**, *45*, 1607.
(42) van Langen, L. M.; van Rantwijk, F.; Sheldon, R. A. *Org. Proc. Res. Dev.* **2003**, *7*, 828.
(43) Feliste, R.; Delebassee, D.; Simon, M. F.; Chap, H.; Derfreyn, G.; Vallee, E.; Douste-Blazy, L.; Maffrand, J. P. *Thromb. Res.* **1987**, *48*, 403.

TABLE 2. Relative Racemization Rates of Substrates 1−14, Using *Lactobacillus paracasei* DSM 20207

substrate	R	rel rate [%]
(*S*)-**1**	CH_3-	100
(*S*)-**2**	CH_3-CH_2-	85
(*R*)-**3**	CH_3-$(CH_2)_3-$	80
(*S*)-**4**	$(CH_3)_2CH-$	2
(*S*)-**5**	$(CH_3)_2CH$-CH_2-	1.5
(*S*)-**6**	*c*-C_6H_{11}-CH_2-	3[a]
(*R*)-**7**	C_6H_5-CH_2-	59
(*S*)-**7**	C_6H_5-CH_2-	21
(*R*)-**8**	C_6H_5-$(CH_2)_2-$	47
(*S*)-**8**	C_6H_5-$(CH_2)_2-$	35
(*S*)-**9**	*p*-F-C_6H_4-CH_2-	19
(*S*)-**10**	*p*-Cl-C_6H_4-CH_2-	22
(*S*)-**11**	*p*-Br-C_6H_4-CH_2-	27
(*S*)-**12**	*p*-HO-C_6H_4-CH_2-	8
(*S*)-**13**	C_6H_5-	25
(*R*)-**14**	*o*-Cl-C_6H_4-	24

[a] Strain DSM 20008 was used.

In general, straight-chain α-hydroxycarboxylic acids (**2**, **3**) gave better results than bulky branched-chain or alicyclic analogues (**4**−**6**), which can be explained by steric hindrance. Among the aryl−alkyl derivatives, phenyl lactate (**7**) was a better substrate than 2-hydroxy-4-phenylbutanoate (**8**). *p*-Halo-substituents on **7** were tolerated rather well, albeit at slight reduced racemization rates. Low values were obtained for the polar (and thus heavily hydrated) *p*-hydroxy analogue **12**. (*S*)-Mandelate (**13**) and even its sterically demanding *o*-chloro analogue (**14**) were converted at fair rates (25%).

Overall, good racemization rates of up to 85% for aliphatic and up to 59% for arylaliphatic compounds were achieved, relative to lactate. In addition, the reactions proved to be essentially clean and less than ∼5% of side products could be detected. It should be noted that attempts to racemize **7** and **8** under conventional conditions (aq pH 2 to 12, 100 °C, 48 h) were unsuccessful.

Close monitoring of the progress of racemization over time for both enantiomers of substrates **7** and **8** with *Lactobacillus paracasei* DSM 20207 revealed that in both cases, the (*R*)-enantiomers were racemized more rapidly than the corresponding (*S*)-counterparts. Such "nonsymmetric" kinetics are not uncommon for enzyme-catalyzed racemization reactions.[44] Due to the diastereomeric interaction of substrate enantiomers with the chiral (bio)-catalyst, the individual K_M and k_{cat} values of enantiomers may differ to a significant extent. However, since these kinetic effects are opposite, they cancel out with respect to the overall racemization rate, and as a consequence, the respective values for the reaction rates of enantiomers (expressed as k_{cat}/K_M) become very similar[45−48] and the equilibrium of the reaction is close to[49] (or at[50]) the racemate.

In summary, clean biocatalytic racemization of a range of pharmaceutically important aliphatic, arylaliphatic, and aromatic α-hydroxycarboxylic acids was accomplished under mild conditions with use of resting cells of *Lactobacillus* spp., most noteworthy *Lactobacillus paracasei* DSM 20207. The full potential of this novel biocatalytic activity is currently being explored in the context of the development of novel deracemization techniques. In addition, the nature of the actual enzyme(s) involved and the mechanism of action responsible for this racemization is currently being studied in detail.

Experimental Section

General. The following chemicals were purchased. (*R*)-(+)- and (*S*)-(−)-lactic acid sodium salt (**1**), (*R*)-(+)- and (*S*)-(−)-2-hydroxybutanoic acid (**2**), (D)-(−)- and (L)-(+)-3-cyclohexylalanine, (L)-(−)-tyrosine, (*R*)-(−)-2-hydroxy-3-methylbutanoic acid (**4**), (D,L)- and (L)-(−)-4-fluoro-, (L)-(−)-4-chloro- and (L)-(−)-4-bromophenylalanine, (*S*)-(+)-2-hydroxy-3-methylbutanoic acid (**4**), (*R*,*S*)- and (*S*)-(+)-2-hydroxy-4-methylpentanoic acid (**5**), (*R*)-(+)- and (*S*)-(−)-2-hydroxy-3-phenylhydroxypropanoic acid (**7**), (*R*,*S*)-2-hydroxyhexanoic acid (**3**) and (*S*)-mandelic acid (**13**), (D,L)-4-chloro- and (D,L)-4-bromophenylalanine, (*R*)-(−)- and (*S*)-(+)-2-hydroxy-4-phenylbutanoic acid ethyl ester, and (*R*,*S*)-2-hydroxy-3-(4-hydroxyphenyl)propanoic acid (**12**). (*R*)-*o*-Chloromandelic acid (**14**) was a gift from R. Gaisberger (Graz). (*S*)-2-Hydroxy-3-(4-hydroxyphenyl)propanoic acid (*S*)-**12**) was synthesized from L-tyrosine according to ref 39.

Lactobacilli were obtained from the DSMZ (Braunschweig, Germany), *Lactococci* were obtained from D. Haltrich (Vienna, Austria) and BASF AG, respectively, *Halobacteria* and *Halococci* were obtained from H. Stan-Lotter (Salzburg) and the DSMZ, respectively FCC stands for our in-house culture collection. TLC plates were run on silica gel Merck 60 (F_{254}) and compounds were visualized by spraying with Mo-reagent [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (100 g/L), $Ce(SO_4)_2\cdot 4H_2O$(4 g/L) in H_2SO_4 (10%)]. Compounds were purified by flash chromatography on silica gel Merck 60 (230−400 mesh). Optical rotation values were measured at 589 nm (Na-line) in a 1dm cuvette and are given in units of 10 deg cm^2 g^{-1}. NMR spectra were recorded in $CDCl_3$ 360 (^{1}H) and 90 (^{13}C) MHz. Chemical shifts are reported in ppm relative to TMS (δ 0.00) as internal standard, and coupling constants (J) are given in Hz. The degree of conversion (expressed as % of racemization with 100% corresponding to the racemate) and enantiomeric excesses were determined via GC or HPLC on a chiral stationary phase. HPLC analyses were carried out with a Chiralpak AD column (column A, Daicel, 0.46 cm × 25 cm). GC analyses were performed with a CP-Chirasil-DEX CB column (column B, 25 m, 0.32 mm, 0.25 μm film) or an Astec Chiraldex B-TA (column C, 30 m, 0.25 mm). H_2 was used as carrier gas.

Strains. The following strains showed racemization-activity toward one or more substrates: *Lactobacillus paracasei* DSM 20008, DSM 20207, DSM 2649, *Lactobacillus sakei* DSM 20017, *Lactobacillus halotolerans* DSM 20190, *Lactobacillus delbrueckii* DSM 20074, *Lactobacillus brevis* DSM 20054, *Lactobacillus acetotolerans* DSM 20749, *Lactobacillus oris* DSM 4864.

No racemization activity was found in the following strains: *Haloferax volcanii* DSM 5716, *Haloarcula vallismortis* DSM 3756, *Lactobacillus acidophilus* DSM 20079, *Lactobacillus piscicola* DSM 20722, *Lactobacillus confusus* DSM 20196, *Lactobacillus farciminis* DSM 20184, *Lactobacillus gasseri* DSM 20243, *Lactobacillus alimentarius* DSM 20249, *Lactobacillus jensenii* DSM 20557, *Lactobacillus kandleri* DSM 20593, *Lactobacillus fructosus* DSM 20349, *Halococcus morrhuae* DSM 1307, *Halococcus saccharolyticus* DSM 5350, *Halococcus dombrowskii* DSM 14522, *Halobacterium salinarum* DSM 3754, *Halobacterium* sp. *NRC-1* ATCC 700922, *Halococcus salifodinae* DSM 8989, *Halobacterium* sp. *A* FCC

(44) For an interesting example of an "overshoot"-reaction observed in the enzymatic racemization of D- and L-alanine using a bacterial pyridoxyl-dependent alanine racemase see: Spies, M. A.; Woodward, J. J.; Watnik, M. R.; Toney, M. D. *J. Am. Chem. Soc.* **2004**, *126*, 7464.
(45) Shibata, K.; Shirasuna, K.; Motegi, K.; Kera, Y.; Abe, H.; Yamada, R. *Comp. Biochem. Physiol. B* **2000**, *126*, 599.
(46) Watanabe, A.; Yoshimura, T.; Lim, Y. H.; Kurokawa, Y.; Soda, K.; Esaki, N. *J. Mol. Catal. B*: *Enzymol.* **2001**, *12*, 145.
(47) Weil-Malherbe, H. *Biochem. J.* **1966**, *101*, 169.
(48) Verseck, S.; Bommarius, A.; Kula, M.-R. *Appl. Microbiol. Biotechnol.* **2001**, *55*, 354.
(49) Yamada, M.; Furuhashi, K. *J. Biochem.* **1968**, *63*, 59.
(50) Hiyama, T.; Fukui, S.; Kitahara, K. *J. Biochem.* **1968**, *64*, 99.

086, *Lactococcus lactis* sp. *Cremoris* DSM 20388, *Lactococcus lactis* sp. *Cremoris* DSM 20069, *Lactococcus lactis* DSM 20729, *Lactococcus lactis* DSM 20481, and *Lactococcus* FCC 093, FCC 094, FCC 095, FCC 096, FCC 097.

FCC numbers refer to our in-house culture collection.

Medium for Active Strains. *Lactobacillus paracasei* DSM 20008, DSM 20207, DSM 2649, *Lactobacillus sakei* DSM 20017, *Lactobacillus halotolerans* DSM 20190, *Lactobacillus delbrueckii* DSM 20074, *Lactobacillus brevis* DSM 20054, and *Lactobacillus oris* DSM 4864 were grown on medium #11 as suggested by DSMZ. The following components of the medium were sterilized in five separate groups: Group I: Pepticase (10 g/L, Sigma), bacteriological peptone (10 g/L, Oxoid), yeast extract (5 g/L, Oxoid). Group II: Glucose (20 g/L, Fluka). Group III: Tween 80 (polyoxyethylene-sorbitan-monooleate, 1 g/L, Aldrich), Group IV: K_2HPO_4 (2 g/L, Merck). Group V: Na-acetate trihydrate (8.3 g/L, Fluka), $(NH_4)_2$-citrate (2 g/L, Fluka), $MgSO_4 \cdot 7H_2O$ (0.20 g/L, Fluka), $MnSO_4$ (0.05 g/L, Fluka). *Lactobacillus acetotolerans* DSM 20749 was grown in medium DSMZ #231, which is identical with medium DSMZ #11 with adjustment of the pH value to pH 5.2.

Strain Maintenance. *Lactobacilli* were maintained on agar plates, using the above-described media with the addition of agar (20 g/L). Subculturing was performed every 4 weeks, the plates were left in the incubator for 48 h at 30 °C, and long-term storage was at +4 °C. It should be noted that a strong loss of activity was observed when the cells were frozen, even in the presence of cryo-protectants, such as (i) NaCl (0.7%) and DMSO (20%) or (ii) glycerol/water (95:5 or 1:1).

Growth of Microorganisms. Strains were grown in flask cultures without shaking at 30 °C (*Lactobacillus paracasei* DSM 20008, DSM 20207, DSM 2649, *Lactobacillus sakei* DSM 20017, *Lactobacillus halotolerans* DSM 20190, *Lactobacillus brevis* DSM 20054, and *Lactobacillus acetotolerans* DSM 20749) and at 37 °C (*Lactobacillus delbrueckii* DSM 20074 and *Lactobacillus oris* DSM 4864). After transfer from agar plates, the microorganisms were grown for 3 d (*Lactobacillus sakei* DSM 20017, *Lactobacillus paracasei* DSM 20008, DSM 20207, DSM 2649, *Lactobacillus halotolerans* DSM 20190, *Lactobacillus brevis* DSM 20054, *Lactobacillus oris* DSM 4864, *Lactobacillus delbrueckii* DSM 20074) and for 11 d (*Lactobacillus acetotolerans* DSM 20749). Then the cells were harvested by centrifugation (18 000 × g), washed twice with BIS-TRIS buffer (50 mM, 10^{-2} M $MgCl_2$, pH 6), lyophilized, and stored at +4 °C.

General Screening Procedure for Biocatalytic Racemization. For the screening, 50 mg of whole lyophilized cells was rehydrated in 0.5 mL of aqueous BIS-TRIS buffer (50 mM, 10^{-2} M $MgCl_2$, pH 6) for 1 h at 42 °C with shaking at 150 rpm. Substrate (5 mg) was added followed by shaking of the reaction mixture with 150 rpm at 42 °C for 24 h. Then the reaction mixture was acidified with 2 M HCl (1 drop) and the cells were removed by centrifugation. The supernatant was extracted with Et_2O and the organic phase was dried with Na_2SO_4. The determination of conversion and enantiomeric excess was carried out by GC or HPLC on a chiral stationary phase. For HPLC determination, the organic phase was evaporated under reduced pressure and the residue was dissolved in HPLC eluent (without trifluoroacetic acid).

Acknowledgment. This study was performed in cooperation with BASF AG (Ludwigshafen) within the Competence Center *Applied Biocatalysis* and financial support from the FFG, the City of Graz, the Province of Styria, and BASF AG is gratefully acknowledged. R. Gaisberger (Graz) and Ciba Specialty Chemicals (Basel) are thanked for the generous supply with chiral materials. D. Haltrich (Vienna) and H. Stan-Lotter (Salzburg) are thanked for the generous donation of *Lactococci* and *Halococci*, respectively.

Supporting Information Available: Synthesis of substrates and reference materials and procedures for GC and HPLC analyses and retention times. This material is available free of charge via the Internet at http://pubs.acs.org.

JO050156N