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Biocatalytic racemisation of α-hydroxycarboxylic acids at physiological conditions†

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Biocatalytic racemisation of aliphatic, aryl-aliphatic and aromatic α-hydroxycarboxylic acids was accomplished using whole resting cells of *Lactobacillus paracasei* DSM 20207; the mild (physiological) reaction conditions ensured an essentially 'clean' isomerization in the absence of side reactions, such as elimination or decomposition.

Racemisation is an entropy-driven 'downhill-reaction' going in hand with a loss of enantiomeric purity[1] and is therefore generally considered as an unwanted side-reaction rather than a synthetically useful transformation. As a consequence, the controlled racemisation of organic compounds has been scarcely studied.[2] It was only recently that the need for 'clean' racemisation protocols had been recognized, in particular due to the increasing demand for so-called deracemisation processes, which allow the transformation of a racemate into a single stereoisomeric product in 100% theoretical yield.[3,4] Detailed analysis of the (chemical) racemisation protocols published so far[2] reveal that typical reaction conditions employ strongly acidic or basic conditions, which are incompatible with a stereoselective (catalytic) *in situ* transformation and are thus of limited use for dynamic kinetic resolutions. In order to circumvent this limitation, enzymatic racemisation (taking place under ambient reactions conditions) holds great potential.[5]

During our studies directed towards the deracemisation of α-hydroxycarboxylic acids by coupling lipase-catalyzed acyl-transfer to the racemisation of the non-reacted substrate enantiomer using mandelate racemase [EC 5.1.2.2][6] we encountered a stringent substrate limitation for the latter enzyme: although mandelate racemase was very tolerant towards various β,γ-unsaturated α-hydroxycarboxylic acids,[7] 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.[8]

In order to extend the applicability of our deracemisation protocol towards saturated α-hydroxycarboxylic acids, a matching isomerase enzyme was required. Analysis of the biochemical literature revealed the existence of a promising candidate: lactate racemase [EC 5.1.2.1]. The respective enzymatic activity was reported in various microbial strains;[9] however, detailed knowledge of the properties of this enzyme and its mode of action[10] is rather scarce. Furthermore, the biocatalytic racemisation of saturated α-hydroxycarboxylic acids other than lactate has not been studied to date.

† Electronic supplementary information (ESI) available: experimental section and chemical structures. See http://www.rsc.org/suppdata/cc/b4/b418786e/

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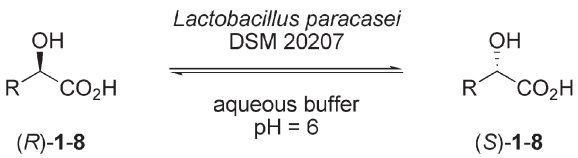
Prompted by reports on lactate racemase activity in anaerobic rumen bacteria (*Clostridium butylicum*, *Selenomonas ruminantium* and *Megasphaera elsdenii*[10b]), halophiles (*Halobacterium* and *Haloferax* spp.[11]) and *Lactobacillus* spp.,[12] we initiated a screening for the respective enzymatic activity among both of the latter genera for their ease of cultivation.†[13] Whereas no activity could be found among halophiles, several positive hits were identified among *Lactobacilli*, the most active strain being *Lactobacillus paracasei* DSM 20207.

The racemisation of substrates **1–8** was tested using rehydrated lyophilized (resting) cells of *Lactobacillus paracasei* DSM 20207 (Scheme 1, Table 1) in aqueous buffer at pH 6. Fast rates were observed with the 'natural' substrate, lactate (*S*)-**1**. For reasons of comparison, its relative rate was arbitrarily set as standard (100%). Almost equally fast racemization of the straight-chain hexanoic acid analogue (*R*)-**2** (relative rate 80%) indicated a highly desirable relaxed substrate tolerance of the system. However, reduced rates were found with sterically demanding aliphatic α-hydroxycarboxylic acids (*S*)-**3** and (*S*)-**4**[14] bearing branched or alicyclic side chains. On the other hand, good racemisation rates were obtained with aryl-alkyl derivatives **5** and **6** (up to 59% relative to lactate). 3-Phenyl-lactate (**5**) and aryl-substituted derivatives thereof are frequently used chiral components of pharmaceuticals, such as rhinovirus protease inhibitors[15] and



Scheme 1 Biocatalytic racemisation of α-hydroxycarboxylic acids.

Table 1 Relative rates of racemisation of substrates **1–8**

Substrate	R	Relative rate (%)[a]
(*S*)-**1**	$-CH_3$	100
(*R*)-**2**	$-(CH_2)_3CH_3$	80
(*S*)-**3**	$-CH(CH_3)_2$	2
(*S*)-**4**	$-CH_2$-cC_6H_{11}	~1
(*R*)-**5**	$-CH_2Ph$	59
(*S*)-**5**	$-CH_2Ph$	21
(*R*)-**6**	$-(CH_2)_2Ph$	47
(*S*)-**6**	$-(CH_2)_2Ph$	35
(*S*)-**7**	$-C_6H_5$	25
(*R*)-**8**	-*o*-Cl-C_6H_4	24

[a] Relative rates were calculated from initial progress curves at conversion of ⩽5%; the racemisation rate of the 'natural' substrate (*S*)-**1** was arbitrarily set as standard (100%).

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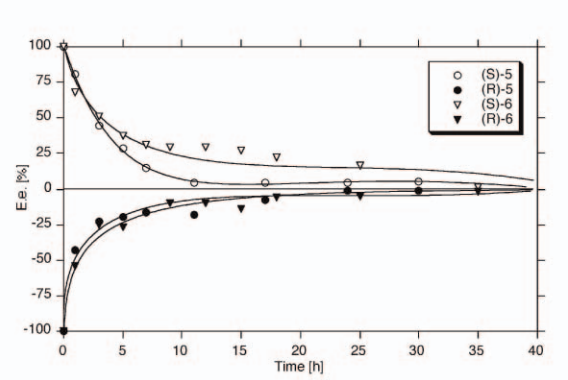

Fig. 1 Time course of the biocatalytic racemisation of the enantiomers of **5** and **6**.

natural antibiotic agents,[16] and are particularly difficult to racemise using conventional methods due to the ease of their elimination, forming cinnamic acid. Compound (*R*)-**6** is an important building block for the synthesis of anti-hypertensive agents and ACE-inhibitors.[17] The remarkable flexibility of this biocatalytic system was demonstrated by the fact that also (*S*)-mandelate (**7**) was accepted at a fair rate (25%). Most importantly, *o*-chloromandelate (**8**), which is a non-substrate for mandelate racemase,[7] was similarly well accepted (24%). The (*R*)-enantiomer of the latter compound is a key intermediate for the synthesis of anticoagulant agents used in cardiovascular therapy (Clopidogrel/Plavix).[18]

Close monitoring of the progress of racemisation over time for both enantiomers of substrates **5** and **6** revealed that both of the (*R*)-enantiomers were racemised more rapidly than the corresponding (*S*)-counterparts (Fig. 1). Such 'nonsymmetric' kinetics is not uncommon for enzyme-catalysed racemisation and is caused by the difference in K_M and k_{cat} values of enantiomers.[19] Overall, the reactions proved to be essentially clean and less than $\sim$5% of side products could be detected. It should be noted that attempts to racemise **5** and **6** under conventional conditions (aq. pH 2–12, 100 °C, 48 h) were unsuccessful.

In summary, clean biocatalytic racemisation of structurally diverse aliphatic, aryl-aliphatic and aromatic α-hydroxycarboxylic acids was accomplished under mild conditions using resting cells of *Lactobacillus paracasei* DSM 20207. The scope and limitations of this novel biocatalytic activity, the nature of the actual enzyme(s) involved and the mechanism of action responsible for this racemisation is currently being studied in detail.

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