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Biocatalytic racemization of synthetically important functionalized α-hydroxyketones using microbial cells

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Abstract—Biocatalytic racemization of straight-chain and cyclic acyloins bearing (halo)alkyl, alkenyl and functionalized (hetero)aryl moieties was accomplished using whole resting cells of bacteria, fungi and yeasts. Mild physiological reaction conditions ensured the suppression of undesired side-reactions, such as elimination or condensation. This biocatalytic protocol represents a useful tool for the clean racemization of unwanted enantiomers of synthetically important α-hydroxyketones derived from kinetic resolution.

1. Introduction

Racemization is an irreversible entropy-driven isomerization reaction, leading to a loss of enantiomeric purity, as discovered by Pasteur in 1853.[1] Since it usually goes in hand with a loss of the 'chiral value' of materials, it has generally been considered as an unwanted side-reaction rather than a synthetically useful transformation. As a consequence, the controlled racemization of organic compounds has scarcely been studied.[1,2] It was only until recently, that the need for 'clean' racemization protocols had been recognized due to the increasing demand for the 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 increasing pressure to enhance the economic balance of chemical processes on an industrial scale, starting from racemates.

Detailed analysis of the (chemical) racemization protocols published so far[2] reveals that the vast majority of protocols employed for racemization strongly favour acidic or basic media, which are incompatible with the presence of an enantioselective (catalytic) transformation of the racemic substrate. As a consequence, traditional chemical protocols for racemization are of limited use for dynamic kinetic resolutions.[4–7] In order to circumvent this limitation, enzymatic racemization, which takes place under ambient reactions conditions, typically at room temperature, atmospheric pressure and neutral pH, holds great potential, since it largely avoids the formation of by-products and thus renders a 'clean' racemization.[8]

Although a number of stereochemically labile compounds, such as hydantoins, cyanohydrins, hemi-(thio)acetals and -aminals were successfully subjected to dynamic kinetic resolution due to the ease of (spontaneous) substrate racemization; strong racemization catalysts, which are usually dependent on transition metals,[4,6,7] aluminium complexes[9] and zeolites,[10] are required for the isomerization of chirally stable hydroxy compounds, such as *sec*-alcohols or hydroxycarboxylic acids.

Due to the high specificity of biochemical processes in general, Nature has little need for racemization and therefore the number of 'true' racemases is very limited.[2,8,11,12] The best studied 'true' racemase so far, mandelate racemase, was shown to be very tolerant towards a wide range of β,γ-unsaturated α-hydroxycarboxylic acids.[13,14] However, saturated (aliphatic) analogues were not accepted at all. This limitation was successfully overcome by the use of

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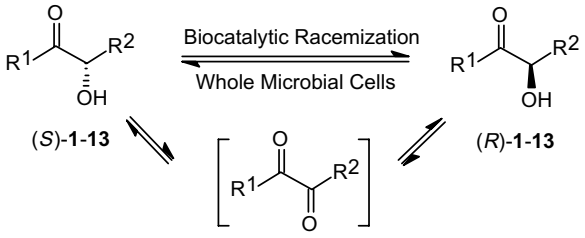
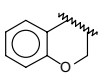
whole resting cells of *Lactobacillus* spp., which allowed the clean racemization of a wide range of aliphatic, (hetero)aryl- and aryl-aliphatic α-hydroxycarboxylic acids.[15,16] However, attempts to identify a distinct 'α-hydroxyacid racemase' related to lactate racemase[17,18] in these strains were unsuccessful. In contrast, subsequent inhibitor-studies suggested that in *Lactobacillus* spp., the racemization of α-hydroxycarboxylic acids proceeded through an equilibrium-controlled oxidation–reduction sequence via the corresponding α-ketoacid as a non-chiral intermediate.[16] Surprisingly, *Lactobacillus* spp. also accepted a few simple non-functionalized α-hydroxyketones (acyloins) to a certain extent.[19] Biochemical studies on benzoin showed that the racemization predominantly proceeded via a redox-process involving the corresponding α-diketone (benzil) as intermediate[20] rather than through the action of a single isomerase enzyme in relation to acetoin racemase.[21]

In order to exploit the functional group tolerance of this useful biotransformation, we initiated a search for organisms, which would racemize a wide range of functionalized acyloins, that possess either interesting bioactivity themselves or serve as chiral building blocks for the synthesis of pharmaceuticals (Scheme 1).

Compound **2** and the closely related derivatives thereof have been used as building blocks for the synthesis of epothilones, which exhibit cytotoxic effects resembling those of paclitaxel (Taxol), and are active against multi-drug resistant cell lines.[22] Compound **4** is a key intermediate for norephedrine derivatives.[23] Haloaryl-substituted acyloins **5** and **6** are used for the synthesis of 2-HPP derivatives, such as the antidepressant Bupropion and the smoking cessation agent Zyban. Compound **6** is a starting material for antifungal agents Ro 09-3355 and SM 8668/Sch 39304 and the norephedrine inhibitor 1555U88.[24] Compound (*S*)-**7** is used in the preparation of pharmaceuticals, which induce cell apoptosis on human oral tumour cell lines and are thus regarded as lead compounds in anticancer therapy.[25,26] The enantiomers of α-furoin **8** and α-pyridoin **9** are frequently encountered as structural subunits in many natural products, such as farnesyltransferase inhibitors kurasoin A and B, antitumour antibiotics olivomycin A and chromomycin A_3 and inhibitors of amyloid-β (Aβ) protein production and inducers of tumour apoptosis.[26] Non-racemic 2-hydroxypropiophenone derivatives **10** and **11** were used for the stereoselective syntheses of antifungal agents[27] and the cytokine modulator cytoxazone, the taxol side chain and the multidrug pump inhibitor 5′-methoxyhydrocarpin.[28] The cyclic derivative **12** was used for the synthesis of the insect deterrent compounds haplophytine[29] and quinoxaline derivatives used as agrochemical and pharmaceutical agents.[30] Compound **13** is an important precursor for the synthesis of HIV inhibitors[31] and benzopyrane type potassium channel openers.[32,33]



Substrate	R^1	R^2
(*R*)-**1**	CH_3-	CH_3-
(*R*)-**2**	CH_3-	$(CH_3)_2C{=}CH\text{-}CH_2$-
(*R*)-**3**	*n*-C_3H_7-	*n*-C_3H_7-
(*R*)-**4**	CH_3-	4-*t*-BuO-C_6H_4-
(*R*)-**5**	3-Cl-C_6H_4-	CH_3-
(*R*)-**6**	3,5-di-F-C_6H_3-	CH_3-
(*S*)-**7**	Ph-	CF_3-
(*R*)-**8**	2-furyl-	2-furyl-
(*R*)-**9**	2-pyridyl-	2-pyridyl-
(*R*)-**10**	2-MeO-C_6H_4-	2-MeO-C_6H_4-
(*R*)-**11**	4-MeO-C_6H_4-	4-MeO-C_6H_4-
(*R*)-**12**	-$(CH_2)_4$-	
(*R*)-**13**		

Scheme 1. Biocatalytic racemization of functionalized acyloins **1**–**13**.

2. Results and discussion

In order to elucidate the full potential of this mild biocatalytic racemization, we investigated the substrate spectrum of various microbial cells, which emerged as top candidates from our initial screening for racemization activity towards *sec*-alcohols and α-hydroxyketones.[20] In order to cover a reasonably broad range of substrates, straight-chain and cyclic acyloins **1**–**13** bearing (halo)alkyl, alkenyl and functionalized (hetero)aryl moieties at both ends were chosen.

In order to obtain a set of quantitative data of comparable activities, the relative rates of racemization using *Lactobacillus paracasei* DSM 20207 were calculated from the slope of initial progress curves of the decline of ee versus time at a conversion of <5%. For the sake of comparison, the activity of the fastest substrate acetoin (*R*)-**1** was arbitrarily set as standard (100%).[19]

The relative activities depicted in Table 1 show that steric requirements play a dominant role: The smallest straight-chain substrate (acetoin, **1**) was racemized at the fastest rate (100%). The introduction of a single (branched) alkenyl chain (substrate **2**) was tolerated, but led to a decreased rate of 42%. A further increase of the steric bulk by shifting the functional group towards the centre of the molecule caused a dramatic drop in activity thus compound **3** turned out to be a non-substrate (~1%). On the other hand, sterically demanding aromatic groups R^2 bearing various substituents were tolerated, as long as the opposite substituent R^1 remained small (e.g., methyl, trifluoromethyl) and substrates **4**–**7** were racemized at encouraging levels with relative rates ranging from 31% to 75%. To

Table 1. Racemization of acyloins **1**–**13** using *Lactobacillus paracasei* DSM 20207

Substrate	Time [h]	ee [%]	Time [h]	ee [%]	Rel. rate[a] [%]
(*R*)-**1**	6	19	48	6	100
(*R*)-**2**	24	52	72	6	42
(*R*)-**3**	24	99	72	98	1
(*R*)-**4**	24	73	72	8	31
(*R*)-**5**	24	16	72	2	75
(*R*)-**6**	24	23	72	5	45
(*S*)-**7**	24	64	72	4	36
(*R*)-**8**	24	8	72	1	34
(*R*)-**9**	24	99	72	98	1
(*R*)-**10**	24	86	72	77	2
(*R*)-**11**	24	53	72	2	20
(*R*)-**12**	24	40	72	6	13
(*R*)-**13**[b]	24	66	72	65	8[c]

[a] Relative racemization rates were determined from the steady slope of the decline of enantiomeric excess versus time during the onset of the reaction at a conversion of <5%. The activity of acetoin (*R*)-**1** was arbitrarily set as standard (100%).
[b] Compound **13** had an ee 82% at the start.
[c] The relative rate was corrected by extrapolation of the initial ee of 82% to 100%.

our delight, two furyl moieties were tolerated rather well (substrate **8**, relative rate 34%), whereas the sterically more demanding bis-pyridyl derivative **9** was not accepted. Again, strong steric effects were observed for aryl-substituted benzoin derivatives: whereas substituents at the *o*-position were detrimental (substrate **10**, relative activity ~2%), *p*-substituents were nicely tolerated (substrate **11**, relative rate 20%). The rigid cyclic derivative **12** was accepted at a modest rate of 13%, and α-hydroxychromanone **13** was slowly racemized at a rate of 8%.

Since our previous studies suggested that the racemization of α-hydroxycarboxylic acids proceeded through the action of stereo-complementary α-ketoacid reductases/α-hydroxyacid dehydrogenases,[34–36] we anticipated that the redox-enzymes required for acyloins would be presumably α-diketone reductases.[37–40] Along these lines, we identified several promising candidates among bacteria, fungi and yeasts, belonging to the genus *Pseudomonas*, *Alcaligenes*, *Bacillus*, *Agrobacterium*, *Helminthosporium*, *Rhizopus*, *Syncephalastrum* and *Candida* spp., which showed enhanced racemization rates compared to *Lactobacillus* spp. In order to investigate their functional group tolerance, they were tested on substrates **1**–**13**. The best activities from this screening are shown in Table 2.[41]

Complete racemization of substrate **2** was obtained with two bacteria (*Rhodococcus ruber*, *Nocardia*, entries 1 and 3) and the fungus *Beauveria bassiana* (entry 13). Surprisingly, the 'internal' acyloin **3**, which was not accepted by *Lactobacillus paracasei* DSM 20207 due to steric hindrance, was racemized nicely by *Bacillus megaterium* (entry 6) and two fungi (*Aspergillus niger* and *Helminthosporium*, entries 10 and 14). Substrate **5** was not only racemized by *Lactobacillus* DSM 20207, but also by *Agrobacterium* FCC 148, *A. niger* DSM 821 and *Botrytis cinerea* DSM 877. Compound **9** (a non-substrate for *L. paracasei*) was also difficult to racemize with the majority of strains. However, acceptable rates were obtained with *R. ruber* (entry 1). Similar positive results were obtained for the bulky *o*-substituted bis-aryl derivative **10**, which was racemized at a fair rate using *Pseudomonas putida* (entry 4). Complete racemization of the cyclic substrate **12** (a slow substrate for *L. paracasei*) was achieved by several strains of bacteria and fungi (entries 1, 4, 8, 11 and 15). The activities of yeast

Table 2. Screening of whole lyophilized microbial cells for the racemization of selected acyloins

Entry	Substrate	ee [%][a]						
	Organism	(*R*)-**2**	(*R*)-**3**	(*R*)-**5**	(*R*)-**9**	(*R*)-**10**	(*R*)-**12**	(*R*)-**13**[b]
1	*Rhodococcus ruber* DSM 44540	**5**	69	40	**56**	86	**6**	**6**
2	*Nocardia H* FCC 147[c]	29	70	31	83	52	28	34
3	*Nocardia G* FCC 146[c]	**6**	91	52	82	82	33	42
4	*Pseudomonas putida* ATCC 47054	19	73	53	83	**36**	**4**	**18**
5	*Alcaligenes faecalis* DSM 13975	33	93	68	91	57	82	41
6	*Bacillus megaterium* DSM 32	18	**8**	53	89	89	18	87
7	*Streptomyces caeruleus* DSM 40088	24	89	45	86	87	61	42
8	*Agrobacterium tumefaciens* FCC 148[c]	63	91	**2**	89	84	**3**	46
9	*Geotrichum candidum* DSM 6401	94	91	58	77	83	32	78
10	*Aspergillus niger* DSM 821	21	**9**	**2**	89	89	31	73
11	*Penicillium simplicissimum* FCC 072[c]	67	90	45	87	77	**4**	53
12	*Botrytis cinerea* DSM 877	52	93	**1**	70	90	19	73
13	*Beauveria bassiana* ATCC 1344	**9**	87	53	89	89	28	73
14	*Helminthosporium* sp. NRRL 4671	24	**9**	51	87	87	91	61
15	*Rhizopus oryzae* DSM 906	53	91	49	87	74	**8**	76
16	*Syncephalastrum racemosum* ATCC 18192	38	65	52	90	90	83	67
17	*Candida parapsilosis* DSM 70125	26	90	35	87	87	39	38
18	*Kluyveromyces lactis* DSM 3795	90	91	50	91	91	18	**24**

[a] Residual enantiomeric excess of the substrate under standard conditions after 72 h.
[b] Compound **13** had an ee 82% at the start.
[c] FCC stands for our in-house strain collection.

strains (entries 17 and 18) were modest in comparison to bacteria and fungi. Even the bulky compound **13** was completely isomerized by *R. ruber* (entry 1) and was racemized with acceptable rates by a bacterium and a yeast strain (entries 4 and 18). Overall, the extended set of microbial strains depicted in Table 2 offered superior racemization activities, in particular for substrates **3**, **9**, **10** and **13**, which previously turned out to be slow substrates for our champion, *L. paracasei*.

In order to test whether the two adjacent *sec*-alcohol centres could be isomerized through biocatalytic racemization and epimerization as well, (*R*,*R*)-1,2-diphenylethane-1,2-diol (hydrobenzoin) was tested. Unfortunately, the solubility of this solid compound in aqueous systems was too low for practical tests. Attempts to use solubilizing agents, such as *N*-methylpyrrolidone or Tween 20, or organic solvents, such as hexane, DMF and *t*-BuOMe, in one- or two-phase systems, respectively, did not yield any useful activities. Finally, we tested eight different ionic liquids as non-conventional solvents.[42] The latter were found to represent useful alternatives to organic solvents, mainly for the class of hydrolases.[43,44] To our regret, no reproducible results could be obtained, which can be explained by the fact that the polar nicotinamide cofactors involved in the oxidation–reduction process cannot be exchanged between the dehydrogenase enzymes responsible for the racemization.

3. Conclusion

In conclusion, biocatalytic racemization of synthetically important functionalized acyloins was accomplished using whole lyophilized cells of various bacteria, fungi and yeast strains. Due to the mild reaction conditions, isomerizations proved to be essentially 'clean' and undesired side-reactions commonly observed in chemical racemization protocols, such as elimination and/or condensation reactions were largely absent. The only 'side products' detected in trace amounts during the course of the racemization were the corresponding α-diketones, which represent the achiral intermediate, and (in rare cases) regio-isomeric acyloins, which are presumably formed via an enzymatic hydride addition onto the 'wrong' carbonyl moiety of the α-diketone intermediate.[45] Overall, this biocatalytic racemization protocol represents a useful tool for the clean racemization of unwanted enantiomers of synthetically important acyloins derived from kinetic resolution.

4. Experimental

4.1. General

The following chemicals were purchased and used as received: (Alfa Aesar) 3′,5′-difluoropropiophenone, *meso*-hydrobenzoin; (Sigma Aldrich) $Mn(OAc)_3{\cdot}2H_2O$, 3′-chloropropiophenone, α-furoin, α-pyridoin, anisoin, 2-hydroxycyclohexanone, 5-hydroxy-4-octanone, (*S*,*S*)-hydrobenzoin, (*R*,*R*)-hydrobenzoin, hydrobenzoin, 6-methyl-5-hepten-2-one, 4-chromanone. *rac*-2,2′-Dimethoxybenzoin (*rac*-**10**) was a gift from B. Larissegger-Schnell (Graz). Substrate **4** was kindly provided by BASF AG (Ludwigshafen).

NMR spectra were recorded in $CDCl_3$ using a Bruker AMX 360 at 360 (^1H) and 90 (^{13}C) MHz. Chemical shifts are reported relative to TMS (δ 0.00) and coupling constants (J) are given in Hz. 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%)]. The degree of conversion (expressed as % of racemization with 100% corresponding to the racemate) and enantiomeric excess were determined via GC or HPLC on a chiral stationary phase. GC analyses were carried out on a Varian 3900 gas chromatograph equipped with an FID detector using a Chrompack Chirasil-DEX CB column (VARIAN, 25 m × 0.32 mm × 0.25 μm, 1.0 bar H_2). HPLC analyses were carried out on a Shimadzu HPLC system equipped with DGU-20A5 degasser, LC 20AD liquid chromatograph, SIL 20AC autosampler, CBM 20A communications bus module, SPD M20A diode array detector and CTO 20AC column oven using a Chiralpak AD column (0.46 × 25 cm, DAICEL). Melting points were obtained on a Gallenkamp melting point apparatus MFB-595 in open capillary tubes. Optical rotation values ($[\alpha]_D^{20}$) were measured on a Perkin–Elmer polarimeter 341 at 589 nm (Na-line) in a 1 dm cuvette and are given in units of 10 deg cm^2 g^{-1}.

4.2. General procedure for the biocatalytic racemization of acyloins

Lyophilized cells (50 mg) were rehydrated in buffer: bacteria, fungi and yeasts (TRIS buffer, 0.5 mL, 100 mM, pH 7.5, 30 °C) and *Lactobacillus* sp. (BIS–TRIS buffer, 50 mM, 10^{-2} M $MgCl_2$, pH 6, 42 °C) for 1 h with shaking at 170 rpm. Substrate (5 mg) was then added followed by the shaking of the reaction mixture with 150 rpm at 30 °C and 42 °C (*Lactobacillus* sp.), respectively. After a given time, the biotransformation was stopped by the addition of ethyl acetate (0.8 mL), extracted and the organic phase dried with sodium sulfate. The determination of conversion and the enantiomeric excess was carried out by GC or HPLC on a chiral stationary phase. For HPLC-analysis, the organic phase was evaporated under reduced pressure and the residue dissolved in HPLC eluent.

4.3. Synthesis of substrates

4.3.1. Synthesis of (*R*)-3-hydroxy-butan-2-one (*R*)-1.[46] Vinyl acetate (5 mL) and lipase powder (Lipase Amano PS, 320 mg) were added to a solution of *rac*-3-hydroxy-butan-2-one (1 g, 11.3 mmol) in *t*-BuOMe (30 mL). The mixture was vigorously shaken at 30 °C and after 48 h, the enzyme was removed by filtration and the solvent was evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**1** (410 mg, 41%, ee >99%). $[\alpha]_D^{20} = -61.4$ (*c* 0.2, EtOH); $[\alpha]_D^{20} = -58.6$ (*c* 0.12, H_2O).[47] ^1H NMR (360 MHz, $CDCl_3$) $\delta = 1.40$ (3H, d, $J = 7.0$ Hz, CH_3), 2.14, (1H, s, OH), 2.17 (3H, s, CH_3), 5.08 (1H, q, $J = 7.1$ Hz, C*H*–OH).

4.3.2. Derivatization of (*R*)-1 to the corresponding benzoyl ester for HPLC analysis. A sample of (*R*)-**1** (5 mg) in dichloromethane was derivatized with benzoic acid anhydride (5 mg) in the presence of a catalytic amount of *p*-dimethylaminopyridine (DMAP). After 15 min of stirring at room temperature, the reaction mixture was quenched with water. The organic layer was dried over sodium sulfate, the solvent was evaporated and the product was dissolved in a mixture of *n*-heptane/*i*-propanol 90:10, the solution was directly used for HPLC analysis. ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.42$ (3H, d, $J = 5.8$ Hz, CH_3), 2.16 (1H, s, OH), 5.38 (1H, q, $J = 6.3$ Hz, CH), 7.27–7.50 (3H, m, $J = 5.3$ Hz, aryl), 8.14 (2H, d, $J = 8.0$ Hz, aryl).

4.3.3. Synthesis of (*R*)-1-hydroxy-1-(4-*t*-butoxyphenyl)-2-propanone (*R*)-4. A technical-grade sample provided by BASF AG was purified by flash chromatography (petroleum ether/ethyl acetate 9:1) to give (*R*)-**4** as white crystals. $[\alpha]_D^{20} = -49.3$ (*c* 1.8, $CHCl_3$); mp 36 °C, ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.3$ (9H, s, CH_3), 2.1 (1H, s, OH), 2.2 (3H, s, C*H*$_3$–C═O), 5.1 (1H, s, C*H*–OH), 7.0 (2H, d, $J = 8.5$ Hz), 7.21 (2H, d, $J = 8.5$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 25.2$, 28.8, 78.8, 79.6, 124.5, 127.9, 132.6, 155.9, 207.3.

4.3.4. General procedure for the α-acetoxylation of ketones. Compounds *rac*-3-acetoxy-6-methyl-5-hepten-2-one *rac*-**2a**, *rac*-1-(3-chlorophenyl)-2-acetoxypropan-1-one *rac*-**5a** *rac*-1-(3,5-difluorophenyl)-2-acetoxypropan-1-one *rac*-**6a** and *rac*-3-hydroxychroman-4-one acetate *rac*-**13a** were obtained from the corresponding ketones using the following procedure adapted from the literature.[48,49]

A solution of α,β-unsaturated ketone (5 mmol) and $Mn(OAc)_3{\cdot}2H_2O$ (20 mmol) in cyclohexane (50 mL) was stirred under reflux using a Dean-Stark apparatus, during which the dark brown colour of $Mn(OAc)_3{\cdot}2H_2O$ gradually disappeared. After the starting material was consumed, the reaction mixture was diluted with diethyl ether and washed with brine. The resulting organic phase was directly filtered through a pad of silica, dried over sodium sulfate and concentrated under vacuum. The residue was purified by flash chromatography (petroleum ether/ethyl acetate 9:1).

4.3.4.1. *rac*-3-Acetoxy-6-methyl-5-hepten-2-one *rac*-2a. 6-Methyl-5-hepten-2-one (1 g, 8 mmol) and $Mn(OAc)_3{\cdot}2H_2O$ (2.66 g, 10 mmol) gave *rac*-**2a** as yellow oil (0.92 g, 58%); ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.14$ (3H, t, $J = 7.1$ Hz, CH_2–*C*H_3), 1.61 (3H, s, CH_3), 1.67 (3H, s, CH_3), 2.09 (1H, s, OH), 2.21–2.27 (1H, m), 2.42–2.47 (2H, m), 4.82 (1H, t, $J = 7.1$ Hz), 5.18 (2H, t, $J = 7.1$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 10.2$, 17.6, 22.5, 25.6, 27.7, 29.8, 93.6, 122.6, 132.7, 174.8, 208.8.

4.3.4.2. *rac*-1-(3-Chlorophenyl)-2-acetoxypropan-1-one *rac*-5a. 3′-Chloropropiophenone (1 g, 6 mmol) and $Mn(OAc)_3{\cdot}2H_2O$ (1.99 g, 7.4 mmol) gave *rac*-**5a** as yellow oil (1.34 g, 94%). ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.20$ (3H, t, $J = 7.2$ Hz), 1.62 (3H, d, $J = 7.1$ Hz), 2.93 (2H, q, $J = 7.4$ Hz), 5.64 (1H, q, $J = 3.5$ Hz), 7.29 (1H, t, $J = 3.1$ Hz), 7.51 (1H, d, $J = 7.1$ Hz), 7.60 (1H, d, $J = 7.3$ Hz), 7.96 (1H, s); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 9.2$, 14.0, 28.9, 70.4, 127.0, 128.7, 129.9, 133.0, 134.2, 137.8, 190.1, 206.9.

4.3.4.3. *rac*-1-(3,5-Difluorophenyl)-2-acetoxypropan-1-one *rac*-6a. 3′,5′-Difluoropropiophenone (1 g, 6 mmol) and $Mn(OAc)_3{\cdot}2H_2O$ (3.3 g, 12.3 mmol) gave *rac*-**6a** as yellow oil (1.05 g, 75%). ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.58$ (3H, d, $J = 6.8$ Hz, CH_3), 2.01 (3H, s, OCH_3), 5.80 (1H, m, C*H*–OH), 6.74–7.32 (2H, tt, $J_1 = 8.2$ Hz, $J_2 = 2.4$ Hz, aryl), 7.74–8.15 (1H, m, aryl); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 17.1$, 20.4, 83.9, 109.1 (d, $J = 25$ Hz), 111.7 (d, $J = 20$ Hz), 112.4, 138.2, 165.2 (d, $J = 248$ Hz), 172.3, 198.9.

4.3.5. General procedure for lipase catalyzed kinetic resolution. Compounds 3-hydroxy-6-methyl-5-hepten-2-one (*R*)-**2**, 1-(3-chlorophenyl)-2-hydroxypropan-1-one (*R*)-**5** and 1-(3′,5′-difluorophenyl)-2-hydroxypropan-1-one (*R*)-**6** were obtained from the corresponding racemic acetoxy ketones *rac*-**2a** *rac*-**5a** and *rac*-**6a** using the following procedure adapted from the literature.[48]

A sample of either *rac*-**2a,** *rac*-**5a** or *rac*-**6a** was dissolved in phosphate buffer (100 mM, pH 7) and CH_3CN. Lipase (Amano PS) was added after which the mixture was vigorously stirred for 48 h at 30 °C. The enzyme was removed by filtration. The products were extracted with ethyl acetate, dried over sodium sulfate and the solvent was evaporated under reduced pressure. Flash chromatography (petroleum ether/ethyl acetate 9:1) yielded enantiomerically pure 2-hydroxyketones (*R*)-**2**, (*R*)-**5** and (*R*)-**6**.

4.3.5.1. (*R*)-3-Hydroxy-6-methyl-5-hepten-2-one (*R*)-2. *rac*-3-Acetoxy-6-methyl-5-hepten-2-one *rac*-**2a** (0.92 g, 4.9 mmol) was treated with lipase (Amano PS, 130 mg) in phosphate buffer (250 mL) and CH_3CN (80 mL) for 24 h to give (*R*)-**2** as yellow oil (20 mg, 60%, ee >99%). $[\alpha]_D^{20} = +3.8$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.61$ (3H, s, CH_3), 1.67 (3H, s, CH_3), 2.09 (1H, s, OH), 2.13 (3H, s, CH_3), 2.23–2.47 (2H, dt, $J_1 = 68.9$ Hz, $J_2 = 7.3$ Hz), 4.12 (1H, t, $J = 7.5$ Hz), 5.06 (1H, m); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 19.6$, 24.1, 25.6, 29.7, 83.1, 113.6, 141.2, 205.3.

4.3.5.2. (*R*)-1-(3-Chlorophenyl)-2-hydroxypropan-1-one (*R*)-5. *rac*-1-(3-Chlorophenyl)-2-acetoxypropan-1-one *rac*-**5a** (1.34 g, 5.6 mmol) was treated with lipase (Amano PS, 200 mg) in phosphate buffer (350 mL) and CH_3CN (115 mL) for 24 h to give (*R*)-**5** as yellow oil (48 mg, 93%, ee >99%). $[\alpha]_D^{20} = +72.8$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) $\delta = 1.26$ (3H, d, $J = 7.2$ Hz, CH_3), 2.0 (1H, s, OH), 4.12 (1H, q, $J = 7.1$ Hz), 7.48–7.54 (2H, m), 7.58–7.64 (1H, m), 7.96 (1H, d, $J = 1.4$ Hz), 8.02 (1H, d, $J = 1.4$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 14.2$, 60.4, 128.0, 128.7, 130.2, 133.4, 134.2, 136.6, 171.4.

4.3.5.3. (*R*)-1-(3′,5′-Difluorophenyl)-2-hydroxypropan-1-one (*R*)-6. *rac*-1-(3′,5′-Difluorophenyl)-2-acetoxypropan-1-one *rac*-**6a** (1.05 g, 4.3 mmol) was treated with lipase (Amano PS, 150 mg) in phosphate buffer (280 mL) and CH_3CN (90 mL) for 24 h to give (*R*)-**6** as yellow oil

(30 mg, 80%, ee >99%). $[\alpha]_D^{20} = +50.0$ (*c* 1.0, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$) $\delta = 1.53$ (3H, d, $J = 7.0$ Hz, CH_3), 2.53 (1H, s, OH), 5.80 (1H, q, $J = 7.1$ Hz, C*H*–OH), 7.32 (1H, t, $J = 6.1$ Hz), 7.60 (2H, d, $J = 5.7$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 32.0$, 71.2, 108.4, 110.8, 139.8, 164.5, 198.1.

4.3.6. *rac*-3-Hydroxychroman-4-one acetate *rac*-13a was obtained as previously described.[49] (*R*)-3-Hydroxychroman-4-one (*R*)-**13**: was obtained from *rac*-**13a** by PPL catalyzed hydrolysis using xylene as co-solvent as described in literature.[49,50] Under these conditions, (*R*)-**13a** was obtained in 82% ee $[\alpha]_D^{20} = +72.9$ (*c* 0.82, $CHCl_3$); $[\alpha]_D^{20} = +54.0$ (*c* 2, $CHCl_3$).[49]

4.3.7. Synthesis of *rac*-3,3,3-trifluoro-2-hydroxy-1-phenylpropan-1-one *rac*-7. *rac*-3,3,3-Trifluoro-2-hydroxy-1-phenylpropan-1-one *rac*-**7** was obtained from the corresponding α-hydroxycarboxylic acid using the following procedure adapted from the literature.[51] Trifluoroacetic anhydride (3.6 mL, 24 mmol) was added to a stirred solution of phenyllactic acid (1 g, 6 mmol) and pyridine (2.92 mL, 36 mmol) in dry benzene (21 mL) at room temperature under an inert atmosphere and the mixture refluxed for 4 h. HCl (5%) was then added to the mixture and the solution was stirred at 60 °C for 15 min. After standard workup, the crude product was purified by column chromatography on silica gel eluting with dichloromethane to give the product *rac*-**7** as a white solid (1.02 g, 78%). ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.20$ (1H, s), 5.44 (1H, q, $J = 6.6$ Hz, C*H*–OH), 7.56 (2H, t, $J = 7.8$ Hz), 7.71 (1H, t, $J = 7.4$ Hz), 8.0 (2H, d, $J = 7.6$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 94.3$, 116.1, 128.9, 129.5, 133.4, 135.3, 193.1.

4.3.8. Synthesis of (*S*)-3,3,3-trifluoro-2-hydroxy-1-phenylpropan-1-one (*S*)-7. Vinyl acetate (5 mL) and lipase powder (lipase CAL-B Novozyme 435, 0.5 g) were added to a solution of *rac*-3,3,3-trifluoro-2-hydroxy-1-phenylpropan-1-one *rac*-**7** (1 g, 6.6 mmol) in *t*-BuOMe (60 mL). The mixture was vigorously stirred at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*S*)-**7** (267 mg, 27%, ee >99%). $[\alpha]_D^{20} = +8.6$ (*c* 0.2, $CHCl_3$); mp = 81 °C; ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.20$ (1H, s), 5.44 (1H, q, $J = 6.6$ Hz, C*H*–OH), 7.56 (2H, t, $J = 7.8$ Hz), 7.71 (1H, t, $J = 7.4$ Hz), 8.0 (2H, d, $J = 7.6$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 94.3$, 116.1, 128.9, 129.5, 133.4, 135.3, 193.1.

4.3.9. General procedure for lipase mediated kinetic resolution.[46,52] The acyl donor (6 mL) and lipase powder (2.5 g) were added to a solution of *rac*-**3**, *rac*-**8** and *rac*-**9** (1 g) in solvent (30 mL). The mixture was vigorously shaken at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent evaporated under reduced pressure. Column chromatography on silica gel afforded optically active α-hydroxyketones (*R*)-**3** and (*R*)-**8**–(*R*)-**12**.

4.3.9.1. (*R*)-5-Hydroxyoctan-4-one (*R*)-3. Vinyl acetate (6 mL) and lipase powder (Lipase Amano PS, 2.5 g) were added to a solution of *rac*-5-hydroxyoctan-4-one (1 g, 7 mmol) in *n*-hexane (30 mL). The mixture was vigorously shaken at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent was evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**3** (332 mg, 32%, ee >99%). $[\alpha]_D^{20} = -13.8$ (*c* 0.5, $CHCl_3$); ^1H NMR (360 MHz, $CDCl_3$) $\delta = 0.91$–0.95 (6H, m, CH_3), 1.37–1.44 (2H, m), 1.56–1.66 (2H, m), 1.67–1.74 (2H, m), 2.13 (1H, s, OH), 2.38–2.49 (2H, m), 4.15 (1H, q, $J = 3.1$ Hz, C*H*–OH), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 13.2$, 13.8, 17.1, 18.1, 35.8, 39.7, 76.2, 212.4.

4.3.9.2. (*R*)-α-Furoin (*R*)-8. Vinyl acetate (6 mL) and lipase powder (Lipase Amano TL, 2.5 g) were added to a solution of *rac*-α-furoin (1 g, 4 mmol) in THF (50 mL). The mixture was vigorously shaken at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**8** (450 mg, 45%, ee >99%). $[\alpha]_D^{20} = +62.7$ (*c* 0.9, $CHCl_3$); mp = 128 °C; ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.18$ (1H, s, OH), 5.81 (1H, s, C*H*–OH), 6.35–6.41 (2H, dd, $J_1 = 3.2$ Hz, $J_2 = 14.4$ Hz), 6.54 (1H, t, $J = 2.0$ Hz), 7.26 (1H, d, $J = 3.6$ Hz), 7.38 (1H, d, $J = 1.0$ Hz), 7.62 (1H, d, $J = 1.0$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 69.3$, 109.2, 110.8, 112.6, 120.2, 143.2, 147.7, 149.3, 151.2, 184.5.

4.3.9.3. (*R*)-α-Pyridoin (*R*)-9. Vinyl acetate (6 mL) and lipase powder (Lipase Amano TL, 2.5 g) were added to a solution of *rac*-α-pyridoin (1 g, 4 mmol) in THF (50 mL). The mixture was vigorously stirred at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent was evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**9** (356 mg, 36%, ee >99%). $[\alpha]_D^{20} = +78.8$ (*c* 0.8, $CHCl_3$); mp = 138 °C; ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.18$ (1H, s), 7.49–7.52 (2H, m), 7.92–7.97 (2H, ddd, $J_1 = J_2 = 1.6$ Hz, $J_3 = 6.1$ Hz), 8.23 (2H, d, $J = 7.8$ Hz), 8.61 (2H, d, $J = 4.2$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 30.9$, 119.4, 121.1, 127.4, 135.9, 137.5, 145.6, 156.6, 206.9.

4.3.9.4. (*R*)-2,2′-Dimethoxybenzoin (*R*)-10. Vinyl acetate (6 mL) and lipase powder (Lipase Amano TL, 2.5 g) were added to a solution of *rac*-2,2′-dimethoxybenzoin (1 g, 4 mmol) in THF (50 mL). The mixture was vigorously stirred at 30 °C and after 96 h, the enzyme was removed by filtration and the solvent evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**10** (110 mg, 11%, ee >99%). $[\alpha]_D^{20} = -70.4$ (*c* 1.0, $CHCl_3$), $[\alpha]_D^{20} = -125$ (*c* 0.9, $CHCl_3$);[53] ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.84$ (1H, s, OH), 3.69 (3H, s, O–CH_3), 3.83 (3H, s, O–CH_3), 6.12 (1H, s, C*H*–OH), 6.84–6.86 (2H, m), 6.93–6.99 (2H, m), 7.16–7.22 (2H, m), 7.50–7.53 (1H, m), 8.12 (1H, d, $J = 3.1$ Hz); ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 54.7$, 54.9, 75.2, 110.8, 111.4, 120.1, 120.2, 128.2, 129.2, 129.5, 130.0, 133.3, 157.3, 158.0, 205.2.

4.3.9.5. (*R*)-4,4′-Dimethoxybenzoin (*R*)-11. Vinyl acetate (6 mL) and lipase powder (Lipase Amano TL, 2.5 g) were added to a solution of *rac*-4,4′-dimethoxybenzoin

(1 g, 4 mmol) in THF (50 mL). The mixture was vigorously stirred at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**11** (391 mg, 39%, ee >99%). $[\alpha]_D^{20} = -87.9$ (*c* 1.3, $CHCl_3$), $[\alpha]_D^{20} = -90.4$ (*c* 1.0, MeOH);[53] mp = 106 °C; ^1H NMR (360 MHz, $CDCl_3$) $\delta = 2.18$ (1H, s, OH), 3.77 (3H, s, O–C*H*$_3$), 3.83 (3H, s, O–C*H*$_3$), 5.86 (1H, s, C*H*–OH), 6.84–6.88 (4H, m), 7.26 (2H, d, $J = 8.7$ Hz), 7.91 (2H, d, $J = 7.1$ Hz), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 55.2$, 55.5, 75.2, 113.9, 114.5, 128.1, 129.0, 131.6, 159.7, 163.9, 197.3.

4.3.9.6. (*R*)-2-Hydroxycyclohexanone (*R*)-12. Vinyl acetate (6 mL) and lipase powder (Lipase Amano PS, 2.5 g) were added to a solution of *rac*-2-hydroxycyclohexanone (1 g, 9 mmol) in *n*-hexane (30 mL). The mixture was vigorously stirred at 30 °C and after 24 h, the enzyme was removed by filtration and the solvent was evaporated under reduced pressure. Column chromatography on silica gel afforded optically active (*R*)-**12** (420 mg, 42%, ee >99%). $[\alpha]_D^{20} = +20.8$ (*c* 0.65, $CHCl_3$); $[\alpha]_D^{20} = +24.1$ (*c* 0.72, $CHCl_3$);[54] mp = 112 °C; ^1H NMR (360 MHz, $CDCl_3$) $\delta = 1.47$–1.75 (4H, m), 2.15 (1H, s, OH), 2.28–2.52 (4H, m), 4.13 (1H, q, $J = 6.3$ Hz, C*H*–OH), ^{13}C NMR (90 MHz, $CDCl_3$) $\delta = 20.2$, 23.8, 27.3, 33.1, 40.7, 76.5, 204.5.

4.4. Racemization experiments using ionic liquids

All racemization experiments were performed in 1.5 mL Eppendorf tubes. Various amounts of water-miscible ionic liquids (10%, 20%, 50% and 80% v/v) were added to the aq buffer solution. Lyophilized *Lactobacillus* spp. was rehydrated in BIS BIS–TRIS buffer (0.5 mL, 50 mM, 10^{-2} M $MgCl_2$, pH 6) for 1 h at 42 °C and shaken at 150 rpm. (*R*,*R*)-Hydrobenzoin (2 mg) was dissolved in each ionic liquid and biocatalyst was added to a total volume of 0.5 mL. The mixture was shaken in a thermomixer at 42 °C and 130 rpm for 24 h. The samples were analyzed by HPLC.

4.5. Biocatalytic procedures

4.5.1. Bacterial strains. All strains were obtained from the Deutsche Sammlung für Mikroorganismen and Zellkulturen (Braunschweig, Germany, http://www.dsmz.de), the American Type Culture Collection (Manassas, USA, http://www.atcc.org) and the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands, http://www.cbs.knaw.nl). FCC numbers stand for our in-house culture collection.

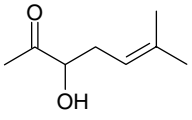
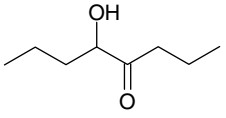
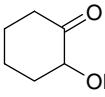
4.5.2. Media for active strains. *R. ruber* DSM 44540, *Agrobacterium tumefaciens* FCC 148, *Streptomyces caeruleus* DSM 40088, *Alcaligenes faecalis* DSM 13975, *B. megaterium* DSM 32, *Nocardia G* FCC 146 and *Nocardia H* FCC 147 were grown on a standard-complex medium. *Rhizopus oryzae* CBS 906, *B. bassiana* DSM 1344, *Penicillium simplicissimum* FCC 072 and *B. cinerea* DSM 877 were grown on an SNLH-medium for fungi. *Candida parapsilosis* DSM 70125 was grown on medium DSMZ #186, *Helminthosporium* sp. NRRL 4671 on tomato-juice medium, *Syncephalastrum racemosum* ATCC 18192 on PEP-medium, *A. niger* DSM 821 on soja-glucose medium, *Geotrichum candidum* DSM 6401 on medium DSMZ #90, *P. putida* ATCC 47054 on Luria broth medium and *Kluyveromyces lactis* DSM 3795 on medium DSMZ #393. *L. paracasei* DSM 20008, DSM 20207, DSM 2649, *Lactobacillus sakei* DSM 20017, *Lactobacillus halotolerans* DSM 20190, *Lactobacillus delbrueckii* DSM 20074, *Lactobacillus confusus* DSM 20196 and *Lactobacillus oris* DSM 4864 were grown on medium #11 as suggested by DSMZ.

4.5.3. Strain maintenance. Bacteria, fungi and yeasts were maintained in frozen stock solutions and long-term storage of lyophilized cells was at +4 °C.

4.5.4. Preparation of lyophilized cells. Strains were grown in flask cultures with shaken at 30 °C (*R. ruber* DSM 44540, *A. tumefaciens* FCC 148, *B. megaterium* DSM 32, *K. lactis* DSM 3795, *A. niger* DSM 821, *Nocardia G* FCC 146 and *Nocardia H* FCC 147, *L. paracasei* DSM 20008, DSM 20207, DSM 2649, *L. sakei* DSM 20017, *L. halotolerans* DSM 20190, *L. confusus* DSM 20196), at 24°C (*B. bassiana* DSM 1344), at 25°C (*C. parapsilosis* DSM 70125, *Helminthosporium* sp. NRRL 4671, *R. oryzae* CBS 906, *S. racemosum* ATCC 18192, *G. candidum* DSM 6401, *B. cinerea* DSM 877), at 26 °C (*P. simplicissimum* FCC 072), at 28°C (*S. caeruleus* DSM 40088, *P. putida* ATCC 47054) and at 37°C (*A. faecalis* DSM 13975, *L. delbrueckii* DSM 20074, *L. oris* DSM 4864).

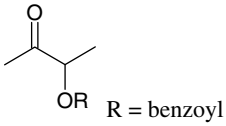
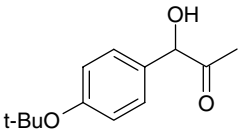
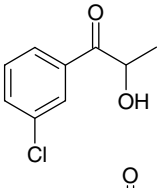
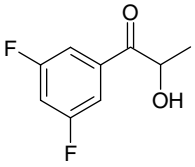
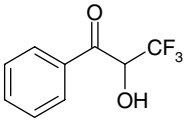
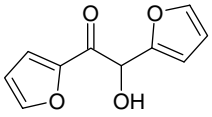
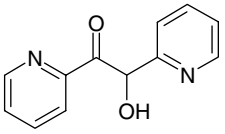
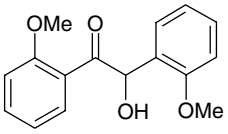
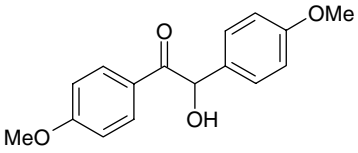
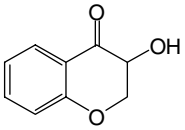
After transfer from agar plates, the microorganisms were grown for 3d (*R. ruber* DSM 44540, *A. tumefaciens* FCC 148, *S. caeruleus* DSM 40088, *A. faecalis* DSM 13975, *B. megaterium* DSM 32, *Nocardia G* FCC 146 and *Nocardia H* FCC 147, *P. simplicissimum* FCC 072, *C. parapsilosis* DSM 70125, *P. putida* ATCC 47054, *K. lactis* DSM 3795, *L. paracasei* DSM 20008, DSM 20207, DSM 2649, *L. sakei* DSM 20017, *L. halotolerans* DSM 20190, *L. delbrueckii* DSM 20074, *L. confusus* DSM 20196, *L. oris* DSM 4864)

Table 3. GC-analyses using a chiral stationary phase[a]

Substrate	Compound	Retention times [min]	
		(*R*)	(*S*)
2		1.8	2.6
3		3.5	3.2
12		3.7	3.6

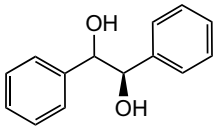
[a] Conditions: Column Chirasil-DEX CB, temperature programme (start temperature [°C]/holding time [min]/heating rate [°C min^{-1}]/plateau temperature [°C]/holding time [min]/heating rate [°C min^{-1}]/final temperature [°C]/holding time [min]): 110/0/3/125/0/25/150/0.

Table 4. HPLC-analyses of functionalized acyloins using a chiral stationary phase

Substrate	Compound	Conditions	Retention times [min]	
			(*R*)	(*S*)
1	 R = benzoyl	[a]	11.5	12.7
4		[a]	16.0	14.8
5		[b]	8.0	8.5
6		[b]	5.0	5.4
7		[b]	6.0	6.9
8		[b]	13.4	15.7
9		[c]	22.9	27.4
10		[b]	17.6	19.3
11		[b]	18.4	20.4
13		[d]	24.4	27.5

[a] Conditions: Column Chiralpak AD, 0.46 × 25 cm, *n*-heptane/*i*- propanol (90/10), flow 0.5 mL min^{-1}; 18 °C.
[b] Conditions: Column Chiralpak OD-H, 0.46 × 25 cm, *n*-heptane/*i*-propanol (90:10), flow 1.0 mL min^{-1}; 18 °C.
[c] Conditions: Column Chiralpak OJ, 0.46 × 25 cm, *n*-heptane/*i*-propanol (80:20), flow 1.4 mL min^{-1}; 18 °C.
[d] Conditions: Column Chiralpak OD-H, 0.46 × 25 cm, *n*-heptane/*i*-propanol (98:2), flow 0.6 mL min^{-1}; 18 °C.

Table 5. HPLC-analysis of hydrobenzoin using a chiral stationary phase[a]

Compound	Retention times [min]		
	(*R*,*R*)	(*S*,*S*)	(*R*,*S*)
	15.6	18.9	22.0

[a] Conditions: Column Chiralpak OJ, 0.46 × 25 cm, *n*-heptane/*i*-propanol (90:10), flow 1.0 mL min^{-1}; 18 °C.

and 5d (*R. oryzae* CBS 906, *B. bassiana* DSM 1344, *B. cinerea* DSM 877, *Helminthosporium* sp. NRRL 4671, *S. racemosum* ATCC 18192, *A. niger* DSM 821, *G. candidum* DSM 6401), respectively. Then the cells were harvested by centrifugation (18,000*g*), washed twice with buffer, lyophilized and stored at +4 °C. Cells were washed using the following buffers: bacteria (sodium/potassium phosphate buffer 50 mM, pH 7.5), fungi and yeast (TRIS buffer 10 mM, pH 7.5) and *Lactobacillus* sp. (BIS–TRIS buffer, 50 mM, 10^{-2} M $MgCl_2$, pH 6).

4.6. Analytical procedures

For analytical procedures see Tables 3–5.

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41. For a complete list of microorganisms tested see Section 4.5, only the most active candidates are listed in Table 2.
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