Biocatalytic Kinetic Resolution of Racemic Hydroperoxides through the Enantioselective Reduction with Free and Immobilized Microorganisms

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Received December 30, 1999

Abstract: A *Bacillus subtilis* strain, isolated from topsoil by a selective screening procedure, is effective for the biocatalytic kinetic resolution of racemic hydroperoxides. A variety of secondary aralkyl hydroperoxides have been resolved by this microorganism in moderate to high enantioselectivities [up to 88% ee for the (*R*)-hydroperoxide]. This topsoil bacterium *B. subtilis* also reduced the racemic tertiary 1-methyl-(1-phenyl)-propyl hydroperoxide enantioselectively to afford the (*R*)-hydroperoxide in 39% ee. In contrast to the bacteria, the fungus *Aspergillus niger* displayed the reverse sense of enantioselectivity in the kinetic resolution of the racemic hydroperoxides, that is, the (*R*) enantiomer was preferably reduced and the (*S*)-hydroperoxide was obtained in enantiomerically enriched form. Such an inverse enantioselectivity has not been observed before for the peroxidases of bacterial versus fungal and plant sources. Furthermore, it was shown that immobilized *B. subtilis* cells may be reused for several catalytic cycles in the kinetic resolution of racemic hydroperoxides without any loss of enzymatic activity. This microbial process offers a promising biocatalytic methodology for the preparation of optically active hydroperoxides.

Introduction

Hydroperoxides are commonly used as oxygen donors in asymmetric oxidation.[1,2] In recent years, optically active derivatives have become important as chiral oxygen sources in the asymmetric synthesis of oxyfunctionalized compounds.[3,4] Since by conventional chemical means it has been difficult to obtain chiral hydroperoxides in high optical purity, enzymes have been successfully employed for this purpose in view of their high degree of enantioselectivity and catalytic activity.[5] In particular, horseradish,[6−8] *Coprinus*,[9] and chloroperoxidase[10] have proven to be efficient biocatalysts for the preparation of optically active hydroperoxides by kinetic resolution of racemic substrates. Besides peroxidases, also lipases[11] and lipoxygenases[12,13] have been used for the kinetic resolution of hydroperoxides. Despite the advantages, large quantities of pure enzyme, necessary for preparative applications, are expensive and not readily accessible.

The latter drawbacks of isolated enzymes may be circumvented by employing microorganisms as biocatalysts. They are inexpensive, do not require costly cofactors, and are available in the desired quantities through replication. Thus, the advantages of regenerative biocatalysts should be evident; however, to date little is known on the enantioselective transformations by microorganisms with peroxidase activity. Some lignin peroxidases of fungal strains have been investigated in detail, but no application in asymmetric synthesis has been reported.[14,15] Furthermore, most of the current work in this area focuses on the genetic control of peroxidases during the response to stress factors.[16,17] For example, the bacterium *Bacillus subtilis* has been shown to display complex behavior toward external stress.[18]

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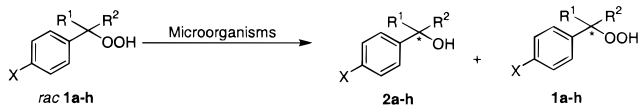
10.1021/ja994530a CCC: $19.00
Published on Web 05/09/2000

Scheme 1. Kinetic Resolution of Hydroperoxides **1** by Asymmetric Reduction with Microorganisms



1, 2	a	b	c	d	e	f	g	h
R^1	Me	Me	Et	Pr	*i*-Pr	Bu	Me	Et
R^2	H	H	H	H	H	H	Me	Me
X	H	Cl	H	H	H	H	H	H

Thus far, no peroxidase of bacterial origin has been explored for the enantioselective reduction of racemic hydroperoxides.

Recently, we have demonstrated[19] for the first time the kinetic resolution of secondary hydroperoxides by a peroxidase-active *B. subtilis* strain. This microorganism was isolated from topsoil by means of a hydrogen-peroxide-mediated selective screening procedure.[19] These preliminary results merited further exploration, and presently we report an in-depth study on the scope and limitations of this biocatalytic process for the kinetic resolution of a variety of racemic hydroperoxides **1** (Scheme 1). Furthermore, to compare the bacterial and fungal peroxidase activity, the commercially available fungal microorganism *Aspergillus niger* was also employed for kinetic resolution.

Results and Discussion

The biotransformations of the racemic substrates **1** were carried out with pregrown microbial cultures by using 0.07 mmol of the particular hydroperoxide. The absolute configurations of the alcohols **2** and the hydroperoxides **1** were assigned by comparison of the chromatographic data with those of authentic reference substances or according to literature data.[6,9] The results of the kinetic resolution of the hydroperoxides **1** with microorganisms are given in Table 1.

The cultures of *B. subtilis*, which contained yeast extract in the growth medium, converted 94% of (1-phenyl)ethyl hydroperoxide (**1a**) within only 30 min; the remaining (*R*)-configured hydroperoxide was enantiomerically pure (entry 1). For a better control of the conversion of the hydroperoxide **1a**, cultures were prepared without yeast extract to yield a fraction of the cell density (enzyme content) compared to the one usually obtained by addition of yeast extract. Thereby, the enzyme activity was significantly decreased, since 64% of the hydroperoxide **1a** was converted only after 240 min to afford the (*R*)-hydroperoxide **1a** in 88% enantiomeric excess (ee) and the (*S*)-alcohol **2a** in 30% ee (entry 2).

To assess whether a chloro substituent would affect the reactivity and selectivity of *B. subtilis* for aralkyl hydroperoxides and thereby display an electronic effect, the biotransformation of the racemic [1-(*p*-chloro)phenyl]ethyl hydroperoxide (**1b**) was carried out. *B. subtilis* showed a lower activity toward the *p*-chloro derivative **1b** compared to the unsubstituted substrate **1a**. Under identical reaction conditions, for **1b** 56% conversion was observed compared to 94% for **1a** (entries 1 and 4). Furthermore, the chloro derivative displays a reduced selectivity, since at similar conversions (64 vs 56% for **1a** and **1b**) the (*R*)-configured hydroperoxide **1a** was obtained in as high as 88% ee in contrast to **1b**, which showed an ee value as low as 16% (entries 2 and 4).

Next, the steric effect was probed by increasing the alkyl chain successively from ethyl to *n*-pentyl as in the hydroperoxides **1a**, **1c**, **1d,** and **1f**. Replacement of the methyl (**1a**) by an ethyl (**1c**) group did not change the enantioselectivity appreciably; thus, at 47% conversion, the (*R*) enantiomer of the hydroperoxide **1c** was obtained in 64% ee and the (*S*)-alcohol **2c** in 36% ee, compared to 88% ee for the (*R*)-hydroperoxide **1a** at 64% conversion (cf. entries 2 and 6). However, a further increase of the alkyl substituent to *n*-propyl in substrate **1d** reduced the enantioselectivity significantly to 25% ee at 41% conversion (entry 8). Nevertheless, the results of the microbial kinetic resolution of **1d** represent a noticeable improvement compared to the horseradish-peroxidase (HRP) system, which did not display any enantioselectivity at 50% conversion of **1d**.[6]

The *n*-butyl group in (1-phenyl)pentyl hydroperoxide (**1f**) marks the limit for the kinetic resolution by *Bacillus subtilis*, since even at 96% conversion of substrate **1f**, the remaining hydroperoxide was racemic (entry 12). In this context, we have previously reported that the biocatalytic kinetic resolution of aralkyl hydroperoxides with longer alkyl chains (*n*-butyl or *n*-pentyl) was also ineffective for horseradish[6] or *Coprinus* peroxidase.[9] Expectedly, the efficacy of the kinetic resolution of aralkyl substrates by peroxidases is severly diminished when the phenyl group and the alkyl chain become sterically equally demanding. Under such circumstances the substrate is positioned randomly in the enzyme cavity and the stereodifferentiation is poor. Necessarily this trend applies rigorously only to substrates without further functionalities capable of strongly interacting electronically with the enzyme, for example, through hydrogen bonding, donor−acceptor complexation, electrostatic attractions/repulsions, which thereby would introduce additional modes of differentiation and override steric effects.

More severe steric effects are operating for branched alkyl groups, as demonstrated by the marked decrease (at 50% conversion only ∼15% ee) observed on the enantioselectivity in the HRP-catalyzed kinetic resolution of (1-phenyl)isobutyl hydroperoxide (**1e**).[6] Therefore, we have tested the microbial transformation of this hydroperoxide, but surprisingly, the soil bacterium *B. subtilis* displayed a quite good enantioselectivity to afford the (*R*)-hydroperoxide **1e** in an ee of 55% at 74% conversion (entry 10). This effective kinetic resolution of the more hindered substrate **1e** by *B. subtilis*, encouraged us to apply this microorganism to tertiary hydroperoxides. Currently known peroxidases either do not accept chiral tertiary hydroperoxides for steric reasons or if some conversion is observed at all, no enantiomeric enrichment of the hydroperoxide has been detected.[6,9]

To determine the reactivity of whole cells toward tertiary hydroperoxides, first the achiral cumene hydroperoxide (**1g**) was employed as model substrate. *B. subtilis* as well as *A. niger* reduced this sterically encumbered substrate without difficulty (entries 14 and 15). Still more pleasing, the racemic tertiary 1-methyl-(1-phenyl)propyl hydroperoxide (**1h**), when treated with *B. subtilis* cells, afforded the (*R*)-hydroperoxide **1h** in 39% ee at 79% conversion (entry 17). This is the first case of biocatalytic kinetic resolution of a tertiary hydroperoxide. Control experiments with autoclaved (dead) cultures of *B. subtilis* and the hydroperoxide **1h** showed no conversion, so that the nonenzymatic degradation of the hydroperoxide may be excluded.

For a comparison of fungal versus bacterial peroxidase activity, the commercially available fungus *A. niger* was employed for the kinetic resolution of the hydroperoxides **1**. With (1-phenyl)ethyl hydroperoxide (**1a**), an ee value of 37%

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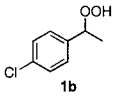
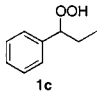
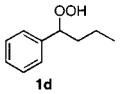
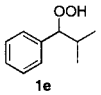
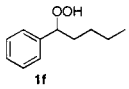
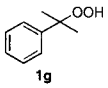
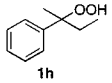
Table 1. Microbial Kinetic Resolution of the Racemic Hydroperoxides **1**

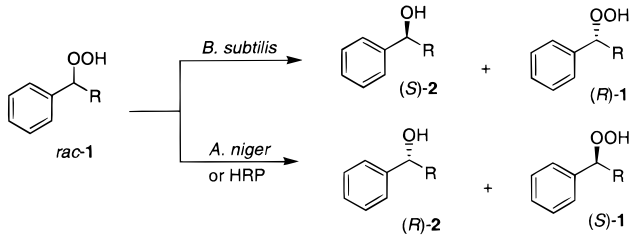
entry	microorganism	substrate	time[a] [min]	convn[b] [%]	enantioselectivity [% ee] ROOH **1**[c]	ROH **2**[c]
1	*B. subtilis*	 **1a**	30	94	> 99 (*R*)	20 (*S*)
2	*B. subtilis*		240[d]	64	88 (*R*)	30 (*S*)
3	*A. niger*		30	67	37 (*S*)	25 (*R*)
4	*B. subtilis*	 **1b**	30	56	16 (*R*)	13 (*S*)
5	*A. niger*		30	65	40 (*S*)	37 (*R*)
6	*B. subtilis*	 **1c**	120	47	64 (*R*)	36 (*S*)
7	*A. niger*		45	55	7 (*S*)	9 (*R*)
8	*B. subtilis*	 **1d**	90	41	25 (*R*)	20 (*S*)
9	*A. niger*		30	83	29 (*S*)	11 (*R*)
10	*B. subtilis*	 **1e**	30	74	55 (*R*)	26 (*S*)
11	*A. niger*		25	59	9 (*S*)	6 (*R*)
12	*B. subtilis*	 **1f**	180	96	-[e]	-[e]
13	*A. niger*		80	53	14 (*S*)	7 (*R*)
14	*B. subtilis*	 **1g**	240	77	-	-
15	*A. niger*		180	100	-	-
16	*B. subtilis*	 **1h**	30	19	5 (*R*)	30 (*S*)
17	*B. subtilis*		13	79	39 (*R*)	7 (*S*)

[a] Incubation time of the substrate **1** with the pregrown culture. [b] Conversion determined by HPLC analysis; error limits ±5% of the stated values. [c] Determined by HPLC analysis on a chiral column [9:1 isohexane:2-propanol, 220 nm, 0.5 mL/ min); error limits ±5% of the stated values. [d] Medium contained no yeast extract. [e] Racemic.

was obtained for the (*S*) enantiomer and 25% ee for the (*R*) alcohol **2a** at 67% conversion (entry 3). Thus, the fungus *A. niger* is significantly less enantioselective in comparison to *B. subtilis* (cf. entry 2); however, in the case of the *p*-chloro derivative **1b**, *A. niger* exhibited higher enantioselectivity than *B. subtilis* (cf. entries 4 and 5). In contrast, a dramatic drop in enantioselectivity (ee values 88 and 16% for **1a** and **1b**, respectively) was encountered with *B. subtilis* for that substrate (cf. entries 2 and 4). These results indicate that, unlike in the case of the bacterium *B. subtilis*, the enzyme expression in *A. niger* is not effected by the chloro-substituted derivative. An increase in the length of the alkyl chain of the aralkyl hydroperoxide **1** has a more pronounced effect on the enantioselectivity of *A. niger* than on *B. subtilis*. Thus, the replacement of the methyl (**1a**) by an ethyl (**1c**) group in the hydroperoxide drastically decreases the ee value from 37% to 7% at 55% conversion (entries 3 and 7). A further increase in the alkyl substituent to *n*-propyl (**1d**) and *n*-butyl (**1f**) or the branching of the alkyl group as in (1-phenyl)isobutyl hydroperoxide (**1e**) did not substantially affect the enantioselectivity in the kinetic resolution by *A. niger* (entries 9, 11, and 13). Clearly, the enantioselectivity of the reduction of aralkyl hydroperoxides **1** by the fungus *A. niger* is lower than that by the bacterium *B. subtilis*.

A remarkable feature in the kinetic resolution of the chiral hydroperoxides **1** with whole cells is the finding that the opposite

Scheme 2. Opposite Configurations in the Asymmetric Reduction of the Hydroperoxides **1** by the Bacterium *B. subtilis* versus the Fungus *A. niger* and the Isolated Enzyme HRP



sense of enantioselectivity was observed for the asymmetric reduction by the bacterium *B. subtilis* in comparison to the fungus *A. niger* (cf. entries 2−11). In the case of *A. niger*, the (*R*)-hydroperoxides are preferably reduced, whereas *B. subtilis* favors the (*S*)-hydroperoxides (Scheme 2). This is the first time that an opposite enantioselectivity has been observed in the kinetic resolution of chiral hydroperoxides with bacterial peroxidases versus fungal and plant ones, such as horseradish and *Coprinus* peroxidase, which both show a preference for the reduction of the (*R*)-hydroperoxides.[6,9]

The comparison of the topsoil-isolated *B. subtilis* strain (Table 2, entries 1 and 2) to commercially avaible *B. subtilis* cultures (entries 3−5) and an *Escherichia coli* strain (entries 6 and 7)

Table 2. Comparative Enantioselectivities in the Biotransformation of (1-Phenyl)Ethyl Hydroperoxide (**1a**) by Commercially Available *B. subtilis* and *E. coli* Strains and Topsoil-Isolated *B. subtilis*

entry	microorganism	time[a] (min)	convn[b] (%)	enantioselectivity [% ee][c] (*R*)-ROOH **1**	(*S*)-ROH **2**
1	*B. subtilis* (topsoil)	240[d]	64	88	30
2	*B. subtilis* (topsoil)	30	94	>99	20
3	*B. subtilis* DSM 10	60	39	23	30
4	*B. subtilis* DB 104	60	26	14	42
5	*B. subtilis* 168	30	12	*e*	4
6	*E. coli* K12 DH5α	15	34	7	18
7	*E. coli* K12 DH5α	30	96	80	10

[a–e] For footnotes *a*−*e* see legends in Table 1.

demonstrates that all the bacterial cultures display the same sense in the enantioselectivity, but the commercial *B. subtilis* strains (entries 3−5) show a significantly lower enantioselectivity and also a lower rate of conversion compared to the bacterium isolated from topsoil (entry 2). This is especially apparent in the case of *B. subtilis* 168, for which hardly any selectivity was observed (entry 5); but also the commercial wild-type strain *B. subtilis* DSM 10 (entry 3) is ineffective. Furthermore, the *E. coli* strain K12 DH5α also displays only a low enantioselectivity, but with the same sense as observed in all of the biotransformations with *B. subtilis* (entries 6 and 7).

It is evident from the data on the incubation times and % conversions in Tables 1 and 2 that the enzyme expression of the microorganisms varies. Even different *B. subtilis* strains (e.g., *B. subtilis* DSM 10 and 168 in Table 2, entries 3 and 5) show substantially distinct enzyme activity and thereby the incubation times to obtain similar conversions changes. Thus, our screening procedure was most effective in isolating a peroxidase-rich bacterial species.

From these results and our previous study on fungal peroxidase activity (*Botrytis cinerea*, *Penicllium verrucosum var. verrucosum*, and *Aspergillus ochraceus*),[20] it becomes evident that the thus-far known bacterial peroxidases show the opposite sense in selectivity compared to the majority of fungal and plant peroxidases. These unprecedented findings constitute a phylogenetic feature worth further investigation, because the peroxidases that are involved in the fungal and bacterial systems must differ fundamentally in the structure and composition of their active sites to explain such opposite sense in the enantioselectivities.

It has been established that the use of immobilized cells offers definite advantages over free-cell cultures, as they may easily be reused by simple filtration of the medium, and at the same time the overall lifetime of the cells is prolonged significantly; moreover, often fewer metabolic byproducts are formed with immobilized cells.[21] To enhance the efficiency of the present biotransformation, the *B. subtilis* cells were harvested during the stationary phase and immobilized in alginate gel. In addition to the above advantages, biotransformations with immobilized *B. subtilis* cells showed a marked increase in peroxidase activity between the first and second catalytic cycle. After the same

Table 3. Biotransformation of (1-Phenyl)ethyl Hydroperoxide (**1a**) with Immobilized *B. subtilis* Cells

entry	time[a] (min)	cycle[b]	convn[c] (%)	(*R*)-ROOH **1**	(*S*)-ROH **2**
1	60	1st	48	36	48
2	60	2nd	98	>95	10
3	60	3rd	100		
4	60	1st	58	49	22
5	40	2nd	70	59	18
6	20	3rd	43	39	29

[a] Incubation time of the substrate **1a** with the pregrown culture. [b] Indicates the number of times gel beads with immobilized bacterial cells were reused on a fresh substrate sample. [c] Conversion determined by HPLC analysis; error limits ±5% of the stated values. [d] Determined by HPLC analysis on a chiral Chiralcel column [9:1 isohexane:2-propanol, 220 nm, 0.5 mL/ min]; error limits ±5% of the stated values.

incubation time of 60 min and addition of equal amounts of hydroperoxide **1a**, the conversion rose from 48% during the first cycle to 98% during the second one (Table 3, entries 1 and 2). Consideration of the fact that peroxidases are expressed as a response to external stress,[16,17] the increased enzymatic activity presumably results from de novo synthesis of peroxidases under immobilized conditions. Indeed, slow cellular growth and, consequently, protein synthesis in immobilized cells have been reported.[22]

To counteract the effect of the increased peroxidase activity, the incubation time was decreased in successive catalytic cycles by 20 min (Table 3, entries 4−6). In the second cycle (entry 5), despite the lower incubation time, the enzyme activity increases compared to the first cycle (entry 4). Further lowering of the incubation time in the third cycle (entry 6) leads to only 43% conversion, but compared to the first cycle (entry 4), almost as much activity is displayed in one-third the time. Thus, the efficiency of the present bioconversion could be increased about 3-fold, since the same batch of immobilized cells was reused at least three times, which amounts to a total incubation time of just 120 min (entries 4−6). Hereby, we were able to convert 30 mg of the racemic hydroperoxide **1a** to afford the (*R*)-hydroperoxide **1a** in moderate ee values. Thus, this biocatalytic process may be applied for the kinetic resolution of hydroperoxides on the semipreparative scale. The only drawback is the lower enantioselectivity (Table 3, entry 4) compared to that of free cells (Table 1, entry 2). This and the observation of the significantly lower enantioselectivity in the case of the *p*-chloro-substituted hydroperoxide **1b** (Table 1, entry 4) imply the presence of more than one peroxidase with different stereoselectivities, whose expression depends on the external growth conditions. It is not unusual for cells to contain several enzymes of the same type (probably isoenzymes) in varying amounts; for instance, at least two catalases (enzymes which are closely related to peroxidase) are known for *B. subtilis*.[23] Furthermore, more than 40 peroxidase genes have been identified in *Arabidopsis thaliana*.[24] The decrease noted in the enantioselectivity for immobilized cells might be alleviated by variation of the medium, as has already been shown for *A. niger*, for which the stereoselectivity was increased up to 4-fold by changing the trace-metal content of the growth medium.[20]

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Conclusions

The kinetic resolution of racemic hydroperoxides has been accomplished with free and immobilized whole cells. Especially effective is the *B. subtilis* strain isolated from topsoil, which converts the aralkyl hydroperoxides **1** rapidly and in moderate to high stereoselectivities. In regard to enantioselectivity, it is striking that *B. subtilis* displays the inverse sense compared to that of the fungal and plant peroxidases known to date. Therefore, topsoil-selected bacteria provide a promising biocatalyts for the kinetic resolution of racemic hydroperoxides. They are convenient to handle, undemanding in the growth medium, environmentally benign, and generally reusable. Still more encouraging, it was demonstrated that the immobilized microorganisms display a higher peroxidase activity than free cells, which offers attractive opportunities for a further development of this concept. The search for improved growth conditions and the application of state-of-the-art genetic engineering might further increase the stereoselectivity and suppress the expression of undesired, less selective peroxidase isoenzymes. The gateway for a broad range of applications of this novel whole-cell biocatalyst has been opened for asymmetric organic synthesis.

Experimental Section

Materials and Chemicals. Plate count agar was purchased from Creatogen (Creatogen Biosciences GmbH, Augsburg, Germany). Cumene hydroperoxide (**1g**) was obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and purified by column chromatography on silica gel. The other racemic hydroperoxides **1** were prepared as described in the literature[25] from the appropriate alcohol and 80% H_2O_2. All other compounds were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Instrumentation. The following instruments were used: Rotary shaker, GFL 3031 and 3033 (Gesellschaft für Labortechnik mbH, Burgwedel, Germany), autoclave for sterilization Wolf SanoClav-MCN (Wolf, Geisslingen, Germany), laminar AirFlow bench (NuAire Inc., Plymouth, MN). GC analysis was performed on a HP 5890A (Hewlett-Packard, Palo Alto, CA); multidimensional gas chromatography (MDGC) with a moving-column-stream-switching-system (MCSS) coupling device was performed on two Fisons GC 8000 instruments (Fisons, Mainz-Kastel, Germany); HPLC analysis was performed on an instrument, which consisted of a Knauer 64A HPLC pump (Knauer, Geretsried, Germany) and a Hewlett-Packard 1040A diode array detector (Hewlett-Packard, Palo Alto, CA) or on a Knauer Maxi-Star HPLC (Knauer, Geretsried, Germany) with a Knauer UV detector and an additional ChiraLyser detector (IBZ Meβtechnik, Hannover, Germany); Chiralcel and Chiralpak columns (J. T. Baker B. V., Deventer, Netherlands) were used for chiral HPLC analysis.

Fungal Cultures. *A. niger* was obtained from the Microbiology Department of the University of Würzburg, and all other fungal cultures were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM, Braunschweig, Germany). The fungal cultures were maintained on agar slants, which contained 10 g/L malt extract, 4 g/L yeast extract, and 8 mL of glucose solution (50% w/v).

Bacterial Cultures. *B. subtilis* DB 104, *B. subtilis* 168, *and E. coli K12 DH5*α were obtained from the Microbiology Department of the University of Würzburg. *B. subtilis* DSM 10 was purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM, Braunschweig, Germany). The other cultures were obtained from topsoil by a selective screening procedure and identified by sequencing of their small-subunit rRNA as described previously.[19] The bacterial cultures were maintained on plate count agar (Creatogen Biosciences GmbH, Augsburg, Germany).

General Procedure for the Biotransformation of the Hydroperoxides by Bacteria. Liquid minimal media (75 mL) were prepared according to Dworkin et al.[26] with the addition of a trace-element solution (5 mL/L)[27] and autoclaved. Glucose (5 g/L) was added in such a way that the culture remained sterile. If not indicated otherwise, 90 mg of yeast extract was added to the flask prior to autoclaving. The cultures for inoculation of liquid media were taken from freshly grown plates. The liquid culture was allowed to grow for ~18 h at 30 °C, and 0.07 mmol of the particular hydroperoxide **1** was added under sterile conditions. After the specified time (Table 1), the culture was worked up by sonication of the bacterial broth for 15 min and centrifugation at 15000*g* for 20 min. The supernatant was extracted with ethyl ether (3 × 50 mL), and combined extracts were dried over Na_2SO_4 and evaporated to dryness (~20 °C/300 mbar).

For the determination of the conversion and the enantiomeric excess of the hydroperoxides, the sample was submitted to HPLC analysis on a chiral column (J. T. Baker B. V., Deventer, Netherlands). A 9:1 mixture of isohexane and 2-propanol was used as eluent, at a flow rate of 0.5 mL/min and 2.4 MPa pressure; UV detection was conducted at $\lambda = 220$ nm. The absolute configurations of the hydroperoxides **1** and the alcohols **2** were assigned by comparison of the elution order (MDGC analysis), and the specific rotations (HPLC analysis, determined on a ChiraLyser), with literature data.[6,9,28] For further verification, a small fraction of the crude product was purified by preparative silica gel TLC (6:4 petroleum ether:ethyl ether). The hydroperoxide was reduced with triphenylphosphine in ethyl ether, and the corresponding alcohol was separated by preparative silica gel TLC. The hydroperoxide **1** (reduced to the alcohol) and alcohol **2** were analyzed by multidimensional gas chromatography on a chiral cyclodextrin column.

General Procedure for the Biotransformation of the Hydroperoxides 1 by Fungi. A modified Czapek-Dox medium was used for the biotransformations. The medium contained 3.0 g of $NaNO_3$ (35 mmol), 1.0 g of K_2HPO_4 (5.7 mmol), 0.5 g of $MgSO_4 \cdot 7H_2O$ (2 mmol), and 0.5 g of KCl (6.8 mmol). The pH was adjusted to 5.4, and 5.0 mL/L of a trace-element solution[27] was added prior to autoclaving, which contained 232 mg of H_3BO_3 (3.7 mmol), 174 mg of $ZnSO_4 \cdot 7H_2O$ (6 mmol), 180 mg of $FeSO_4 \cdot 7H_2O$ (0.65 mmol), 96.0 mg of $CoSO_4 \cdot 7H_2O$ (0.34 mmol), 22.0 mg of $(NH_4)_6Mo_7O_{24}$ (0.019 mmol), 8.0 mg of $CuSO_4 \cdot 5H_2O$ (0.03 mmol), and 6.0 mg of $MnSO_4 \cdot H_2O$ (0.035 mmol). After autoclaving, 6 mL of a sterile glucose solution (50% w/v) was added (final concentration 30 g/L glucose). All cultures were grown at 30 °C on a rotary shaker at 120 rpm.

The spores for inoculation of liquid media (100 mL) were taken from freshly grown plates. The liquid culture was allowed to grow for 8−11 days, dependent on the strain used. Then 0.07 mmol of the hydroperoxide was added under sterile conditions. After the specified time (Table 1), the culture was separated by filtration from the medium. The clear medium was extracted with ethyl ether (3 × 50 mL), and the combined extracts were dried over Na_2SO_4 and evaporated to dryness (~20 °C/300 mbar). Analysis was carried out as described in the bacterial Biotransformation section.

Immobilization of *B. subtilis* Cells and Biotransformation of Hydroperoxides 1 by Immobilized Cells. A liquid culture (75 mL) was grown to the early stationary phase and centrifuged (~3000*g*). The bacterial pellet was resuspended in 2 mL of an isotonic NaCl/$MgSO_4$ solution, and 3 mL of a sodium alginate solution (3% w/v) was added. After mixing, the cell suspension was added dropwise to a new medium of 0.1 M $CaCl_2$, 5 g/L glucose, 1.2 g/L yeast extract, and 5 mL/L of a trace-element solution.[27] The gel beads were allowed to harden for 1 h without agitation, and after an additional 30 min at 30 °C and 120 rpm, the substrate was added. Workup conditions were as described in the Bacterial Biotransformation section except that the beads were collected by filtration and immediately resuspended in fresh liquid medium to initiate a new catalytic cycle.

MDGC Conditions for the Alcohols 2. The chiral columns for the separation of the enantiomers of alcohols **2** were heptakis-(2,6-*O*-dimethyl-3-*O*-pentyl)-β-cyclodextrin column (β) and heptakis-(2,3-*O*-diethyl-6-*tert*-butyl-dimethylsilyl)-β-cyclodextrin column (ethyl).

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(1-Phenyl)ethanol (2a): MDGC; DB Wax: 100−240 °C (10 °C/min); β-cyclodextrin column: 100 °C (15 min isothermic) to 200 °C (2 °C/min).

***p*-Cl-(1-Phenyl)ethanol (2b):** MDGC; DB Wax: 140−240 °C (10 °C/min); β-cyclodextrin column: 100 °C (10 min isothermic) to 200 °C (2 °C/min).

(1-Phenyl)propanol (2c): MDGC; DB Wax: 80−240 °C (10 °C/min); β-cyclodextrin column: 60 °C (15 min isothermic) to 200 °C (2 °C/min).

(1-Phenyl)butanol (2d): DB Wax: 100−240 °C (10 °C/min); β-cyclodextrin column: 60 °C (15 min isothermic) to 200 °C (1 °C/min).

(1-Phenyl)isobutanol (2e): DB Wax: 80−240 °C (5 °C/min); ethylcyclodextrin column: 80 °C (20 min isothermic) to 200 °C (2 °C/min).

(1-Phenyl)pentanol (2f): MDGC; DB Wax: 100−240 °C (5 °C/min); β-cyclodextrin column: 80 °C (24 min isothermic) to 200 °C (2 °C/min).

1-Methyl-1-phenyl-1-propanol (2h): MDGC; DB Wax: 60−240 °C (5 °C/min); β-cyclodextrin column: 60 °C (27 min isothermic) to 200 °C (1 °C/min).

Acknowledgment. We express our gratitude to Dr. U. Rdest (Institute of Microbiology, University of Würzburg, Germany) for her valuable help and advice. The Deutsche Forschungsgemeinschaft (SFB 347 “Selektive Reaktionen Metall-aktivierter Moleküle”) and the Fonds der Chemischen Industrie are thanked for their generous financial support.

JA994530A