Kinetic Resolution of Chiral Hydroperoxides: Hydrogen-Peroxide-Mediated Screening of Peroxidase-Active Soil Bacteria

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Hydroperoxides have been used frequently in asymmetric oxidation;[1,2] in fact, recently, enantiomerically pure hydroperoxides have become important as oxidants in the synthesis of optically active compounds.[3a,b] Since by conventional chemical means it has been difficult to obtain enantiomerically pure products, nowadays the popularly used enzymes in preparative organic chemistry have been successfully employed in view of their high degree of enantioselectivity and catalytic activity.[4] By this means, optically active hydroperoxides were obtained with lipases,[5] lipoxygenase,[6] and horseradish peroxidase.[7] Most recently, a chemically modified subtilisin enzyme, namely selenosubtilisin, was successfully applied to the kinetic resolution of racemic hydroperoxides.[8] Nevertheless, large quantities of pure enzyme, which are necessary for preparative applications, are difficult to come by and expensive.

These disadvantages are to be contrasted with whole cell systems (bacteria, fungi, and plant/animal cells), which circumvent such problems since they are potentially available in large quantities through self-replication. No work along these interdisciplinary lines is currently available on peroxidase systems in bacteria and fungi, certainly not for the kinetic resolution of racemic hydroperoxides.

As a matter of fact, most of the focus in recent years has been set on the elucidation of the genetic expression of peroxidases during the response to stress factors.[9,10] Here, we communicate our results of the *first* kinetic resolution of organic hydroperoxides by soil bacteria and fungi.

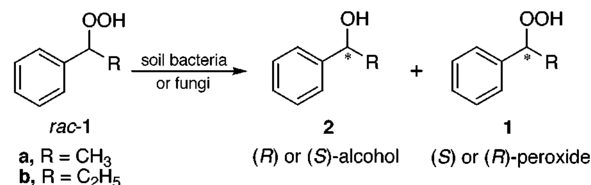
First, an adequate screening procedure to select suitable soil bacteria for the biotransformation of hydroperoxides had been developed. Since approximately 10^8-10^9 bacteria are present in 1 g of soil,[11] this covers a wide genetic diversity suitable for initial screening. Hydrogen peroxide was chosen as selecting agent, because it is a potent inductor of peroxidase transcription; however, it may also select some strains with catalase activity. In contrast to current screening procedures, in which the substrate is administered as the only carbon, sulfur, or nitrogen source at an early stage of the growth cycle, in our protocol, the substrate is added as a selecting agent in addition to all the other nutrients necessary for the normal growth of the bacteria.

Our growth medium was either a full (Plate Count Agar, Difco Laboratories) or a minimal (Dworkin et al.[12]) medium, which was supplemented with the necessary trace elements. The rationale behind this strategy was that most of the substrates will not be accepted as a sole nutrient source by bacteria, but such substances are still toxic enough to induce the detoxification in sufficient bacteria, which have the necessary genes to express the required peroxidase. Moreover, the material may be added at any stage of the bacterial growth cycle to allow for more diversity in the control of the biotransformation under examination.

The present screening procedure with H_2O_2 resulted in four distinct species, which have been subjected to characterization by the sequencing of the first 300 base pairs of their small subunit ribosomal RNA gene (16s/18s) with the Taq-cycle-DyeDeoxy-terminator technique[13] (ABI division of Perkin-Elmer, Weiterstadt). The results show that three *Bacillus* spp. have been isolated that were not identical. Morphological and 18s-rRNA sequence data show that the fourth strain isolated is most probably a *Paecilomyces* sp. The *Bacillus* strain, which gave the best results in regard to the kinetic resolution of the organic hydroperoxides, was more rigorously characterized and determined to be a *Bacillus subtilis* strain.

All of the bacterial species mentioned above were incubated with 0.07 mmol of the particular racemic hydroperoxide for the kinetic resolution of the hydroperoxides (Scheme 1):

Scheme 1. Kinetic Resolution of Hydroperoxides 1 by Soil Bacteria or Fungi



For these experiments, the (1-phenyl)ethyl (**1a**) and 1-(1-phenyl)propyl (**1b**) hydroperoxides were used as model substrates. The results are summarized in Table 1.

Two of the isolated bacterial species showed slow conversion of the hydroperoxides and poor enantiomeric excess for both the alcohol and the hydroperoxide (see entry 6 for an example). Thus, these two strains were not considered for further experimentation. The *Paecilomyces* sp. (entry 4) showed much faster conversions, so that after only 80 min as much as 92% of the hydroperoxide **1a** was converted and an enantiomeric excess (ee) of 79% of the (*R*)-hydroperoxide **1a** was achieved (entry 4). The best results were obtained with *B. subtilis* (entry 1), which converted 64% of hydro-

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(1) Johnson, R. A., Sharpless, B., Ojima, I., Ed. *Catalytic Asymmetric Synthesis*; VCH: Weinheim, 1993; pp 103−158.
(2) Adam, W.; Richter, M. J. *Acc. Chem. Res.* **1994**, *27*, 57−62.
(3) (a) Shum, W. P. S.; Saxton, R. J.; Zajacek, J. G. US Patent 5663384, 1997. (b) Adam, W.; Korb, M. N.; Roschmann, K. J.; Saha-Möller, C. R. *J. Org. Chem.* **1998**, *63*, 3423−3428.
(4) Adam, W.; Lazarus, M.; Saha-Möller, C. R.; Weichold, O.; Hoch, U.; Häring, D.; Schreier, P. Biotransformations with Peroxidases. In *Advances in Biochemical Engineering/Bio-technology/Biotransformations*; Faber, K., Ed.; Springer-Verlag: Heidelberg, in press.
(5) Baba, N.; Mimura, M.; Hiratake, J.; Uchida, K.; Oda, J. *Agric. Biol. Chem.* **1988**, *52*, 2658−2687.
(6) Datechva, V. K.; Kiss, K.; Solomon, L.; Kyler, K. S. *J. Am. Chem. Soc.* **1991**, *113*, 270−274.
(7) Adam, W.; Hoch, U.; Lazarus, M.; Saha-Möller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **1995**, *117*, 11898−11901.
(8) Häring, D.; Herderich, M.; Schüler, E.; Withopf, B.; Schreier, P. *Tetrahedron: Asymmetry* **1997**, *8*, 853−856.
(9) Storz, G.; Tartaglia, L. A.; Ames, B. N. *Science* **1990**, *248*, 189−194.
(10) Demple, B. *Annu. Rev. Genet.* **1991**, *25*, 315−337.
(11) Blaine Metting, F., Jr. *Soil Microbiological Ecology*; Marcel Dekker Inc.: New York, 1993.
(12) Dworkin, M.; Foster, J. W. *J. Bacteriol.* **1958**, *75*, 592−603.
(13) Harmsen, D.; Heesemann, J.; Brabletz, T.; Kirchner, T.; Müller-Hermelink, H. K. *Lancet* **1994**, *343*, 1288.

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Table 1. Biotransformation of Hydroperoxides by Diverse Microorganisms

entry	ROOH	microorganism	precultivation[a] (days)	incubation time[b] (min)	convn[c] (%)	ROH[d] ee (%)	ROOH[e] ee (%)
1	**1a**	*B. subtilis*[f]	3	240	64	30 (*S*)	88 (*R*)
2	**1a**	*B. subtilis*[f]	1	30[g]	94	20 (*S*)	>99 (*R*)
3	**1b**	*B. subtilis*[f]	1	120[g]	47	36 (*S*)	64 (*R*)
4	**1a**	*Paecilomyces* sp.[f]	11	80[g]	92	8 (*S*)	79 (*R*)
5	**1b**	*Paecilomyces* sp.[f]	11	120	36	3 (*S*)	3 (*R*)
6	**1a**	*Bacillus* sp.[f]	3	240	19	0	6 (*R*)
7	**1a**	*A. niger*[h]	8	15	26	24 (*R*)	10 (*S*)
8	**1a**	*A. niger*[h]	8	30	67	25 (*R*)	37 (*S*)
9	**1b**	*A. niger*[h]	8	45	55	9 (*R*)	7 (*S*)
10	**1a**	*Botrytis cinerea*[h]	8	15	57	7 (*R*)	24 (*S*)
11	**1a**	*Penicillium v.*[h,i]	8	60	23	23 (*R*)	9 (*S*)

[a] Time at which the culture has been pregrown. [b] Actual time for the incubation of the substrate with the pregrown culture. [c] Conversion determined by HPLC analysis (error limit ± 5%). [d] Determined by multidimensional gas chromatography on a 2,6-dimethyl-3-*O*-pentyl-β-cyclodextrin column; **1a**: (a) DB−Wax 100−10 °C/min to 240 °C, (b) cyclodextrin column, 100 °C (15 min isocratic) to 2 °C/min to 200 °C; **1b**: (a) DB-Wax 80−10 °C/min to 240 °C, (b) cyclodextrin column, 60 °C (15 min isocratic) to 2 °C/min to 200 °C) (error limit ±1%). [e] Determined by HPLC analysis on a chiral Chiracel OD-H column (isohexane:2-propanol 9:1, 220 nm, 0.5 mL/min) (error limit ±5%). [f] Bacteria isolated from topsoil by a selective screening procedure. [g] Yeast extract was added to the growth medium. [h] Fungi obtained from Deutsche Sammlung von Mikroorgansimen und Zellkulturen (DSM). [i] *Penicillium verrucosum* var. *verrucosum*.

peroxide **1a** within 240 min with an enantiomeric excess of 88% for the (*R*) hydroperoxide **1a** and 30% for the (*S*)-alcohol **2a**.

When the sterically more demanding 1-(1-phenyl)propyl hydroperoxide (**1b**) was applied to the *Paecilomyces* sp. and *B. subtilis*, the former showed a sharp decline in the conversion rate and the enantiomeric excess (entry 5). Thus, the ee values were only 3% for the (*R*)-peroxide **1b** and 3% for the (*S*)-alcohol **2b** at a conversion of 36%. The conversion was only approximately one-third of that for the hydroperoxide **1a** (entry 4), despite the longer incubation time for the propyl derivative **1b**. In contrast, *B. subtilis* still gave 47% conversion after 120 min to afford an enantiomeric excess of 64% for the (*R*)-hydroperoxide **1b** and 36% for the (*S*)-alcohol **2b** (entry 3). The results show a moderate decrease in enantioselectivitiy, which has also been observed with horseradish peroxidase,[7] because the propyl derivative **1b** is sterically more demanding and presumably does not fit into the enzyme cavity as well as the ethyl homologue **1a**. A decrease in conversion rate was observed, but the drop is smaller than for horseradish peroxidase.[7]

For comparison, arbitrarily chosen commercially available fungal systems were subjected to the hydroperoxides **1a,b**, administered in the same amount as for the bacterial incubations. The fungal cultures showed an even faster conversion, i.e., more than 50% after 15−30 min incubation (Table 1, entries 8 and 10). Unfortunately, due to the much higher biomass present in fungal systems, these results cannot be readily compared to the bacterial ones. Nevertheless, the enantiomeric excess obtained here was significantly lower than that achieved with the cultures isolated from topsoil. The best fungal system was *Aspergillus niger,* which gave an ee value of 37% for the (*S*)-hydroperoxide **1a** and 25% for the corresponding (*R*)-alcohol **2a** (entry 8). The biotransformation of hydroperoxide **1b** with *A. niger* (entry 9) showed again a decrease in the conversion rate and a substantial diminution of the enantiomeric excess (entry 9) compared to what was achieved with (1-phenyl)ethyl hydroperoxide (**1a**).

The results described herein for the microorganism *A. niger* and *B. subtilis* are compared with those of the isolated enzyme horseradish peroxidase reported earlier[7] (Figure 1). Clearly, *B. subtilis* (Table 1, entries 1−3) not only provides a good alternative to horseradish peroxidase, but the additional advantage is the inversion of the sense of the enantioselectivity vs that observed for horseradish peroxidase. Thus, while the fungal systems, with the exception of the *Paecilomyces* sp., and horseradish peroxidase yield the *S* enantiomer of the peroxide and the *R* enantiomer of the alcohol, the bacterial strains afford the opposite enantiomers, namely *R* for the peroxide and *S* for the corresponding alcohol. Therefore, there appear to exist structurally distinct peroxidase enzymes in nature that differ fundamentally in their substrate recognition to provide such differential enantioselectivities.

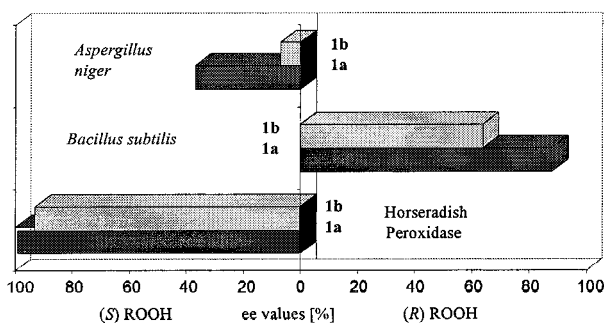

Figure 1. Comparison of the enantioselectivities (ee values) for the kinetic resolution of racemic 1-phenylethyl (**1a**) and 1-phenyl-1-propyl hydroperoxides (**1b**) by *A. niger* (cf. Table 1, entry 8), *B. subtilis* (cf. Table 1, entry 1), and horseradish peroxidase.[7]

For the first time, the kinetic resolution of racemic hydroperoxides has been successfully performed with whole cell systems, in the present case, bacteria. *B. subtilis*, especially, affords high enantioselectivities, allows for easy handling, is environmentally acceptable, and is a readily accessible peroxidase-producing system with little demand on the growth medium. Indeed, our preliminary efforts have already shown novel and encouraging results, which should initiate the further use of whole-cell biocatalysis in the asymmetric synthesis of cumbersome to prepare organic substances such as optically active peroxides.

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Supporting Information Available: Experimental details (5 pages).

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