Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 983–985

A highly enantioselective biocatalytic sulfoxidation by the topsoil bacterium *Pseudomonas frederiksbergensis*

Waldemar Adam,[a,*] Frank Heckel,[b] Chantu R. Saha-Möller,[a] Marcus Taupp[b] and Peter Schreier[b,*]

[a]*Institute of Organic Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany*
[b]*Institute of Food Chemistry, University of Würzburg, Am Hubland, 97074 Würzburg, Germany*

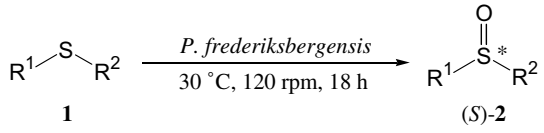
Received 12 December 2003; accepted 23 December 2003

Abstract—The biocatalytic oxidation of various organic sulfides with whole cells of the commercially available topsoil bacterium *Pseudomonas frederiksbergensis* DSM 13022 affords the corresponding (*S*)-sulfoxides in high (up to >99% ee) enantiomeric excess.

1. Introduction

Enantiomerically pure sulfoxides play an important role in asymmetric synthesis either as chiral building blocks or stereodirecting groups.[1] Recently, metal- and enzyme-catalyzed asymmetric sulfoxidations have been developed for the preparation of optically active sulfoxides.[2,3] Enzymes, especially peroxidases, catalyze efficiently the enantioselective sulfoxidation of a variety of sulfides.[4–6] Despite this success, asymmetric transformations with isolated enzymes are tedious and expensive, a major disadvantage for preparative applications.[7] In contrast, whole-cell systems (fungi and bacteria) are available as biocatalysts in sufficient quantities for preparative purposes through self-replication. Thus, several bacterial strains have been successfully employed for the biocatalytic sulfoxidation.[8–10] These studies revealed that intact-cell bacterial oxidation is very promising for the development of environmentally benign, efficient asymmetric sulfoxidation. Therefore, in continuation of our investigations on the biocatalytic oxidation with bacteria,[11–13] we have searched for new bacterial strains with broad scope of substrate tolerance for the enantioselective oxidation of sulfides.

Recently, a topsoil bacterium was isolated as a *Pseudomonas* biovar at a coal gasification site in Frederiksberg, Copenhagen, Denmark,[14] a site propitious for the occurrence of elemental sulfur and its organic derivatives. The strain was phenotypically and genotypically characterized. DNA–DNA hybridization data showed that this strain JAJ28 (DSM 13022) belongs to a new species, which was named *P. frederiksbergensis* sp. Nov.[15] Although, this bacterial strain is known since 1996,[14] biocatalytic applications of this microorganism are essentially unknown. To date, only the degradation of phenanthrene by this topsoil bacterium has been carried out.[14] Herein, we report on the very first application of *P. frederiksbergensis* in asymmetric oxidation, in particular, the enantioselective oxidation of alkyl aryl sulfides (Scheme 1).



1 (*S*)-**2**

Scheme 1. Enantioselective sulfoxidation by the topsoil bacterium *P. frederiksbergensis*.

2. Results and discussion

To assess optimum reaction conditions for the asymmetric sulfoxidation by *P. frederiksbergensis*, screening experiments were carried out with methyl phenyl sulfide **1a** as the model substrate. For this purpose, sulfide **1a** was treated with pregrown bacteria (for details see Experimental) for particular periods of time and, subsequently, the yield and ee value of methyl phenyl sulfoxide were determined (Fig. 1). As shown in Figure 1, the yield of the sulfoxide increased gradually for reaction times of up to 70 h, while high enantioselectivity

* Corresponding authors. Tel.: +49-931-8885480; fax: +49-931-8885-484; e-mail: schreier@pzlc.uni-wuerzburg.de

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2003.12.030

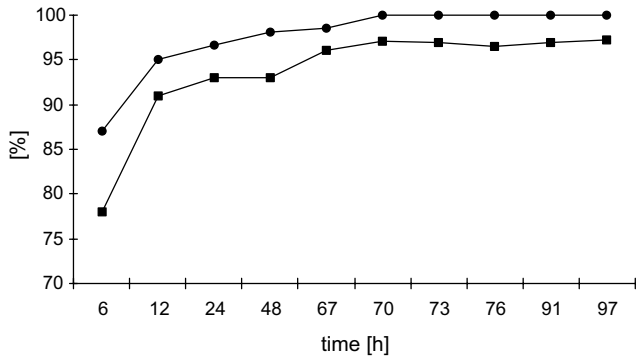


Figure 1. Time profile of the oxidation of methyl phenyl sulfide (**1a**) with pregrown *P. frederiksbergensis*; conversion (–●–) of methyl phenyl sulfide and enantiomeric excess (–■–) of methyl phenyl sulfoxide.

(>90% ee) was achieved already after 12 h of conversion. After about 18 h, the enantioselectivity did not increase any more for the model sulfide **1a** and, therefore, this reaction time was applied for the oxidation of the other sulfide substrates. To assess the appropriate pH value in the bacterial sulfoxidation, a pH profile was run for the sulfoxidation of the methyl phenyl sulfide **1a** by *P. frederiksbergensis*. Essentially enantiopure sulfoxide was obtained at the broad pH range of 6–8 (data not shown). Thus, an adjusted Dworkin mineral nutriment solution of neutral pH value (pH = 7) was used as the basic medium for the bacterial sulfoxidation. A 50% aqueous solution of glucose was chosen as the carbon source and optimal bacterial growth was achieved by using 15–30 g glucose per liter of growth medium.

The optimized conditions were applied in the biocatalytic oxidation of a broad series of sulfides with the soil bacterium *P. frederiksbergensis* (Table 1). The yields of the sulfoxides range between 4% and 48% (data not shown in Table 1), but have as yet not been optimized, since the emphasis of this work lies on the enantioselectivity of the sulfoxidation. The standard model substrate, methyl phenyl sulfide **1a**, was sulfoxidized in an enantiomeric excess of 91% (entry 1). In the case of *para* substitution, as in the sulfides **1b**–**d** (entries 2–4), essentially enantiopure sulfoxides were obtained. Interestingly, the enantioselectivity is independent of the electronic properties of the *para* substituents, since for both electron-donating (Me, MeO) and electron-withdrawing (Cl) groups excellent enantiodifferentiation by the topsoil bacterium *P. frederiksbergensis* was achieved. To assess the effect of the alkyl side chain on the enantioselectivity, the *n*-propyl derivative **1e** was treated with the microorganism. As shown in entry 5, significantly lower enantioselectivity (81% ee of **2e**) was observed compared to that for the methyl derivative **1a** (91% ee, entry 1); thus, the chain length has a pronounced effect on the enantioselectivity of sulfoxidation by this bacterium. Moreover, a branched alkyl chain dramatically lowers the substrate acceptance of *P. frederiksbergensis*; for example, the isopropyl derivative **1f** was barely consumed by this topsoil bacterium (entry 6). Not only alkyl aryl sulfides **1a**–**e**, but also dialkyl sulfides such as cyclohexyl methyl sulfide **1g** are sulfoxidized in good enantioselectivity (70% ee, entry 7). In contrast, the benzyl methyl sulfide **1h** was not accepted by *P. frederiksbergensis* (entry 8).

For all enantioenriched sulfoxides described herein, the *S* enantiomer predominates and in no case was the corresponding sulfone formed in these bacterial transformations. The observed sense of the enantioselectivity in the sulfoxidation by *P. frederiksbergensis* is opposite to that of peroxidases, for example, chloroperoxidase.[16]

3. Conclusions

In summary, we have described for the first time the enantioselective sulfoxidation of a series of organic sulfides by *P. frederiksbergensis*, whose biocatalytic potential has so far been hardly explored for oxidation purposes. These encouraging results should stimulate further work on this readily available soil bacterium, such as the identification of its oxidation enzyme and the utilization of this microorganism for large-scale application in sulfoxidation and other oxidative transformations.

4. Experimental

4.1. General procedure for the biotransformation of sulfides by *P. frederiksbergensis*

The *P. frederiksbergensis* strain (DSM13022) was obtained from the DSMZ (Deutsche Sammlung für

Table 1. Enantioselective oxidation of sulfides **1** to sulfoxides **2** by *P. frederiksbergensis*

Entry	Substrate	R^1	R^2	Conversion (%)	(*S*)-**2** ee (%)[a]
1	**1a**	Ph	Me	87	91
2	**1b**	4-Me–Ph	Me	100	>99
3	**1c**	4-MeO–Ph	Me	98	>99
4	**1d**	4-Cl–Ph	Me	86	96
5	**1e**	Ph	*n*-Pr	27	81
6	**1f**	Ph	*i*-Pr	<5	—
7	**1g**	c-Hex	Me	98	70
8	**1h**	Ph–CH_2	Me	<5	—

[a] Enantiomeric excess determined by a multidimensional gas-chromatography/mass spectrometry combination on a 2,3-diethyl-6-*tert*-butyldimethylsilyl-β-cyclodextrin column, error ±1.5% of the stated value; the *S* absolute configuration was assessed from CPO data (Ref. 16).

Mikroorganismen und Zellkulturen, Braunschweig, Germany) and maintained at 4 °C on Standard Methods Agar (from Bio Mérieux, Marcy l'Etoile, France). To elucidate the scope of sulfoxidation activity by *P. frederiksbergensis*, several organic sulfides [analytical grade obtained from Fluka (Seelze, Germany), Sigma-Aldrich (Taufkirchen, Germany), or Lancaster (Morecambe, England)] were used for biotransformation. The bacterial sulfoxidation experiments were carried out in 300-mL Erlenmeyer flasks, which were charged with 100 mL Dworkin mineral nutriment solution[17] and 375 μL *Pseudomonas* trace element solution.[18] This medium was sterilized at 121 °C for 16 min prior to the addition of 750 μL 50% steril glucose solution (standard conditions). After inoculation with bacterial material, the culture was pregrown for 24 h and then shaken in the presence of 100 μmol of the appropriate sulfide for 18 h at 30 °C with 120 rpm. Subsequently, the bacterial cells were treated by ultrasound to release the sulfoxides that are retained in the cells. After centrifugation (9000 rpm, 15 min), the bacterial suspension was successively extracted twice with 100 mL ethyl ether and 100 mL dichloromethane (all solvents were distilled before use). Finally, the products were submitted to gas-chromatographic analysis and mass spectrometry (GC–MS). The chiral analysis of these products was performed by a multidimensional GC–MS combination on a 2,3-diethyl-6-*t*-butyldimethylsilyl-β-cyclodextrin column. For GC analysis, Thermo Finnigan Gaschromatographs 8000 series were utilized and for chiral analysis a moving column switching system (MCSS) was used for peak transfer. High-resolution mass spectra were obtained on a Finnigan MD800. The absolute configurations of the sulfoxides were determined by comparison with authentic samples (prepared by enzymatic sulfoxidation with chloroperoxidase (CPO) according to the previously reported procedure[4]) and literature data.[16]

References and notes

1. Mata, E. G. *Phosphorus, Sulfur Silicon Relat. Elem.* **1996**, *117*, 231–286.
2. Adam, W.; Lazarus, M.; Saha-Möller, C. R.; Weichold, O.; Hoch, U.; Häring, D.; Schreier, P. *Adv. Biochem. Eng. Biotechnol.* **1999**, *63*, 73–108.
3. Colonna, S.; del Sordo, S.; Gaggero, N.; Carres, G.; Pasta, P. *Heteroat. Chem.* **2002**, *13*, 467–473.
4. Colonna, S.; Gaggero, N.; Casella, L.; Carrea, G.; Pasta, P. *Tetrahedron: Asymmetry* **1992**, *3*, 95–106.
5. van Deurzen, M. P. J.; Remkes, I. J.; van Rantwijk, F.; Sheldon, R. A. *J. Mol. Catal. A: Chem.* **1997**, *117*, 329–337.
6. Adam, W.; Mock-Knoblauch, C.; Saha-Möller, C. R. *J. Org. Chem.* **1999**, *64*, 4834–4839.
7. Adam, W.; Heckel, F.; Saha-Möller, C. R.; Schreier, P. *J. Organomet. Chem.* **2002**, *661*, 17–29.
8. Holland, H. L. *Chem. Rev.* **1988**, *88*, 473–485.
9. Kelly, D. R.; Knowles, C. J.; Mahdi, J. G.; Taylor, I. N.; Wright, M. A. *Tetrahedron: Asymmetry* **1996**, *7*, 365–368.
10. Boyd, D. R.; Sharma, N. D.; Haughey, S. A.; Kennedy, M. A.; McMurray, B. T.; Sheldrake, G. N.; Allen, C. C. R.; Dalton, H.; Sproule, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1929–1933.
11. Adam, W.; Lukacs, Z.; Kahle, C.; Saha-Möller, C. R.; Schreier, P. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 377–385.
12. Adam, W.; Lukacs, Z.; Harmsen, D.; Saha-Möller, C. R.; Schreier, P. *J. Org. Chem.* **2000**, *65*, 878–882.
13. Adam, W.; Lukacs, Z.; Saha-Möller, C. R.; Schreier, P. *J. Am. Chem. Soc.* **2000**, *122*, 4887–4892.
14. Johnsen, K.; Andersen, S.; Jacobsen, C. S. *Appl. Environ. Microbiol.* **1996**, *62*, 3818–3825.
15. Andersen, S. M.; Johnsen, K.; Sørensen, J.; Nielsen, P.; Jacobsen, C. S. *Int. J. Syst. Evolut. Microbiol.* **2000**, *50*, 1957–1964.
16. Colonna, S.; Gaggero, N.; Manfredi, A.; Casella, L.; Gullotti, M.; Carrea, G.; Pasta, P. *Biochemistry* **1990**, *29*, 10465–10468.
17. Dworkin, M.; Forster, J. W. *J. Bact.* **1958**, *75*, 592–603.
18. Atlas, R. M. In *Handbook of Microbiological Media*; Parks, L. C., Ed.; 2nd ed.; CRC: Boca Raton, 1993; p 1.