ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 17 (2002) 235–240

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

Acetic acid bacteria as enantioselective biocatalysts

A. Romano [a], R. Gandolfi [a], P. Nitti [b], M. Rollini [a], F. Molinari [a,*]

[a] *Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Sezione Microbiologia Industriale, Università degli Studi di Milano, via Celoria 2, 20133 Milano, Italy*

[b] *Dipartimento di Scienze Chimiche, Università di Trieste, via L. Giorgieri 1, 34127 Trieste, Italy*

Received 4 December 2001; received in revised form 8 February 2002; accepted 8 February 2002

Abstract

Acetic acid bacteria (five strains of *Acetobacter* and five strains of *Gluconobacter*) were used for the biotransformation of different primary alcohols (2-chloropropanol and 2-phenylpropanol) and diols (1,3-butandiol, 1,4-nonandiol and 2,3-butandiol). Most of the tested strains efficiently oxidized the substrates. 2-Chloropropanol and 1,3-butandiol were oxidized with good rates and low enantioselectivity (enantiomeric excess = 18–46% of the *S*-acid), while microbial oxidation of 2-phenylpropanol furnished (*S*)-2-phenyl-1-propionic acid with enantiomeric excess (e.e.) >90% with 10 strains. The dehydrogenation of 2,3-butandiol was strongly dependent on the stereochemistry of the substrate; the *meso* form gave *S*-acetoin with all the tested strains, the only exception being a *Gluconobacter* strain. The formation of diacetyl was observed only by using *R,R*-2,3-butandiol with *Acetobacter* strains. Oxidation of 1,4-nonandiol gave γ-nonanoic lactone in one step, although with moderate enantioselectivity.

Keywords: *Acetobacter*; *Gluconobacter*; Carboxylic acids; Resolution; Oxidation

1. Introduction

Biotransformations may offer distinct advantages over classical chemical synthetic technology; they can be carried out under mild and ecologically compatible conditions, being often chemo-, regio- and enantioselective [1]. Application of whole microbial cells can be very attractive when oxidations are involved, since cofactor and recycle systems form part of the cells' metabolism [2].

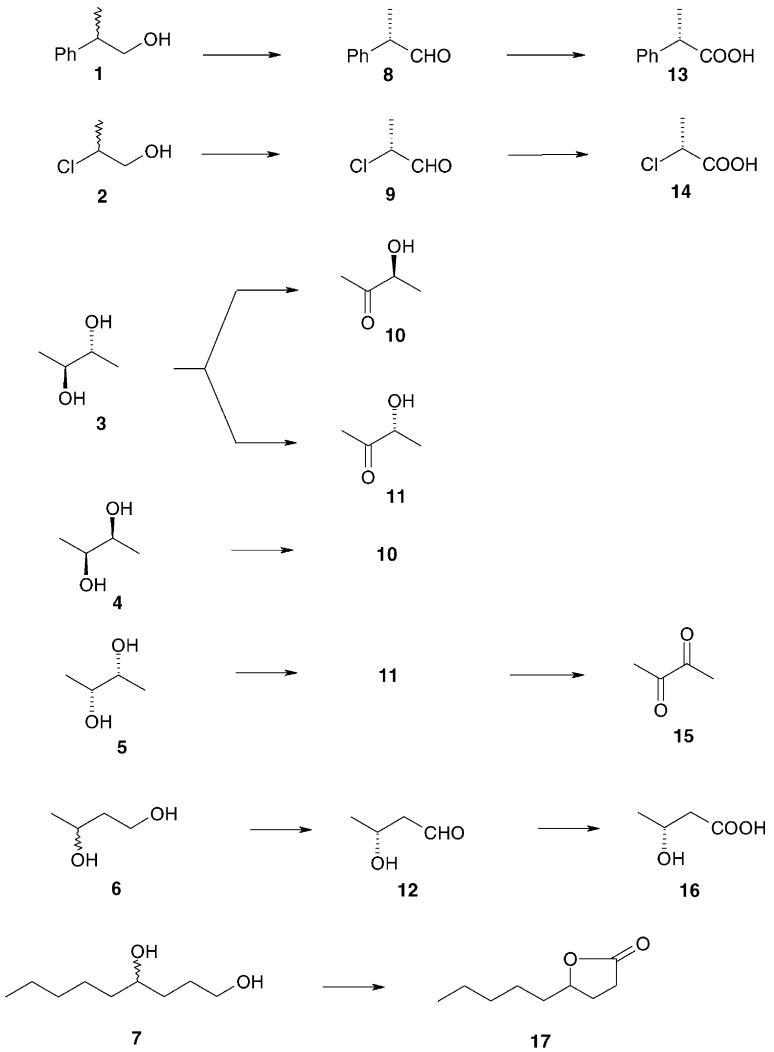
Acetic acid bacteria have a respiratory metabolism characterized by incomplete oxidation in which partially oxidized organic compounds are excreted as end-products [3]. Oxidation of primary alcohols with acetic acid bacteria has proven an easy and efficient means for the production of structurally diverse carboxylic acids [4–7]. Moreover, they can be employed for the enantioselective oxidation of different racemic primary alcohols for the production of optically pure carboxylic acids [8,9]. The enantioselectivity of quinoehaemoprotein dehydrogenases belonging to acetic acid bacteria has been recently reviewed and mechanisms accounting for their stereopreference have been proposed [10].

The dehydrogenation of diols with acetic acid bacteria had been the object of few studies [11–13], but no recent systematic information about the stereochemical outcomes of these oxidations is available.

In this work, we have employed acetic acid bacteria for the oxidation of racemic primary alcohols and chiral and prochiral diols (Scheme 1) with strains belonging to the genus *Acetobacter* and *Gluconobacter*.

* Corresponding author. Tel.: +39-02-50316695; fax: +39-02-50316694.
E-mail address: francesco.molinari@unimi.it (F. Molinari).

1381-1177/02/$ – see front matter
PII: S1381-1177(02)00013-9



Scheme 1. Oxidation of chiral and prochiral alcohols and diols.

The selection of biocatalysts capable of performing enantioselective oxidation of racemic alcohols or diols is of preparative interest.

2. Results and discussion

2.1. Oxidation of 2-phenylpropanol and 2-chloropropanol

Twelve strains belonging to different species of acetic acid bacteria were used for gaining information about the enantioselective potential of this microbial group. Acetic acid bacteria were grown in submerged cultures using GlyY medium and tested for oxidation at 28 °C (Scheme 1). Biotransformations were performed with cells grown 24 h; Table 1 reports the average dry weights obtained.

The oxidation of racemic 2-phenyl-1-propanol (**1**) and 2-chloropropanol (**2**) was firstly investigated. These compounds were chosen as model substrates due to the importance of optically pure 2-arylpropionic acids [14] and 2-chloropropionic acid [15]. Two strains failed to oxidize the substrates (*Acetobacter*

Table 1
Dry weight of acetic acid bacteria grown for 24 h in GlyY medium

Strains	Dry weight ($g\,l^{-1}$)
A. aceti DSM 3508	1.8
A. aceti MIM 2000/28	4.1
A. aceti MIM 2000/50	4.4
A. hansenii MIM 2000/5	3.8
A. liquefaciens DSM 5603	2.6
A. pasteurianus DSM 3509	1.0
A. xylinus MIM 2000/13	4.2
G. asaii MIM 1000/9	2.7
G. cerinus DSM 9534	2.0
G. frateurii DSM 7146	2.3
G. oxydans DSM 2343	1.4
G. oxydans MIM 1000/14	2.9

liquefaciens Deutsche Sammlung von Mikroorganismen (DSM) 5603 and *A. pasteurianus* DSM 3509), while the other strains furnished the correspondent carboxylic acid with no significant accumulation of the intermediate aldehyde (Table 2).

The oxidation of (*R,S*)-2-phenyl-1-propanol generally proceeded with high enantioselectivity, while the oxidation of the racemic mixture of 2-chloropropanol occurred with much lower enantioselectivity, the highest enantiomeric excess (e.e.) being below 50%. Higher rates were generally observed with 2-chloropropanol, which may indicate that less bulky substituents (e.g. chloro versus phenyl) have a positive effect on velocity and a negative effect on enantioselectivity.

Low e.e. observed with whole cells may be due to a number of factors, such as the presence of various dehydrogenases with different enantioselectivity, the action of a single enzyme with low enantioselectivity or racemization of the formed product. Racemization was ruled out by carrying out experiments in which optically pure *S*-chloropropanoic acid was added to the cells and observing that no significant change in the enantiomeric composition took place. Biotransformations were also carried out at various substrate concentrations using cells resuspended in different buffers with pH values from 4 to 9. The variation of these parameters did not influence significantly the stereochemical outcome with e.e. ranging between 45 and 55%. The action of the involved dehydrogenases seems, therefore, stereo-convergent, whatever, the conditions of the biotransformations.

2.2. Oxidation of chiral and prochiral diols

Dehydrogenation of 2,3-butandiol was firstly investigated. It had been reported that oxidation of 2,3-butandiol with acetic acid bacteria occurred with formation of acetoin, but not diacetyl [3]. It had also been observed that strains of *A. xylinus*, *A. aceti* and *Gluconobacter oxydans* oxidized *meso*-2,3-butandiol to (*S*)-acetoin and general preference for the oxidation of the hydroxyl in the *R*-configuration was encountered [10,11].

The different stereoisomers of 2,3-butandiol were used as substrates. No diacetyl formation could be detected with *meso*-2,3-butandiol (**3**); biotransformations performed in closed vessels or with vapour traps could rule out the disappearance of diacetyl by evaporation.

Table 2
Molar conversions and e.e. obtained in the oxidation of 2-chloropropanol and 2-phenylpropanol ($2.5\,g\,l^{-1}$) with acetic acid bacteria

Strains	2-Chloropropanol			2-Phenylpropanol		
	Conversion	e.e. (%)	Time (h)	Conversion	e.e. (%)	Time (h)
A. aceti DSM 3508	35	40 (*R*)	1	40	95 (*S*)	24
A. aceti MIM 2000/28	20	35 (*R*)	1	25	92 (*S*)	24
A. aceti MIM 2000/50	37	43 (*R*)	2	43	97 (*S*)	24
A. hansenii MIM 2000/5	50	39 (*R*)	4	38	91 (*S*)	24
A. xylinus MIM 2000/13	25	45 (*R*)	1	34	90 (*S*)	24
G. asaii MIM 1000/14	23	46 (*R*)	4	33	>97 (*S*)	24
G. cerinus DSM 9534	27	44 (*R*)	4	20	95 (*S*)	24
G. frateurii DSM 7146	35	28 (*R*)	3	22	91 (*S*)	24
G. oxydans DSM 2343	20	45 (*R*)	4	<5	–	24
G. oxydans MIM 1000/9	45	18 (*R*)	4	45	97 (*S*)	24

Table 3
Molar conversion and e.e. of acetoin production after 1 h by oxidation of *meso*-2,3-butandiol (**3**) with acetic acid bacteria

Strains	Conversion (%)	e.e. (%)
A. aceti DSM 3508	30	>97 (*S*)
A. aceti MIM 2000/28	22	80 (*S*)
A. aceti MIM 2000/50	15	76 (*S*)
A. hansenii MIM 2000/5	80	>97 (*S*)
A. xylinus MIM 2000/13	89	97 (*S*)
G. asaii MIM 1000/9	100	>97 (*S*)
G. frateurii DSM 7146	50	40 (*S*)
G. cerinus DSM 9534	15	72 (*R*)
G. oxydans DSM 2343	40	>97 (*S*)
G. oxydans MIM 1000/14	50	>97 (*S*)

The biotransformation was sometimes very fast (*A. hansenii* MIM 2000/5, *A. xylinus* MIM 2000/13, *G. asaii* MIM 1000/9) and highly enantioselective with a general preference for the reaction of the *R*-stereocenter, the only exception being *G. cerinus* DSM 9534 (Table 3).

Optically pure (*S*,*S*)- and (*R*,*R*)-2,3-butandiol were also tested. No diacetyl was formed with (*S*,*S*)-2,3-butandiol (**4**), while *A. hansenii* MIM 2000/5 oxidized (*R*,*R*)-2,3-butandiol (**5**) with significant production of diacetyl (Table 4).

The (*R*,*R*) stereoisomer was always consumed much faster and complete specificity for the oxidation of the *R*-stereocenters was observed with two *Gluconobacter* strains which were also very fast.

The same strains were employed for the oxidation of (*R*,*S*)-1,3-butandiol (Table 5). The reaction proceeded with absolute chemoselectivity, since only the primary alcohol was oxidized; the initial reaction rates were often quite high, although complete molar conversion after 24 h was never observed. Small amounts of the intermediate aldehyde could be detected in few cases.

The enantioselectivity was generally very low; one reason for the high rates and low enantiospecificity may be the distance between the stereocenter and the reacting site. Similar rates could be observed in the oxidation of *n*-butanol with acetic acid bacteria [6].

Finally the oxidation of (*R*,*S*)-1,4-nonadiol was investigated. Also in this case, the selective oxidation of the primary hydroxyl function was observed, since nonanoic lactone was the only product detectable in significant amounts (Table 6).

The highest e.e. of the lactone were obtained with two *Gluconobacter* strains, but enantioselectivity was generally quite low. The direct formation of the corresponding lactone is an interesting feature; the biotransformation was carried out also with cells resuspended in phosphate buffer at pH in the range of 5–8 and in all the cases the lactone was the only product. No improvement of the rates and enantioselectivity was observed.

3. Experimental section

3.1. Microorganisms

Strains from an official collection (*A. aceti* DSM 3508, *A. hansenii* DSM 5602, *A. liquefaciens* DSM 5603, *A. pasteurianus* DSM 3509, *A. xylinus* DSM 2004, *G. asaii* DSM 7148, *G. cerinus* DSM 9534, *G.*

Table 4
Molar conversion of acetoin and diacetyl production after 1 h by oxidation of (*R*,*R*)- and (*S*,*S*)-2,3-butandiol with acetic acid bacteria

Strains	(*S*,*S*)-2,3-Butandiol **(4)**		(*R*,*R*)-2,3-Butandiol **(5)**	
	Acetoin	Diacetyl	Acetoin	Diacetyl
A. aceti DSM 3508	<5	–	78	–
A. aceti MIM 2000/28	15	–	78	–
A. aceti MIM 2000/50	9	–	45	–
A. hansenii MIM 2000/5	<5	–	70	30
A. xylinus MIM 2000/13	85	–	87	5
G. asaii MIM 1000/9	10	–	45	–
G. cerinus DSM 9534	<5	–	<5	–
G. frateurii DSM 7146	22	–	>97	–
G. oxydans DSM 2343	27	–	50	–
G. oxydans MIM 1000/14	<5	–	>97	–

Table 5
Molar conversions and e.e. obtained in the oxidation of (*R,S*)-1,3-butandiol (**6**) (2.5 g l^{-1}) with acetic acid bacteria

Strains	1 h		24 h	
	Aldehyde **(12)**	Acid (**16**) (e.e.)	Aldehyde (**12**)	Acid (**16**) (e.e.)
A. aceti DSM 3508	5	50 (12 *S*)	–	90 (<5)
A. aceti MIM 2000/28	5	35 (7 *S*)	–	70 (<5)
A. aceti MIM 2000/50	10	30 (15 *S*)	5	65 (7 *S*)
A. hansenii MIM 2000/5	10	40 (18 *S*)	–	95 (<5)
A. xylinus MIM 2000/13	5	25 (12 *S*)	–	90 (<5)
G. asaii MIM 1000/9	10	5	10	30 (10 *S*)
G. cerinus DSM 9534	–	55 (22 *S*)	–	75 (<5)
G. frateurii DSM 7146	–	25 (5 *S*)	–	30 (<5)
G. oxydans DSM 2343	10	<5	25	5 (<5)
G. oxydans MIM 1000/14	5	30 (< 5)	–	70 (<5)

frateurii DSM 7146, *G. oxydans* DSM 50049; DSM) were used with microorganisms from our collection. Microorganisms were routinely maintained on GYC solid medium. Submerged cultures were carried out in a GlyY medium (glycerol 25 g l^{-1}, yeast extract 10 g l^{-1}, pH 5) into 1 l Erlenmeyer flasks containing 150 ml of medium on a reciprocal shaker (100 spm). After centrifugation of 100 ml of submerged cultures, cells were washed with distilled water and dried at 110 °C for 24 h for determining dry weight. The results are the means (±0.3 g l^{-1}) of 10 replicates.

3.2. Biotransformations

Experiments were carried out with 24 h submerged cultures. Submerged cultures were carried out in a GlyY medium (glycerol 25 g l^{-1}, yeast extract 10 g l^{-1}, pH 5) into 1 l Erlenmeyer flasks containing 150 ml of medium on a reciprocal shaker (100 spm). Biotransformations were directly carried out with growing cells or with cells centrifuged and suspended in phosphate buffers (0.1 M) at different pH values. Neat substrates were directly added to the suspensions and flasks were shaken on a reciprocal shaker (100 spm). The results were expressed as molar conversion, defined as the number of converted moles per number of starting moles.

Table 6
Formation of γ-nonanoic lactone after 3 h by oxidation of (*R,S*)-1,4-nonandiol (**7**) (2.5 g l^{-1}) with acetic acid bacteria

Strains	Molar conversion (%)	e.e. (%)
A. aceti DSM 3508	30	35 (*S*)
A. hansenii MIM 2000/5	50	5 (*S*)
A. xylinus MIM 2000/13	28	12 (*S*)
A. aceti MIM 2000/28	35	20 (*S*)
A. aceti MIM 2000/50	38	30 (*S*)
G. asaii MIM 1000/9	<5	–
G. cerinus DSM 9534	<5	–
G. frateurii DSM 7146	12	44 (*S*)
G. oxydans DSM 2343	<5	–
G. oxydans MIM 1000/14	20	58 (*S*)

3.3. Analytical methods

Samples (0.5 ml) were taken at intervals, brought to pH 1 by addition of 0.5 M HCl and extracted with an equal volume of $CHCl_3$. The conversion of 2-phenyl-1-propanol (**1**) into phenylpropanoic acid (**13**) and 1,4-nonandiol (**7**) into γ-nonanoic lactone (**17**) were routinely determined by GLC analysis. In the case of **13**, the analysis was performed after conversion of the acid to the corresponding methyl ester by treatment with CH_2N_2; the organic extracts were then dried and dissolved in a $CHCl_3$ solution containing an internal standard (phenethyl alcohol). GLC was carried out on a Carlo Erba Fractovap G1 gaschromatograph equipped with a hydrogen flame ionization detector. The column (3 *mm* × 2000 mm) was packed with Carbowax 1500 (10% on Chromosorb W 80–100 mesh) with the column temperature kept at 195 °C.

The biotansformations of 2-chloro-1-propanol (**2**), 2,3-butandiol (**3**–**5**) and 1,3-butandiol (**6**) were followed by HPLC analysis using a Polyspher OA

HY column (Merck) and an aqueous acidic solution (H_2SO_4 0.005 N) as eluent which allowed for the determination of the concentration of the substrates and products.

The enantiomeric composition was routinely determined by gas chromatographic analysis using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 m, DMePeBeta-CDX-PS086, MEGA, Legnano, Italia). Acetoin (**10**) and γ-nonanoic lactone (**17**) could be directly resolved, while the acids **13**, **14** and **16** were converted into the corresponding methyl esters. The absolute configuration of the products was determined by comparison with the optical rotation of authentic samples of the optically pure enantiomers.

4. Conclusions

Acetic acid bacteria can be advantageously used as enantioselective biocatalysts for the oxidation of primary alcohols or diols. The stereobias of acetic acid bacteria mediated oxidations appears to be mainly dependent on the substrate and not on the strain employed. Complete chemoselectivity was observed when 1,3 or 1,4-diols were employed, with oxidation only of the primary hydroxy group; it is noteworthy that nonanoic lactone was produced in one step by oxidation of 1,4-nonandiol.

Acknowledgements

This work was supported by the C.N.R. Target Project on Biotechnology (n 97.01019. PF 115.08601).

References

[1] D. Vasic-Racki, History of industrial biotransformations-dreams and reality, in: A. Liese, K. Seelbach, C. Wandrey (Eds.), Wiley, Weinheim, Germany, 2000.
[2] H.L. Holland, Enzymes as bioconversion catalyst, in: Organic Synthesis With Oxidative Enzymes, VCH Publishers, New York, 1992.
[3] T. Asai, Biochemical activities of acetic acid bacteria, in: Acetic Acid Bacteria, University of Tokyo Press, Tokyo, pp.103–314, 1968.
[4] I. Gatfield, T. Sand, European Patent Application 289,822 (1988).
[5] J. Svitel, E. Sturdik, Enzym. Microb. Technol. 17 (1995) 546.
[6] F. Molinari, R. Villa, F. Aragozzini, P. Cabella, M. Barbeni, J. Chem.Technol. Biotechnol. 70 (1997) 294.
[7] R. Gandolfi, N. Ferrara, F. Molinari, Tetrahedron Lett. 42 (2001) 513–514.
[8] A. Geerlof, J.A. Jongejan, T.J.G.M. Van Dooren, P.C. Raemakers-Franken, W.J.J. Van Den Tweel, J.A. Duine, Enzym. Microb. Technol. 16 (1994) 1059.
[9] F. Molinari, R. Villa, F. Aragozzini, R. Lèon, D.M.F. Prazeres, Tetrahedron: Asymmetry 10 (1999) 3003.
[10] A. Jongejan, S.S. Machado, J.A. Jongejan, J. Mol. Catal. B Enzym. 8 (2000) 121.
[11] E. Grivsky, Bull. Soc. Chem. Belge. 51 (1942) 63.
[12] E.I. Fullmer, L.A. Underfloker, A.C. Bantz, J. Am. Chem. Soc. 65 (1943) 1425.
[13] P. Adlercreutz, Appl. Microbiol. Biotechnol. 30 (1989) 257.
[14] G.P. Stahly, R.M. Starrett, Production method for chiral non-steroidal anti-infiammatory profen drugs, in: A.N. Collins, G.N. Sheldrake, J. Crosby (Eds.), Chirality in Industry, 2nd Edition, Wiley, Chichester, 1997.
[15] S.J.C. Taylor, (*S*)-2-chloropropanoic acid: developments in its industrial manufacture, in: A.N. Collins, G.N. Sheldrake, J. Crosby (Eds.), Chirality in Industry, 2nd Edition, Wiley, Chichester, 1997.