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An easy and efficient method for the production of carboxylic acids and aldehydes by microbial oxidation of primary alcohols

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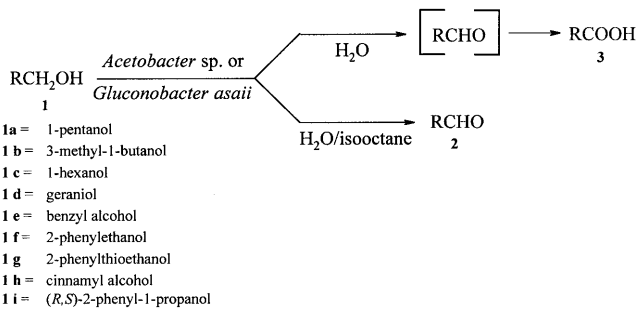
Abstract—An easy and efficient methodology for obtaining aldehydes or carboxylic acids by oxidation of the corresponding primary alcohols with acetic acid bacteria is reported. When the biotransformation is performed in water the acids are obtained; aldehydes can be accumulated by using a water/isooctane two-liquid phase system. © 2001 Elsevier Science Ltd. All rights reserved.

Biotransformations are a useful and complementary tool for preparative organic chemistry. In the course of a biotransformation the chemo-, regio- and stereoselective transformation of a substrate often takes place under mild and ecologically-compatible conditions. The oxidation of primary alcohols by acetic acid bacteria to the respective acids is a widespread transformation which proceeds firstly through the action of alcohol dehydrogenases and secondly through aldehyde dehydrogenase.[1] The overall dehydrogenase activities are generally not very specific and aldehydes are not normally accumulated.[2–4] The use of two-liquid phase systems (composed of water and an organic solvent) may provide a method for the production of sufficiently hydrophobic aldehydes, since they can be extracted in situ from the aqueous phase, avoiding further oxidation.[5] Two strains of acetic acid bacteria were used in this work: *Acetobacter* sp. ALEG MIM, which has previously shown the highest yields of phenylacetaldehyde production[5] and *Gluconobacter asaii* MIM 1000/14.[6] The oxidation of different alcohols was carried out in water and in a two-liquid phase system composed of water and isooctane (vol/vol 1/1) (Scheme 1).[7]

As shown in Table 1, the oxidation of primary alcohols in water by the two strains tested furnished the respective carboxylic acids as main products, while aldehydes were only transiently observed. The transformation which proceeds through the action of two dehydrogenases is not very specific and, therefore, aldehydes are not accumulated. *Acetobacter* sp. ALEG oxidized all the substrates with high rates and yields, the only exception being benzyl alcohol; also the *Gluconobacter* showed good selectivities, although sometimes (**1d** and **1h**) with lower yields.

The transformations carried out in the two-liquid phase system composed of water and isooctane (vol/vol 1/1) showed that the use of an apolar extractive solvent allowed the accumulation of the aldehydes which are mostly found in the organic phase. Over prolonged reaction times the aldehydes were further oxidized to carboxylic acids, with the exception of geranial which was not further transformed by *Acetobacter* sp. ALEG. With *Gluconobacter asaii* we observed a higher conversion of geraniol in the two-liquid phase system than in water; this may be due to an inhibitory effect of geraniol on the microbial dehydrogenases, which is reduced



Scheme 1.

Keywords: biotransformation; carboxylic acid; aldehyde; alcohol oxidation; acetic acid bacteria; two-liquid phase system.

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Table 1.

	Acetobacter						*Gluconobacter*					
	Water			Water/isooctane			Water			Water/isooctane		
Substrate	Product	Yield[a]	Time (h)	Product	Yield[b]	Time (h)	Product	Yield	Time (h)	Product	Yield	Time (h)
1a	**3a**	>97	3	**2a**	74	1	**3a**	>97	4	**2a**	93	45 min
1b	**3b**	>97	3	**2b**	90	1	**3b**	>97	4	**2b**	90	1
1c	**3c**	>97	3	**2c**	87	1	**3c**	>97	3	**2c**	91	45 min
1d	**3d**	>97	24	**2d**	72	4	**3d**	16	24	**2d**	29	5
1e	**3e**	25	24	**2e**	<5	24	**3e**	<5	24	**2e**	<5	24
1f	**3f**	>97	3	**2f**	90	45 min	**3f**	>97	5	**2f**	85	2
1g	**3g**	>97	2	**2g**	93	45 min	**3g**	>97	5	**2g**	96	1
1h	**3h**	>97	8	**2h**	77	45 min	**3h**	20	24	**2h**	24	4
1i	**3i**	40	24	**2i**	<5	24	**3i**	33	24	**2i**	<5	24

[a] Yields (%) determined by standard GLC analysis; carboxylic acids were analysed after conversion to the corresponding methyl ester after treatment with CH_2N_2.
[b] The yields of the aldehydes are related to the sum of the products detected in the aqueous and organic phase.

in the presence of an organic phase where the hydrophobic substrate mostly accumulates. The aldehydes produced by these biotransformations were easily purified from the organic phase.[8]

The racemic mixture of **1i** was subjected to a kinetic resolution, furnishing the (*S*)-alcohol with high ee. (95% at 40% molar conversion); the use of acetic acid bacteria to perform enantioselective oxidation of racemic mixtures of primary alcohols has been already reported.[9–11] The enantiomeric composition of 2-phenylpropanoic acid was determined as previously described.[11]

The use of a two-liquid phase system composed of water and isooctane appears, therefore, to be suited to the production of various aldehydes by oxidation of primary alcohols using acetic acid bacteria. This microbial biotransformation seems a promising and possibly general method to furnish aldehydes under mild and ecologically compatible conditions.

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6. Strains (*Acetobacter* sp. ALEG and *Gluconobacter asaii* 1000/14) were from our collection (MIM, Microbiologia Industriale Milano) and routinely maintained on GYC solid medium (glucose 50 g l^{-1}, yeast extract 10 g l^{-1}, $CaCO_3$ 30 g l^{-1}, agar 15 g l^{-1}, pH 6.3) at 28°C. 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 200 ml of medium on a reciprocal shaker (100 spm).
7. Experiments were carried out with 24 hour submerged cultures. In experiments with two-liquid phase systems, solvents were added to reach the desired volumes. Neat substrates (2.5 g l^{-1}) were directly added to the suspensions and flasks were shaken on a reciprocal shaker (100 spm).
8. The work-up of the production of phenylthioacetaldehyde is reported as an example. Biotransformation was carried out starting from 500 mg of the alcohol in 200 ml of cultural broth containing 200 ml of isooctane and after 50 minutes the reaction mixture was centrifuged (15000 g, 10 min) to remove the bacterial cells, the surnatant was extracted with ethyl acetate. The organic extracts were dried over Na_2SO_4 and the solvent removed; the crude product was purified by flash chromatography (hexane/ethyl acetate, 7/3) to give 415 mg of phenylthioacetaldehyde. All compounds were characterized by a combination of ^1H NMR, GC/MS and molar conversions determined by GC or reversed phase HPLC analysis.
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