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Chemoselective oxidation of primary alcohols to aldehydes with *Gluconobacter oxydans*

Raffaella Villa,[a] Andrea Romano,[a] Raffaella Gandolfi,[a] José V. Sinisterra Gago[b] and Francesco Molinari[a,]*

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

[b]*Departamento de Química Orgánica y Farmacéutica, Facultad de Farmacia, Universidad Complutense de Madrid, Plaza Ramon y Cajal, 28040 Madrid, Spain*

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Abstract—The production of aliphatic and aromatic aldehydes by oxidation of primary alcohols was achieved with *Gluconobacter oxydans* DSM 2343. The biotransformation was optimised studying the oxidation of 2-phenyl-1-ethanol to 2-phenylacetaldehyde. A high molar conversion (95% chromatographic conversion, 83% of isolated yield) was obtained using cells grown on glycerol as the main carbon source and directly used in the cultural medium after 24 h at 28°C, pH 4.5 and 5 g L^{-1} substrate concentration. The conversion of structurally different primary alcohols was performed under these conditions allowing the chemoselective production of aldehydes, sometimes with very good yields. © 2002 Published by Elsevier Science Ltd.

The enzymatic oxidation of primary alcohols for the production of aldehydes is attractive since it can be carried out under mild conditions, suited also for labile products. The use of isolated enzymes is often complicated by the requirements for cofactors and systems for their regeneration and whole microbial cells have been used.[1] The oxidation of alcohols by acetic acid bacteria is an old and established microbial method for obtaining production of vinegar and various carboxylic acids,[2] sometimes with remarkable chemo- and enantioselectivity,[3] but the accumulation of aldehydes is only seldom observed. It has been previously reported that a strain of *Gluconobacter oxydans* was able to accumulate isovaleraldehyde by oxidation of 2-methyl-1-butanol with good molar conversions, but its substrate specificity was limited to aliphatic substrates.[4] 2-Phenyl-1-ethanol could be also oxidised by *Gluconobacter* and *Acetobacter* allowing for maximum accumulation of 50% molar conversion into the corresponding aldehyde, before its further oxidation to phenylacetic acid.[5] Two-liquid phase systems proved suitable for the production of phenylacetaldehyde by oxidation of 2-phenylethanol using acetic acid bacteria.[6]

We have carried out further screening experiments observing that selective oxidation of 2-phenyl-1-ethanol to 2-phenylacetaldehyde can be obtained using *Gluconobacter oxydans* DSM 2343 and the potential of this strain is evaluated in this work. The microorganism was grown in submerged cultures in various media (glucose, glycerol, mannitol and sorbitol were employed as main carbon sources) and cells grown for 24 or 48 h were used for the oxidation of 2-phenyl-1-ethanol (2.5 g L^{-1}).[7] No significant differences in the oxidative activity were observed if the cells were harvested after 24 or 48 h. The oxidative activity towards 2-phenyl-1-ethanol was higher with cells grown on glycerol and yeast extract and gave high molar conversions into phenylacetaldehyde (80–85%). No detectable production of phenylacetic acid was observed even after 24 h. The optimum temperature and initial pH were 28°C and 4.5, respectively. These results offer a notable simplification of the whole procedure, since the temperature for obtaining optimal growth and biotransformation coincide and the pH of 24 h submerged cultures of *G. oxydans* DSM 2343 (pH 4.3) was already close to the optimum. Fig. 1 shows the profile of reactions carried out starting from different substrate concentrations.

The highest rate and product yield was observed at 5 g L^{-1} of substrate and no significant further oxidation could be observed even at prolonged times. Nevertheless, molar conversions above 30% were obtained with higher concentrations (7.5 and 10 g L^{-1}) after 5 h.

Keywords: Gluconobacter oxydans; biotransformation; aldehyde production; alcohol oxidation; flavour.

* Corresponding author. Tel.: 0039-0250316695; fax: 0039-0250316694; e-mail: francesco.molinari@unimi.it

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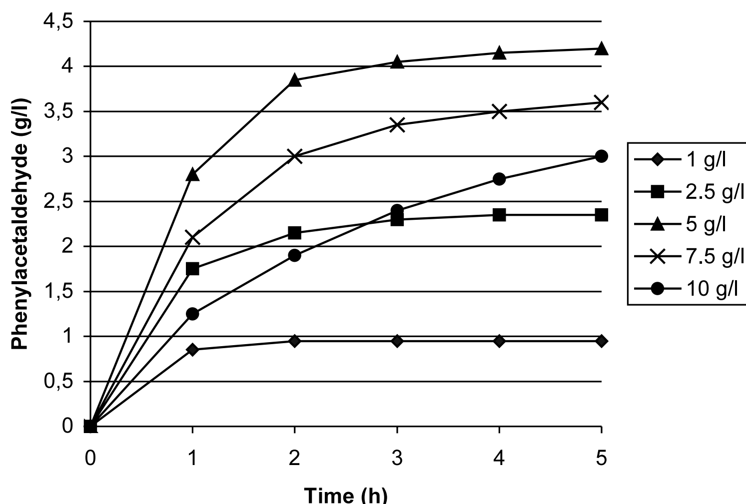


Figure 1. Phenylacetaldehyde production by *G. oxydans* DSM 2343 using different substrate concentrations.

G. oxydans DSM 2343 was utilised for testing the oxidation of different primary alcohols under the conditions optimised for the production of phenylacetaldehyde (Scheme 1).[8]

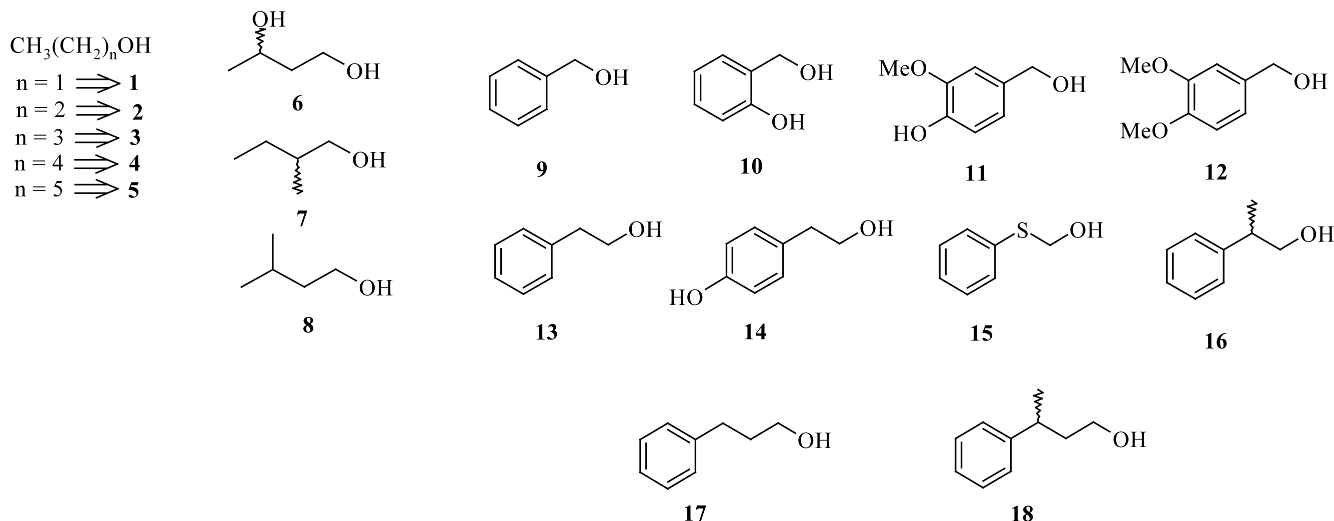
Substrates (5 g L^{-1}) were directly added to submerged 24 h cultures and biotransformations performed at 28°C. *Gluconobacter o.* DSM 2343 was able to produce several aldehydes, sometimes in very good yields (Table 1).

The production of carboxylic acids (not reported in the table) was observed at prolonged times only with aliphatic substrates. Maximum production of aromatic aldehydes was achieved in the first h of the reaction and they were not transformed even after 24 h.

The biotransformation of 1,3-butanediol proceeded with complete chemoselective oxidation of the primary alcohol producing 3-hydroxy butanal. The racemic substrates employed were generally transformed with low enantioselectivity (*E* in the range of 2–5).

The low conversions observed in the case of benzyl alcohols can be explained by the stability of the carbocation (generated by the cofactor-assisted removal of the hydride) which is stabilised by conjugation with the aromatic ring. The good activity observed with 2-hydroxy-benzyl alcohol can be due to the steric hindrance of the hydroxy group in *o*-position which disturbs the conjugation, giving a less stable and more reactive carbocation. Therefore, a higher yield is obtained in the case of **10** than with benzyl alcohol.

In conclusion, the use of *G. oxydans* DSM 2343 appears to be a very promising method for the production of aldehydes. The procedure is very simple as the alcohols are added directly to 24 h submerged cultures without



Scheme 1.

Table 1. Maximum production of aldehydes with *G. oxydans* DSM 2343

Entry	Substrate	Molar conversion (%)	Isolated yield (%)	Time (h)
1	Ethanol	65	51	5
2	Propanol	79	62	3
3	Butanol	84	71	2
4	1,3-Butanediol	40	35	4
5	Pentanol	60	50	3
6	Hexanol	84	72	3
7	2-Methyl-1-butanol	74	65	6
8	3-Methyl-1-butanol	45	38	6
9	Benzyl alcohol	15	12	24
10	2-Hydroxy-benzyl alcohol	51	43	5
11	4-Hydroxy-3-methoxy-benzyl alcohol	<5	–	8
12	3,4-Dimethoxy-benzyl alcohol	<5	–	24
13	2-Phenyl-1-ethanol	95	83	24
14	2-(4-Hydroxy)-phenylethanol	5	–	24
15	2-Phenyl-1-thioethanol	70	64	24
16	2-Phenyl-1-propanol	30	25	4
17	3-Phenyl-1-propanol	90	82	3
18	3-Phenyl-1-butanol	50	43	8

any modification of conditions (pH, temperature) and the aldehydes are often produced quickly under mild conditions.

Acknowledgements

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7. *G. oxydans* DSM 2343 (Deutsche Sammlung von Mikroorganismen) was 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 1 L Erlenmeyer flasks containing 150 mL of different media with various carbon sources and incubated at 28°C on a reciprocal shaker (100 spm).
8. Neat substrates were directly added to the submerged cultures or to cells centrifuged and resuspended in phosphate buffers (pH in the range 6.0–8.0) and flasks were shaken on a reciprocal shaker (100 spm). All compounds were characterised by a combination of ^1H NMR, GC/MS and molar conversions determined by GC or reversed phase HPLC analysis. The oxidation of 2-phenyl-1-ethanol was also carried out in 5 L reactor with 1 L working volume with cells grown directly inside the reactor vessel, agitation speed 250 rpm, air flow rate 1 v v^{-1} m^{-1}. The reaction was started with 5 g of the alcohol. The work-up of this biotransformation is reported as an example. After 5 h the reaction mixture was centrifuged (15 000 *g*, 10 min) to remove the bacterial cells and the supernatant was extracted with ethyl acetate. The organic extracts were dried over anhydrous Na_2SO_4 and the solvent removed. The crude product was purified by flash chromatography (hexane/ethyl acetate, 7/3) to give 4.05 g of phenylacetaldehyde.