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Enantioselective oxidation of prochiral 2-methyl-1,3-propandiol by *Acetobacter pasteurianus*

Francesco Molinari,[a,*] Raffaella Gandolfi,[a] Raffaella Villa,[a] Eva Urban[b] and Andreas Kiener[b]

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

[b]*Lonza A.G., CH-3930 Visp, Switzerland*

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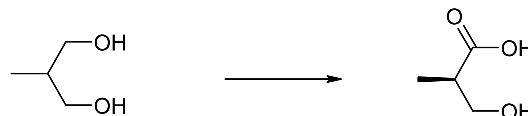
Abstract—The microbial oxidation of prochiral 2-methyl-1,3-propandiol into (*R*)-3-hydroxy-2-methyl propionic acid with *Acetobacter pasteurianus* DSM 8937 is reported. The biotransformation was optimised furnishing (*R*)-3-hydroxy-2-methyl propionic acid with 97% enantiomeric excess and 100% molar conversion of 5 g/L within 2 h. A simple fed-batch procedure allowed for the obtainment of 25 g/L of the enantiomerically enriched acid. (*R*)-3-Hydroxy-2-methyl propionic acid is an important building block for the synthesis of Captopril, a widely used antihypertensive drug. © 2003 Elsevier Ltd. All rights reserved.

1. Introduction

(*R*)-3-Hydroxy-2-methyl propionic acid is a valuable chiral intermediate[1] and it has been obtained through biotransformation by modification of isobutyric acid with a mutant strain of *Candida rugosa*.[2] 3-Hydroxy-2-methyl propionic acid can also be obtained by enantioselective dehydrogenation of prochiral 2-methyl-1,3-propandiol, a cheap and widely available substrate. The transformation of prochiral molecules into enantiomerically pure products is of great relevance in synthetic methods;[3,4] enzymatic reactions might discriminate between prostereogenic groups of prochiral substrates thus giving high yields of enantiopure products (*meso*-trick).[5] The enantioselective oxidation of 2-methyl-1,3-propandiol to give (*R*)-3-hydroxy-2-methyl propionic acid has been reported with *Gluconobacter* and *Acetobacter* species, but complete enantioselectivity has not been achieved.[6,7]

Acetic acid bacteria are efficient enantioselective biocatalysts and can perform the oxidation of structurally different primary and secondary alcohols or diols, provided that suite growth and biotransformation conditions are employed.[8–11]

In this work we have studied the conditions for obtaining high yields and enantioselectivity in the microbial oxidation of prochiral 2-methyl-1,3-propandiol into (*R*)-3-hydroxy-2-methyl propionic with *Acetobacter* and *Gluconobacter* strains.



2. Results

Several acetic acid bacteria (45 strains both *Acetobacter* and *Gluconobacter*) were tested for the oxidation of prochiral 2-methyl-1,3-propandiol; the biotransformation was carried out by directly adding the substrate (2.5 g/L) to liquid cultures of acetic acid bacteria, which were grown for 24 h in different media containing glucose or glycerol as the main carbon source. Most of the strains furnished 3-hydroxy-2-methyl propionic acid as the only product of biotransformation when grown with glycerol and yeast extract, while very low activity was observed with cells grown in the medium containing glucose.

Acetobacter pasteurianus DSM 8937 resulted in being the best candidate for further development: this strain completely converted the substrate into (*R*)-3-hydroxy-

* Corresponding author. Tel.: +39-0250316695; fax: +39-0250316694; e-mail: francesco.molinari@unimi.it

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2-methyl propionic acid within 3 h and no further modification of the product was observed at prolonged times (Scheme 1).

Table 1 reports the results obtained with cells of *Acetobacter pasteurianus* DSM 8937 grown on different media. The reaction was enantioselective (e.e. in the range of 90–97%) when grown with cultural media containing 1,3-dihydroxyacetone, glycerol, mannitol, 1,3-propandiol or sorbitol. The use of glycerol gave a high concentration of biomass and this was employed as main carbon source in further experiments.

Experiments with resting cells of *Acetobacter pasteurianus* DSM 8937 in different aqueous systems showed that the removal of the cells from the growth medium did not improve the performances; the whole cultural medium was, therefore, employed in further experiments aimed at the optimization of the bioconversion; the growth and the biotransformation were performed in the same reactor.

The initial optimization was carried out following the Multisimplex experimental design, which has previously been employed for enantioselective oxidation with acetic acid bacteria.[8] The response variables were the growth time of the microbial cells and the more crucial parameters of the biotransformation (pH, temperature and aeration rate). The best results were obtained with cells grown for 36 h and employed at pH 7.2, 32°C with an aeration rate of 1.5 vvm. The influence of the substrate concentration was evaluated under these conditions (Fig. 1). Similar reaction rates were observed in the range of 5–10 g/L and showed that the stereobias was not affected by the different reaction conditions. Experiments with a semi-continuous addition of the substrate (5 g/L after 2 h) were also performed. Parallel experiments were carried out with or without adjustment of the pH to a constant value of 7.0 (Fig. 2).

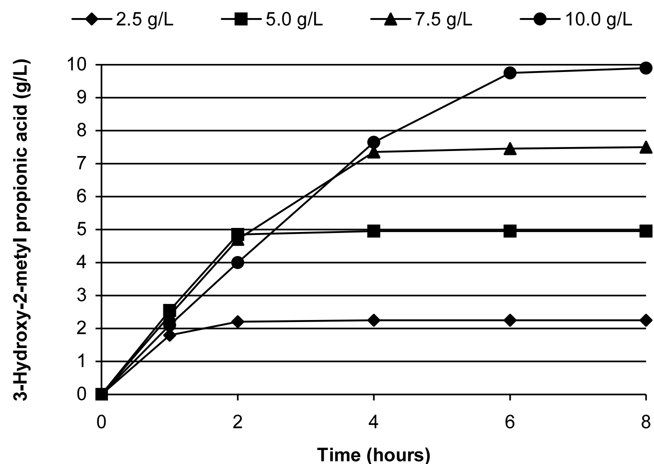
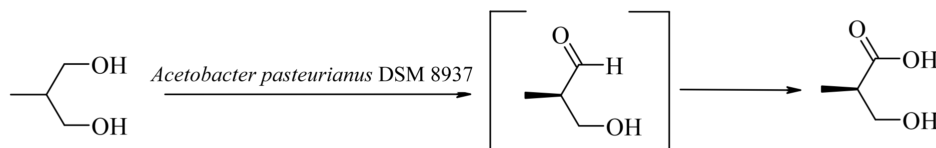


Figure 1. Biotransformation of 2-methyl-1,3-propandiol at different concentrations with *Acetobacter pasteurianus* DSM 8937.

Bioconversion carried out without pH adjustment showed a general decrease of microbial activity after 4–5 h when the acid concentration was above 12 g/L, while the control of the pH allowed for the accumulation of 25 g/L of the product (e.e. 96–97%) within 10 h.

3. Conclusions

The key intermediate of the synthesis of Captopril is (*R*)-3-hydroxy-2-methyl propionic acid that has been obtained by microbial oxidation of prochiral 2-methyl-1,3-propandiol. The bioconversion occurred with high molar conversion and enantioselectivity using *Acetobacter pasteurianus* DSM 8937, a wild type and generally regarded as a safe microorganism. A simple fed-batch procedure allowed for the obtainment of 25 g/L of the enantiomerically enriched (97% e.e.) acid.



Scheme 1.

Table 1. Growth and biotransformations with *Acetobacter pasteurianus* DSM 8937 grown for 24 h with different carbon sources and substrates (5 g/L) added directly to submerged cultures. Dry weights of the cells and pH of the culture after 24 h; molar conversion and enantiomeric excess of (*R*)-3-hydroxy-2-methyl propionic after 3 h

Carbon source	Dry weight (g/L)	pH	Molar conversion (%)	E.e. (%)
1,3-Dihydroxyacetone	3.7	6.0	75	90
Ethanol	2.4	3.5	<5	–
Glucose	4.0	3.7	5	–
Glycerol	4.4	6.1	>95	97
3-Glycerophosphate	1.2	7.0	10	80
Mannitol	2.0	6.5	80	94
1,3-Propandiol	0.9	4.0	90	92
Sorbitol	1.9	6.5	65	92

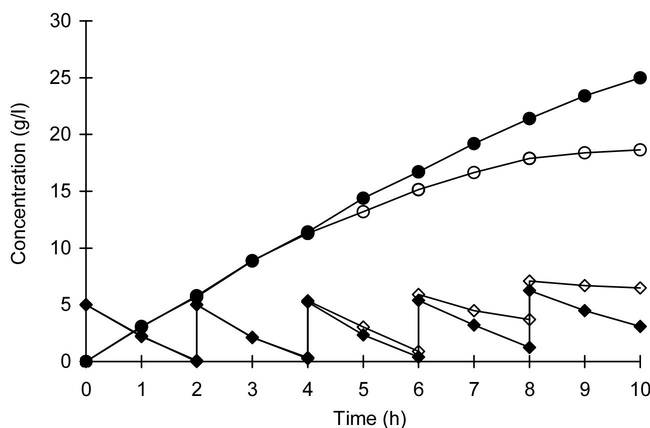


Figure 2. Batch-fed biotransformation of 2-methyl-1,3-propandiol to (*R*)-HIBA with *Acetobacter pasteurianus* DSM 8937. The reactions were carried out with the pH maintained at 7.0 (◆, diol; ●, acid) or without the adjustment of the pH (◇, diol; ○, acid).

4. Experimental

Microorganisms, growth and biotransformation conditions. Acetic acid bacteria were routinely maintained on GYC slants (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. The strains, grown on GYC slants for 24 h at 28°C, were inoculated into 500 ml Erlenmeyer flasks containing 50 ml of the liquid medium containing yeast extract (10 g L^{-1}) and different carbon sources (25 g L^{-1}) at pH 5 in distilled water and incubated on a reciprocal shaker (100 spm). *Acetobacter pasteurianus* DSM 8937 (Deutsche Sammlung von Mikroorganismen) was employed in optimization studies accomplished using cultures grown in a 1 L air-lift reactor.[10] Biotransformations were accomplished using bacteria grown directly inside the reaction vessel. Neat substrate was directly added onto suspensions. The control of the pH was performed by continuous addition of aqueous NaOH via a multichannel Watson-Marlow 503 U/R peristaltic pump connected to a pH controller (pH/ORP Controller 3675, Jenco Electronics). The dry weights were determined after centrifugation of 100 mL of cultures, cells were washed with distilled water and dried at 110°C for 24 h.

Optimization by sequential simplex method. The simplex optimization method was based on the sequential experimental trials guided by the systematic search strategies of the Multisimplex® 2.0 program (Multisimplex AB, Karlskrona, Sweden). The four starting experiments were selected with levels of each control variable (growth time, pH, temperature and aeration rate) within the following ranges: pH 4–8, temperature 20–35°C, substrate concentration 25–35 mM, speed of agitation 100–200 rpm.

The control responses to be optimized were the molar conversion into acid after 2 h and the enantiomeric excess of the product obtained. Each experiment was carried out in triplicate.

Analytical methods. The molar conversion was routinely determined by HPLC analysis using a Polyspher OA HY column (Merck, Darmstadt, Germany) and an aqueous acidic solution (H_2SO_4 0.005N) as eluent which allowed for the determination of the concentration of the substrates and products. Samples (0.5 mL) were taken at intervals, brought to pH 1 by the addition of 1 M HCl and extracted with an equal volume of ethyl acetate. The enantiomeric composition was routinely determined by gas chromatographic analysis of the corresponding methyl ester using a chiral capillary column (diameter 0.25 mm, length 25 m, thickness 0.25 μ, DMePeBeta-CDX-PS086, MEGA, Legnano, Italia). The absolute configuration of the obtained acid was determined after methylation by comparison with the specific rotation of the commercially available (Sigma-Aldrich) enantiomerically pure (*R*)-3-hydroxy-2-methyl propionic methyl ester.

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