ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 9 (2000) 209–217

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

www.elsevier.com/locate/molcatb

# Microbiological transformations 44. Optimisation of a new Baeyer–Villigerase activity: application to the stereospecific oxidation of 3-phenylcyclobutanone

Véronique Alphand, Roland Furstoss *

*Groupe Biocatalyse et Chimie Fine, ESA CNRS 6111, Université de la Méditerranée, Faculté des Sciences de Luminy, Case 901, 13288 Marseille Cedex 9, France*

Received 25 July 1999; received in revised form 28 September 1999; accepted 28 September 1999

**Abstract**

A screening was achieved out of 80 microbial strains in order to detect new ''Baeyer–Villigerase'' activities, using bicyclo[3.2.0]hept-2-en-6-one **1** as a test substrate. Such a new and interesting activity was discovered in the fungus *Cunninghamella echinulata*. Using this strain, oxidation of prochiral 3-phenyl-cyclobutanone **5** was examined. After an optimisation of the experimental conditions, the corresponding γ-butyrolactone **6** was obtained in 71% yield and 98% ee.


*Keywords:* Microbiological asymmetry; Baeyer–Villiger oxidation; Whole cells; γ-Butyrolactone

## 1. Introduction

The Baeyer–Villiger (BV) oxidation of linear or cyclic ketones, by which a ketone can be transformed into its corresponding ester or lactone, is one of the major oxidation reactions in organic chemistry. Although the stereochemical features of this type of reaction have been extensively studied over the years, and in spite of the nowadays utmost importance of chirotechnology in the manufacturing of various biologically active molecules, ways to carry out asymmetric BV oxidation using conventional chemistry have only been described very recently with some success [1]. However, these procedures, which essentially imply metal catalysed reactions, show a low versatility and often afford products of only moderate enantiomeric purity. On the other hand, the involvement of a BV oxidation step in the metabolic pathway of many microorganisms — mediated by a so-called Baeyer–Villigerase (BVase) enzyme — has been known for a long time. Others and ourselves have explored over the past 10 years the possibility to achieve the asymmetric BV oxidation of various substrates by using such potentially very promising biocatalysts. This proved to be a highly efficient way to obtain enantiopure lactones, starting from either racemic or prochiral cyclic ketones [2–4].

In this context, the bacterium *Acinetobacter calcoaceticus* NCIMB 9871 has been the first

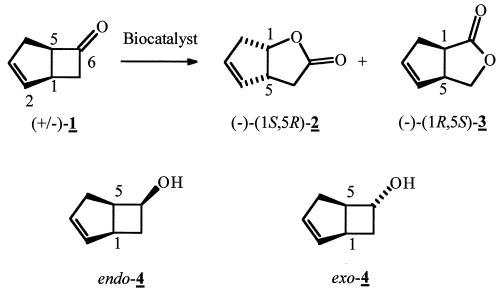
* Corresponding author. Fax: +33-4-91-82-91-45.
*E-mail address:* furstoss@luminy.univ-mrs.fr (R. Furstoss).

1381-1177/00/$ - see front matter 
PII: S1381-1177(99)00098-3

and most studied microorganism [4]. It was shown to contain a cyclohexanone monooxygenase (CHMO) which, when applied to racemic bicyclo[3.2.0]hept-2-en-6-one **1**, leads to the very peculiar reaction depicted in Scheme 1. The originality of this reaction is the fact that, to the contrary of a ''normal'' resolution scheme where only one product enantiomer would be formed, the two regioisomeric lactone **2** and lactone **3** were obtained, surprisingly both in nearly enantiomerically pure form [5]. This reaction is considered by now as being a kind of ''prototype reaction'' for enzymatic — as well as chemical — asymmetric BV oxidation [3,6].

Surprisingly enough, only few other biocatalysts have been described and studied up to now in this context in spite of the promising potentialities of such biooxidations. Thus, it is of high interest to develop a further search for novel BVases which could eventually be either substrate- and/or enantio-complementary as compared to the *A. calcoaceticus* one. Toward this aim, we have recently performed a screening out of 80 different microbial strains using racemic ketone **1** as a test substrate.

In this paper, we described the most interesting results of this screening, as well as the application (and optimised experimental conditions) of one of these new biocatalysts to the oxidation of the prochiral substrate 3-phenylcyclobutanone **5**. We were particularly interested in obtaining γ-butyrolactones in high optical purity, since these are important building blocks for further synthesis of various natural products or therapeutic agents [7–10].




Scheme 1.

## 2. Experimental

### 2.1. Substrates

Ketone **1** was purchased from Merck. Ketone **5** was synthesised in two steps from styrene by a [2 + 2] cycloaddition of in situ generated dichloroketene followed by reduction of the formed dichlorocyclobutanone into cyclobutanone **5**. The cycloaddition was carried out according to a slightly modified Hassner's procedure [11]: phosphoryl trichloride was purified by distillation over $K_2CO_3$ and glassware was flame-dried under nitrogen. To a very well stirred suspension of styrene (14 ml, 120 mmol), zinc dust (39 g, 600 mmol, 5 eq) and copper powder (12 g, 190 mmol, 1.6 eq) in 200 ml of dry diethyl ether, a solution of phosphoryl trichloride (17 ml, 150 mmol, 1.3 eq) and trichloroacetyl chloride (15 ml, 160 mmol, 1.5 eq) in 200 ml of dry diethyl ether was added dropwise over a period of 4 h. During addition, and over 15 h more, reflux must be maintained either spontaneously or by slight heating. After cooling, the reaction mixture was filtered over Celite, concentrated under reduced pressure, affording an orange oil which was washed with $6 \times 100$ ml pentane. The supernatant was decanted and washed with cold saturated aqueous solution of $NaHCO_3$ (until basic pH) then with brine before drying over $MgSO_4$. Concentration under reduced pressure afforded 18 g of crude 3-phenyl-2,2-dichlorocyclobutanone as a light yellow solid (70% yield) which was reduced with zinc in acetic acid to lead to pure **5** in 89% yield [11].

### 2.2. Microorganisms

*Cunninghamella echinulata* NRRL 3655 was obtained from the Northern Regional Research Laboratory (USA); *C. echinulata* LCP 66.1901, LCP 73.2203 and LCP 58.1543 were obtained from the Laboratoire de Cryptogamie de Paris (France) and *Syncephalastrum racemosum*

MUCL 28766 from the Mycothèque de l'Université Catholique de Louvain (Belgium). *C. verticillata* VKM F430 and *Scopulariopsis brevicaulis* VKM F406 were a generous gift from Profs. Terentyev and Parchikov (Moscow, Russia). These fungi were maintained on corn steep liquor (CSL)/glucose agar slopes at 26°C, stored at 4°C and subcultured at six monthly intervals.

### 2.3. Screening

In a reciprocating shaker (150 spm, 28°C), 500 ml shake-flasks filled with 100 ml of a CSL/glucose culture medium (20 g/l CSL (Roquette), 5 g/l glucose, 1 g/l $KH_2PO_4$, 2 g/l $K_2HPO_4$, 1 g/l $NaNO_3$, 0.5 g/l KCl, 0.5 g/l $MgSO_4$, 0.02 g/l $FeSO_4$) were inoculated with a piece of agar supporting mycelium. After 48–72 h growth, 0.5 ml of an ethanolic solution of **1** (100 g/l EtOH) were added directly into the culture medium. After 48–120 h biotransformation, the culture medium was continuously extracted with $CH_2Cl_2$ for 24 h, then analysed by GC.

### 2.4. Biotransformations of ketone **5** using *C. echinulata* NRRL 3655

#### 2.4.1. Spore suspension

The fungus was grown on bacto yeast malt extract agar medium in large flasks for 3–4 weeks. The spores were collected in a 0.5% Tween 80 solution by shaking with glass beads. Final concentration of this spore suspension was determined using a Malassez cell. Volumes corresponding to $5 \cdot 10^7$ spores were stored at −18°C before being used as inoculum.

#### 2.4.2. Cell culture

Spore suspension was used to inoculate a 5-l complex medium in a 7-l Setric fermentor under the following conditions: 27°C, 450 rpm, 30 l/h air. Unless otherwise mentioned, the complex medium was composed of: 50 g tryptone (Difco), 15 g malt extract (Difco), 15 g yeast extract (Difco), 25 g glucose, 12.5 g $K_2HPO_4$, 25g NaCl, 1 ml Pluronic PE 8100 (BASF), 0.25 ml Antifoam Silicon 426R (Prolabo) and adjusted to pH 5.5 using a 3 N HCl solution.

#### 2.4.3. Analytical whole-cell biotransformation

In a typical procedure, 50 ml of culture were sampled after a ca. 96–120 h growth (when pH reached 8.2–8.5), cells were harvested by filtration and washed with water before being suspended in 250 ml shaking flasks containing a 50 ml phosphate buffer (pH 8, 0.83 g/l $NaH_2PO4$, 13.45 g/l $Na_2HPO4$). This cell suspension was incubated with 26 mg of ketone **5** (3.5 mM) at 27°C on a reciprocating shaker at 150 spm for 24 h. The progress of the reaction was monitored by GC. The biotransformation was quenched by addition of a HCl solution until pH 2. The medium was then continuously extracted with $CH_2Cl_2$ for 24 h. Product composition was determined by GC.

#### 2.4.4. Preparative scale biotransformation

When the pH of the culture medium reached 8.3 (ca. 5 days), 1 litre culture was filtrated and cells were re-suspended in 1 litre pH 8 phosphate buffer. The biotransformation was performed in a 1 litre Setric fermentor (450 rpm, 16 l/h aeration) at 27°C. Ketone **5** (0.5 g, 3.4 mmol) was added and the progress of the reaction was monitored by GC. When the yield of alcohol **7** decreased to about 5%, the biotransformation was quenched by addition of a HCl solution until pH 2. The whole medium (aqueous solution and cells) was then continuously extracted with $CH_2Cl_2$ for 48 h. After concentration of the organic phase, γ-butyrolactone **6** was purified by flash chromatography and bulb-to-bulb distillation.

### 2.5. Identification of the metabolites

All metabolites (i.e., lactones **2**, **3** [5] and **6** [12,13] and alcohols **4** [14] and **7** [15]) were

Table 1
Most interesting results obtained from the screening using ketone **1** as a test-substrate

| Microorganisms | | Residual ketone **1** | Lactone | | Alcohol | |
|---|---|---|---|---|---|---|
| | | | Normal **2** | Abnormal **3** | Endo-**4** | Exo-**4** |
| *C. echinulata* NRRL 3655 | yield % (ee %) | 33 (73) | < 1 (4) | 21 (94) | 9 (82) | 7 (95) |
| *C. echinulata* LCP 66.1901 | yield % (ee %) | 21 (29) | 4 (15) | 15 (94) | – | 3 (90) |
| *C. echinulata* LCP 58.1543 | yield % (ee %) | 22 (28) | 3 (27) | 18 (95) | – | 4 (83) |
| *C. echinulata* LCP 73.2203 | yield % (ee %) | 23 (10) | 2 (3) | 7 (84) | – | 3 (84) |
| *C. verticillata* VKM F 430 | yield % (ee %) | 22 (5) | < 1 (48) | 4 (78) | – | 29 (37) |
| *S. brevicaulis* VKM F 406 | yield % (ee %) | 13 (77) | 2 (4) | 18 (93) | – | 5 (77) |
| *S. racemosum* MUCL 28766 | yield % (ee %) | 18 (25) | < 1 (27) | 13 (94) | – | 3 (78) |

identified by comparison of their retention times on two GC columns with those of authentic compounds synthesised by classical chemical reactions.

### 2.6. Quantitative GC analysis

#### 2.6.1. Screening

After continuous extraction of the biotransformation medium, 1 ml of the organic phase was added to 1 ml of ethylacetate containing 0.5 g/l tridecane as an internal standard. The solution was analysed by GC using a capillary OV 1701 or BP 10 column.

#### 2.6.2. Biotransformation of 5

Biotransformation of ketone **5** was monitored by periodic sampling of aliquots (1 ml). These aliquots were acidified at pH 2, heated at 50°C for 5 min then cooled. They were extracted by 1 ml of ethylacetate containing 0.5 g/l octadecane as an internal standard. The organic phase was analysed by GC using a capillary Optima 5 column.

### 2.7. Ee and absolute configuration determination

Ees were determined by GC analysis using chiral columns. The enantiomers of compounds **1**, **2**, **3** and **4** (respectively **6**) were separated on a 25-m capillary column 6-*O*-methyl-2,3-di-*O*-pentyl)-β-cyclodextrin (respectively, a 25 m capillary column (6-*O*-methyl-2,3-di-*O*-pentyl)-γ-cyclodextrin). A preparative scale biotransformation allowing isolation of the formed products was performed in order to measure their optical rotation. Their absolute configuration was assigned by comparison of the sign of their optical rotation with those previously reported (for compounds **1**, **2** and **3**, see Ref. [5], for compound **6**, see Ref. [12]) or, for the analytical scale experiments, by comparison of their GC retention time on chiral columns.

## 3. Results and discussion

### 3.1. Screening

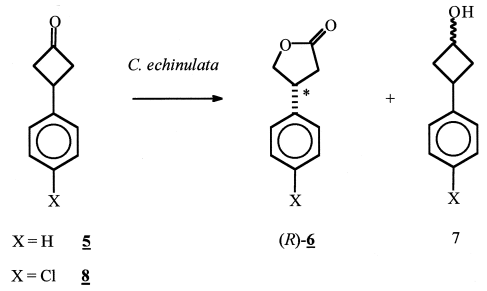
Using racemic **1** as a test-substrate, 80 microorganisms — including various bacteria or fungi — were screened for a new BV activity. The most original outcomes were obtained with seven fungal strains which were essentially from the *Cunninghamella* family (Table 1). All of them led to the highly predominant formation of the ''abnormal'' lactone (1*R*,5*S*)-**3** of good to excellent enantiomeric purity, whereas only traces of the ''normal'' lactone **2** were observed. Ketone (1*R*,5*S*)-**1**, [1] as well as the corresponding *endo*- and *exo*-alcohols **4**, were also observed as by-products, in amounts depending on the progress of the reaction.

[1] The stereochemistry of the residual ketone and ''abnormal'' lactone are opposite, although both are designated as being of (1*R*,5*S*) absolute configuration. This stems from the switch due to the Cahn Ingold Prelog priority rules.

Interestingly, these results differ from those described with some bacterial strains, where an enantiodivergent BV reaction led to both regioisomeric lactones. Two explanations are possible: (i) both lactones were formed but only the (1*R*,5*S*) ''abnormal'' lactone was not further overmetabolised; (ii) only the (1*R*,5*S*) ''abnormal'' lactone was formed, due to the enantio- and regioselectivity of the BVase. In any case, the highly preferential formation of the single regioisomer **3** is an attractive result for synthetic applications, since subsequent chromatographic separation can be avoided. Based on these results, gram-scale production of (1*R*,5*S*)-**3** was performed (using *C. echinulata* NRRL 3655) for achieving the synthesis of biologically active compounds [13,16].

### *3.2. Biotransformation of **5** with C. echinulata NRRL 3655*

We had previously described the synthesis of Baclofen®, an agonist of the γ-aminobutyric acid (GABA), following a strategy based on the biocatalysed oxidation of 3-(*p*-chlorophenyl)-cyclobutanone **8** by the same fungus [17], using experimental conditions identical to those used for biooxidation of **1** [13]. However, we had observed that the fungal growth was not always reproducible and that the yield of lactone was only of about 30%, a fact which obviously could be improved since a theoretical 100% yield should be accessible due to the prochiral nature of the substrate. Similar results were obtained for biooxidation of **5**. Lactone **6** and alcohol **7** were formed in about 20% and 4% yields, respectively. Therefore, we decided to try to optimise this biooxidation by modifying the experimental conditions (Scheme 2).




Scheme 2.

#### *3.2.1. Influence of the growth conditions*[2]

*3.2.1.1. Influence of the carbon source nature.* Experiments were conducted by replacing, in the culture medium, the initially used glucose with various other carbon sources (i.e., fructose, glycerol or cyclohexanediol). After 60 h cell growth, we observed a complete disappearance of the substrate and a concomitant formation of 35–45% lactone **6** and of 35–40% alcohol **7**.[3] The results were similar whatever the medium composition. Thus, it seems that the nature of the carbon source did not influence the outcome of the biotransformation, although in some cases the mycelium showed a different aspect (i.e., formed either smaller or bigger beads).

*3.2.1.2. Influence of the peptone origin.* Peptones of various origins (tryptic soy broth, tryptone, soytone, peptone (Difco), meat peptone (Organotechnie)) were tested in the screening conditions but using the medium described in Section 2.4.2 for an 84-h culture. Results were compared to those obtained using a CSL-based medium as well as a sabouraud medium. The results were similar whatever the origin of the peptone, affording about 55–70% lactone **6** and $< 10\%$ alcohol **7**. The highest lactone yield was obtained using either a tryptone- or a sabouraud-based medium. All further experiments were performed using the tryptone-based medium inoculated with a spore suspension.

[2] In this paragraph, all biotransformations were performed in a pH 7 phosphate buffer and were stopped after 24 h.

[3] The important ratio of alcohol **7** obtained can be explained by the fact that some carbon sources were still present in the culture medium after 60 h growth, an observation already made by Ouazzani-Chahdi et al. [18] with the fungus *Curvularia lunata*.

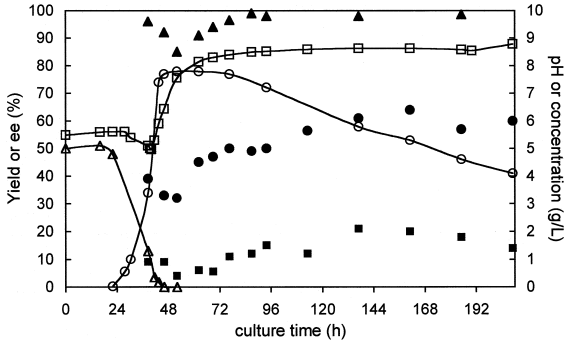



Fig. 1. Time-course of *C. echinulata* growth (points connected by lines) and products of ketone **5** biotransformation carried out using differently aged cells (points only). Growth parameters: pH (□), glucose concentration (△), dry weight (○); biotransformation of **5** (after 24 h reaction): lactone yield (●) and ee (▲), alcohol yield (■).

*3.2.1.3. Influence of the growth period.* A culture, using the tryptone-based medium, was carried out according to the procedure described in Section 2.4.2. Three parameters (pH, glucose concentration, dry weight[4]) were determined regularly. The fungus grew as small yellow beads of ca. 1 mm diameter. The exponential phase was characterised by a slight decrease of pH (down to 4.9) followed by an increase up to 6.5 which was simultaneous to the disappearance of glucose[5] (cf. Fig. 1).

Biotransformation of **5** was performed using whole cells harvested after different growth periods from a 100-ml culture aliquot. Results are shown on Fig. 1.

All over the growth time, formation of lactone and alcohol were concomitant, which unfortunately prevents complete discrimination between these two products by choosing an adequate growth period. However, the end of the stationary phase (ca. 90 h) seems much more favourable to lactone formation, leading to somewhat higher yields and ees (50–65% yield; ee ≥ 98%). Surprisingly enough, the ees were lower when cells harvested at the beginning of the stationary phase were used, an observation which can be tentatively explained by the transient presence of an other ''BVase'' endowed with a different stereoselectivity. On the other hand, we noted it was difficult to get a good reproducibility of the growth with respect to time. Therefore, all further experiments were conducted by using cells prepared from cultures where the pH reached a value of about 8.2–8.5 (this corresponds to a culture time of about 96–120 h).

### *3.2.2. Influence of the biotransformation conditions*

*3.2.2.1. Influence of the experimental process: growing cells vs. resting cells.* Experiments were conducted (i) by adding directly the substrate to the culture medium at pH 8; (ii) by using the ''resting cells'' suspended in a phosphate buffer (pH 8). No noticeable difference was observed as far as the yield and proportions of the alcohol and remaining ketone are concerned. However, the ee of the lactone, determined after 24 h biotransformation, was much better when using resting cells (98% ee) instead of growing cells (75% ee). Therefore, this last technique was used for all further experiments.

*3.2.2.2. Influence of the medium pH.* Grown cells (120 h) were suspended in a buffer solution at different pHs (i.e., using 0.2 M phosphate buffers at pH 6, 7, 8 or 0.1 M carbonate buffers at pH 8, 9 or 10). The time-course dependence of the different products proportions were determined. The results are presented on Fig. 2.

At low pH values (pH 6 and 7), ketone consumption was slow and alcohol formation was favoured (faster formation and slower disappearance) whereas lactone formation was particularly slow. At higher pHs, the yield of lac-

[4] During the culture, mycelium grew as a compact mass on the walls of the fermentor and around the electrodes. Dry weight was determined using only the cells kept in suspension.

[5] A similar behaviour has been previously described for a *Cunninghamella* strain. The increase of pH was due to formation of $NH_3$ after total glucose consumption [19].

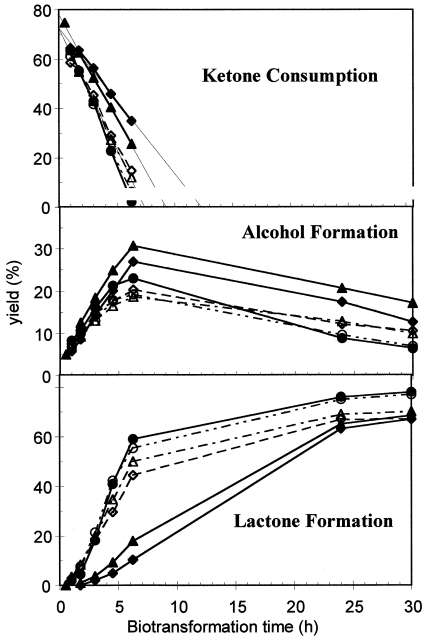



Fig. 2. Effect of pH on ketone **5** disappearance, alcohol **7** and lactone **6** formation. Carbonate buffer: pH 10 (○), pH 9 (△), pH 8 (◇); phosphate buffer: pH 8 (●), pH 7 (▲), pH 6 (♦).

tone increased to about 65–70%, and was best at pH 8 (ca. 80% yield whatever the nature — carbonate or phosphate — of the buffer). Similarly, the ee of the lactone slightly increased from 96 to 98 going from pH 6 to 8 (phosphate buffer) which therefore appears to be the optimal condition for lactone production. As far as ketone **5** and alcohol **7** proportions are concerned, we only observed slight differences between experiments conducted at pH 8, 9 or 10.

*3.2.2.3. Influence of cells and substrate concentrations*. Experiments were carried out using 5-day-old cells, suspended in 50 ml pH 8 phosphate buffer. Cell concentrations of respectively 4.5,[6] 5.2, 9 and 18 g/l (dry weight) and substrate concentrations increasing from 0.25 to 0.5, 1 and 2 g/l were used.

[6] Which corresponds to the cell concentration after 120 h growth.

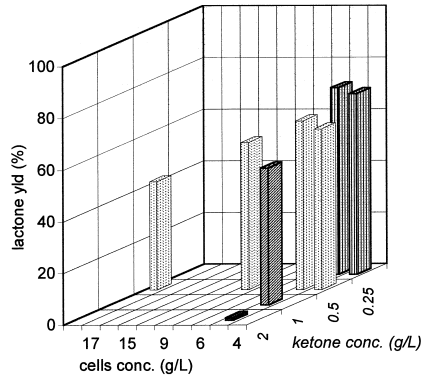



Fig. 3. Maxima lactone yields obtained for biotransformations carried out using different cells and ketone concentration.

The results indicate that (i) low concentrations of cells and ketone led to the best results for lactone formation (Fig. 3). For example, an experiment conducted at 0.5 g/l ketone and 9 g/l cells afforded a lactone yield about 15–20% lower than a similar experiment achieved using 0.25 g/l of ketone and 5 g/l of cells although the ketone/cell concentration ratio was similar; (ii) at a 2 g/l ketone concentration, the reaction was highly inhibited; (iii) decreasing the cell concentration had a beneficial effect on the lactone ee (i.e., 94% at 18 g/l, 97% at 9 g/l and 99% at 4.5 g/l); (iv) at a 5 g/l cell concentration, the ee was high (> 98%) and stayed unchanged whatever the substrate concentration; (v) the highest proportion of alcohol formation was obtained after 5 h, using 1 g/l ketone and 5.2 g/l cell concentrations (Fig. 4).

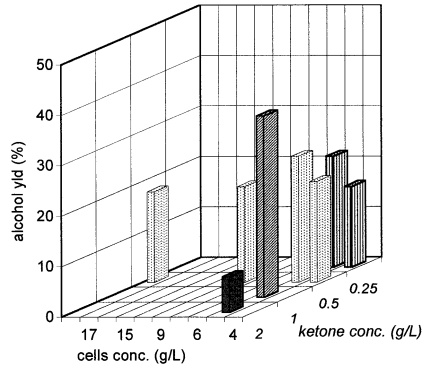



Fig. 4. Maxima alcohol yields obtained for biotransformations carried out using different cells and ketone concentration.

These results led us to consider that concentrations of 0.5 g/l of ketone **5** (3.4 mM) for ca. 5 g/l of cells (dry weight) as the best compromise for routine biotransformation.

*3.2.2.4. Influence of temperature*. Experiments were performed at 21°C and 28°C. The reaction at 21°C was slightly slower with a gap of ca. 5 h but no important variation of the yield or the obtained ees was observed.

*3.2.2.5. Addition of a co-solvent*. In order to improve the solubility of the substrate into the reaction medium, the possibility to use water miscible co-solvents — i.e., ethanol, acetone or DMF — was explored. Thus, 0.25 mg of ketone was dissolved in 0.5 ml of these co-solvents before addition to 50 ml of resting cell suspension. No noticeable variations were observed in terms of yield and ee by comparison with a biotransformation performed without co-solvent addition.

*3.2.3. Optimised preparative scale biotransformation*

Owing to the various results obtained, we carried out the BV oxidation of ketone **5** on a preparative scale. Thus, 500 mg of **5** were transformed by a resting cell process using cells previously grown in a 1 litre tryptone/glucose-based medium and re-suspended in 1 litre of pH 8 phosphate buffer at 28°C. This led, after normal work-up, to 390 mg of (−)-(*R*)-lactone **6** in 71% yield (ee > 98%, $[\alpha]_D^{20} = -50$ (ca. 0.9 $CHCl_3$)).

## 4. Conclusion

We have explored the possibility to find a new ''BVase'' activity by performing a screening on 80 different microbial strains, using **1** as a test substrate. This led us to select the *C. echinulata* NRRL 3655 strain which proved to be endowed with such an interesting, previously unknown, enzymatic activity. Further studies, aimed to optimise the experimental conditions, were achieved using **5**. By choosing the proper parameters, we thus succeeded in improving the yield of this biotransformation from about 20 to 71%. Moreover, the obtained lactone was nearly enantiopure, showing that the enzymatic oxidation of this prochiral substrate can be highly stereospecific, to the contrary of what has been observed using metal catalysts [20,21]. These results illustrate the fact that enzymatic BV oxidation is nowadays undoubtedly still the best way to achieve such reactions in an asymmetric way.

## Acknowledgements

We would like to thank Prof. Terentyev and Dr. Parchikov (University of Moscow, Russia) for their generous gift of both *C. verticilata* and *S. brevicaulis* strains. Part of this work was achieved in the context of the Framework IV EC Biotechnology Contract (PL970267) entitled ''Biotransformations using Baeyer–Villiger monooxygenases''.

## References

[1] G. Strukul, Angew. Chem. Int. Ed. 37 (1998) 1199.
[2] V. Alphand, R. Furstoss, in: K. Drauz, H. Waldmann (Eds.), Enzyme Catalysis in Organic Synthesis, VCH, Weinheim, 1995, pp. 745–772.
[3] A. Willets, Trends in Biotechnology 15 (1997) 55.
[4] J.D. Stewart, Current Organic Chemistry 2 (1998) 195.
[5] V. Alphand, R. Furstoss, J. Org. Chem. 57 (1998) 1306.
[6] C. Bolm, Schilingloff, J. Chem. Soc. Chem. Commun. (1995) 1247.
[7] S.S. Canan Koch, A.R. Chamberlin, J. Org. Chem. 58 (1993) 2725, and references herein.
[8] R. Gagnon, G. Grogan, E. Groussain, S. Pedragosa-Moreau, P.F. Richardson, S.M. Roberts, A.J. Willetts, V. Alphand, J. Lebreton, R. Furstoss, J. Chem. Soc., Perkin Trans. 1 (1995) 2527.
[9] V. Alphand, C. Mazzini, J. Lebreton, R. Furstoss, J. Mol. Catal. B: Enzym. 5 (1999) 219.
[10] C. Mazzini, J. Lebreton, V. Alphand, R. Furstoss, J. Org. Chem. 62 (1997) 3382.
[11] A. Hassner, J.L. Dillon, J. Org. Chem. 48 (1983) 3382.
[12] G.J. Dawson, J.M. Williams, S.J. Coote, Tetrahedron: Assymmetry 6 (1995) 2535.

[13] J. Lebreton, V. Alphand, R. Furstoss, Tetrahedron 53 (1997) 145.
[14] I.C. Cotterill, E.I.A. Macfarlane, S.M. Roberts, J. Chem. Soc., Perkin Trans. 1 (1988) 3387.
[15] H.L. Holland, M. Kindermann, S. Kumaresan, T. Stefanac, Tetrahedron: Asymmetry 4 (1993) 1353.
[16] L. Andrau, J. Lebreton, P. Viazzo, V. Alphand, R. Furstoss, Tetrahedron Lett. 38 (1997) 825.
[17] C. Mazzini, J. Lebreton, V. Alphand, R. Furstoss, Tetrahedron Lett. 38 (1997) 1195.
[18] J. Ouazzani-Chahdi, D. Buisson, R. Azerad, Tetrahedron Lett. 28 (1987) 1109.
[19] B.C. Foster, D.L. Litster, J. Zamecnik, R.T. Coutts, Can. J. Microbiol. 37 (1991) 791.
[20] C. Bolm, T.K. Khank Loung, G. Schlingloff, Synlett (1997) 1151.
[21] T. Kanger, K. Kriis, A. Paju, T. Pehk, M. Loop, Tetrahedron: Asymmetry 9 (1998) 4475.