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Enantioselective benzylic microbial hydroxylation of indan and tetralin

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Abstract

A screening with 15 strains of bacteria and fungi targeted at the production of specific hydroxylated benzylic derivatives of indan **1** and tetralin **2** was carried out. *Mortierella isabellina, Mortierella ramanniana* and *Beauveria bassiana* were shown to mediate the respective conversions to 1-indanol (**3**) and 1-tetralol (**4**), the most satisfactory results being obtained with *M. isabellina*, which gave 78% conversion of **1** to (1*R*)-**3** (64% yield, 86% ee) after a 2-day-incubation, and 52% conversion of **2** to (1*R*)-**4** (38% yield, 92% ee) in a 4-day-incubation. Over-oxidation of alcohols **3** and **4** during the reactions resulted on the formation of 1-indanone and 2-tetralone, respectively.

Keywords: *Mortierella*; *Beauveria*; 1-Indanol; 1-Tetralol

1. Introduction

Biocatalysis has been found to be an important tool in the organic chemist's arsenal to solve synthetic problems. The selectivity (regio-, chemo-, diastereo- and enantioselectivities) and mildness with which biotransformations can be carried out make this approach superior to many chemical-based methods [1]. Such reactions provide efficient, specific and environmentally benign conditions [2] and are of great synthetic and commercial relevance for the pharmaceutical industries, where the production of optically pure molecules free from synthesis contaminants, such as toxic solvents and heavy metals, is of particular importance. Biocatalysis can uniquely perform certain transformations, such as selective hydroxylation of hydrocarbons, reactions which are difficult by chemical methods [3], especially because their specificity and, due to certain unsolved problems, including a certain lack of control and predictability of product structures, as well as the expense of oxidizing reagents [4].

Microbial hydroxylation can be considered a valuable alternative [5,6]. Over the past few decades, a large number of biohydroxylations have been reported, revealing a wide range of biocatalysts and substrates, including cycloalkane carboxylic acids, ketones, amines, amides and alcohols [1]. However, there are almost no methods available that allow an efficient oxidation of hydrocarbons on a preparative scale [3,7]. Problems with substrate acceptance, undesired side reactions, selectivity and the prediction of hydroxylation position all hamper the general synthetic utility of this approach [1].

The possibility of biotransforming indan **1** and tetralin **2** to their respective benzylic alcohols 1-indanol **3** and 1-tetralol **4** has been reported using *Aspergillus niger* (with unspecified yields and stereochemistries) [8], *Cunninghamella echinulata* (3% yield and 33% ee for (1*R*)-**3** and 23% yield and 60% ee for (1*R*)-**4**) [9], *Helminthosporium* sp. (no conversion to (1*R*)-**3** but 3% yield and 75% ee for (1*R*)-**4** [9]), *Mortierella isabellina* (12% yield and 33% ee for (1*R*)-**3** and 17% yield and 33% ee for (1*R*)-**4**) [9,10], *Fusarium moniliforme* (with unspecified yields and 9% ee for (1*R*)-**3** and 4% ee for (1*R*)-**4**) [11], *Pseudomonas stutzeri* (with unspecified yields and stereochemistries)

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[12], mutant *Pseudomonas putida* (with unspecified yield and up to 92% ee for (1*S*)-**3**) [13] and *Escherichia coli* containing cloned genes of *P. putida* F39/D (with unspecified yields and 83% ee for (1*R*)-**3**) [14]. Also, there are reports on the use of hepatic cytochrome P-450 from microsomal incubations (with unspecified yield and 24–52% ee for (1*S*)-**3**) [15]; and purified P-450cam from *P. putida* (with unspecified yields and 87% ee for (1*R*)-**3** and 95% ee for (1*R*)-**4**) [16]. These methods generally comprise non-selective processes, mutant cells, low yields or a number of by-products. Since systems able to hydroxylate the benzylic positions of **1** and **2** with acceptable enantioselective yields provide derivatives that are relevant either in contaminant biodegradation or as synthons for production of drugs, fragrances and insecticides [17–22], we describe herein a screening with 15 different strains of bacteria and fungi.

2. Experimental

2.1. Substrates and chemicals

The substrates **1** (95%) and **2** (99%) were purchased from Acros (Morris Plains, NJ) and Sigma–Aldrich (St. Louis, MO), respectively. Racemic standards of **3** and **4**, required for chiral GC analysis, were prepared by treatment of the corresponding ketones with $NaBH_4$ in MeOH, as described by Aina et al. [21]. Enantiomeric enriched samples of **3** and **4**, used to determine the enantiomer specificity by GC, were prepared by ruthenium-catalyzed reduction of racemic tricarbonyl(η^6-1-indanone or 1-tetralone)chromium complexes, as described by Ursini et al. [23]. The optical rotation values were measured with a Perkin-Elmer 341 Polarimeter and compared with previously reported data for **3** [13,24,25] and **4** [26,27].

2.2. Microorganisms and culture media

Strains of *Arthrobacter globiformis* CCT0193T, *Arthrobacter oxydans* CCT3027T, *Arthrobacter* sp. CCT1875, *Beauveria bassiana* CCT3161 (from ATCC 7159), *Geotrichum candidum* CCT1205, *Mortierella isabellina* CCT3498, *Mortierella ramanniana* CCT4428, *Pichia kluyveri* CCT3365, *Pichia stiptis* CCT2617, *Pichia canadensis* CCT2636, *Pseudomonas oleovorans* CCT1969, *P. putida* CCT2357, *Rhodotorula glutinis* CCT2182, *Rhodotorula minuta* CCT1751 and *Trichosporon cutaneum* CCT1903 were selected on the basis of the related hydroxylation potential and laboratory availability, and tested for benzylic hydroxylations of **1** and **2**. The microorganisms were obtained from and are stored at the Tropical Culture Collection of the André Tosello Research Foundation [28] and cultivated in mixtures of ingredients for cultivation media or in pre-formulated cultivation media purchased from Vetec, Biobrás or Accumedia (Belo Horizonte, Brazil), for biomass acquisition. Alternatively, potatoes, carrots and corn were purchased in a local market and employed to prepare different broths, developed in order to support high biomass concentrations, to improve the enzymatic activity and to be economically more advantageous. Bacteria were grown in nutrient broth (NB): 8 g Biobrás nutrient L^{-1}; tryptic soy broth (TSB): 30 g Accumedia tryptic soy L^{-1}; potato/sucrose broth (PSB): 100 g potato and 30 g commercial sugar L^{-1}; peptone/malt broth (PMB): 3 g Biobrás malt extract, 10 g Biobrás peptone and 20 g de D-(+)-glucose L^{-1}. Filamentous fungi were grown in potato/dextrose broth (PDB): 100 g potatoes and 30 g D-(+)-glucose L^{-1}; potato/sucrose broth (PSB); potato/sucrose/corn broth (PSBM): 250 g potatoes, 30 g commercial sugar and 100 g corn kernels L^{-1}; potato/dextrose/carrot (PCDB) broth: 100 g potatoes, 30 g D-(+)-glucose and 10 g carrot L^{-1}; malt extract broth (MEB): 20 g Biobrás malt extract L^{-1}; malt extract broth (MHB): 5 g Vetec yeast extract, 5 g Accumedia tryptic soy, 40 g de D-(+)-glucose, 5 g NaCl, 5 g Na_2HPO_4 L^{-1}; peptone/malt broth (PMB): 3 g Biobrás malt extract, 10 g Biobrás peptone and 20 g de D-(+)-glucose L^{-1}; yeast/soy broth (YSB): 3 g Vetec yeast extract, 3 g Accumedia tryptic soy, 5 g sodium chloride, 5 g dibasic sodium phosphate and 40 g de D-(+)-glucose L^{-1}; yeast/malt/peptone broth (YMPB): 3 g Vetec yeast extract, 3 g Biobrás malt extract, 5 g Biobrás peptone and 10 g de D-glucose L^{-1}.

2.3. Feeding and time-course experiments

Screening and time-course experiments were performed in conical Erlenmeyers flasks (125 mL) containing 50 mL of medium. Before each experiment, cultures of microorganisms were aseptically transferred to conical Erlenmeyer flasks (500 mL) containing 200 mL of sterile culture media and kept on a rotary shaker (150 rpm) at 25, 28 or 30 °C for 2 (bacteria) or 3 days (fungi) to acquire biomass. After the incubation time, 1 mL of an ethanolic solution (30 mg mL^{-1}) of substrate, without prior sterilization, was added to the growth media. Alternatively, microbial biomass was harvested by centrifugation (5000 rpm, 10 min) and 3 g (wet wt) was transferred to conical flasks containing 50 mL of potassium (0.2 ionic strength) phosphate buffer solutions at pH 5.0 (25.84 g KH_2PO_4 and 0.429 g Na_2HPO_4 L^{-1}), pH 6.0 (18.4 g KH_2PO_4 and 4.025 g Na_2HPO_4 L^{-1}), pH 7.0 (4.71 g KH_2PO_4 and 7.85 g Na_2HPO_4 L^{-1}) and pH 8.0 (0.61 g KH_2PO_4 and 9.3 g Na_2HPO_4 L^{-1}) to which 1 mL of an ethanolic solution of the substrate (30 mg mL^{-1}) was added. For both procedures, the suspensions were then returned to the shaker for 5 days. At the desired time intervals, 5 mL portions of the incubation mixture were harvested from triplicate flasks. After vigorous shaking, the samples were extracted with 5 mL of EtOAc. If necessary, a centrifugation procedure (3 min at 3000 rpm) was used to break the emulsion. The organic fraction was collected and 1 μL of the solution was submitted to GC-MS and GC-FID, for qualitative and chiral analysis, respectively. Experiments were repeated at least twice.

Control experiments without microorganisms were carried out to verify the stability of the substrates, and without substrate, to verify the microbial catabolism. These controls were prepared by the addition of 1 mL of an ethanolic solution of substrate to 50 mL of medium, and, in other flasks, 3 g of microorganism (wet wt) and 50 mL medium. No oxidation products could be observed in control flasks under the assay conditions.

2.4. Analytical procedures

Gas chromatography–mass spectrometry (GC-MS) analyses were carried out using a HP-6890/5973 or a Shimadzu QP-5000 system equipped with J&W Scientific HP-5 (30 m × 0.25 mm × 0.25 μm) or Supelco Simplicity 1 (30 m × 0.25 mm × 0.25 μm) fused silica capillary columns. Injector and detector temperatures were set at 220 and 240 °C, respectively; the oven temperature was programmed from 60 to 230 °C at 40 °C/min. Helium was employed as carrier gas (1 mL min^{-1}). Compound identification was based on a comparison of mass spectra with those of synthetic racemic and enantiomerically enriched samples and literature data [13,16,21,24,26,27,29] and confirmed by infrared and nuclear magnetic resonance spectroscopy [23,30]. The retention times obtained for **1**, **3** and **5** were 4.7, 5.94 and 6.2 min, respectively; while the retention times obtained for **2**, **4** and **6** were 5.58, 6.52 and 6.63 min, respectively. The percent composition of unreacted substrate and products were obtained from electronic integration measurements, without taking into account relative response factors. Chiral Gas Chromatographic analyses were carried out using a HP-6890/5973 GC system, equipped with flame ionization detector and Macherey 212117/91 Hydrodex-β 3P (25 m × 0.25 mm × 0.25 μm) fused silica capillary column. The oven temperature was programmed from 100 to 210 °C at 10 °C/min. Injector and detector temperatures were set at 200 and 240 °C, respectively. Hydrogen was employed as carrier gas (1 mL min^{-1}). Under these conditions, the retention times obtained for (1*S*)-**3** and (1*R*)-**3** were 8.07 and 8.11 min, respectively; while the retention times obtained for (1*S*)-**4** and (1*R*)-**4** were 9.62 and 9.69 min, respectively.

2.5. Preparative biotransformations of *1–3* and *2–4* by M. isabellina

Seventy-two hour-old stage cultures of *M. isabellina* (30 g wet wt), cultivated in PCDB media, were transferred to 500 mL Erlenmeyer flasks, each containing 200 mL of potassium phosphate buffer at pH 6.0 (18.4 g KH_2PO_4 and 4.025 g Na_2HPO_4 L^{-1}) for substrate **1** at pH 7.0 (4.71 g KH_2PO_4 and 7.85 g Na_2HPO_4 L^{-1}) for substrate **2**. A total of 100 mg of **1** or **2** (2 ml of an ethanolic solution at 50 mg mL^{-1}) was placed in the flasks, without prior sterilization. The flasks were incubated at 30 °C in an orbital shaker (150 rpm). After 48 h for substrate **1** or 96 h for **2**, the cultures were harvested and filtered. The filtrates were saturated with saturated aqueous sodium chloride solution and extracted six times with EtOAc. The organic phases were separated and the sodium chloride-saturated aqueous solutions remaining were extracted again by a continuous liquid–liquid process at ca. 50 °C for 24 h. The combined organic extracts were then evaporated to dryness under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (400–200 mesh—Aldrich) using a glass column (2.5 cm × 25 cm) with 50 g of silica gel, eluted with 300 mL portions of hexane:EtOAc (90:10 and 80:20), collecting 10 mL fractions to give **3** and **5** (64 mg, 90:10 ratio) or **4** and **6** (38 mg, 80:20 ratio). The isolated 1-indanone **5** and 1-tetralone **6** were identified by physical data comparison with authentic samples purchased from Sigma–Aldrich.

(*R*)-1-Indanol [(*R*)-**3**] was isolated as a white solid in 64% yield. mp 67–68.0 °C; $[\alpha]_D^{23} = -25°$ (*c* 0.41, $CHCl_3$), 88% ee. Lit. [14,25]: mp 72 °C, $[\alpha]_D^{22} = +34°$ (*c* 1.895, $CHCl_3$) for *S* enantiomer. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.46–7.42 (m, 1H, Ph), 7.24–7.20 (m, 2H, Ph), 7.14–7.10 (m, 1H, Ph), 5.27 (br t, *J* = 5.9 Hz, 1H, C*H*OH), 3.07 [ddd, *J* = 16.2 Hz, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H, C*H*HCH$_2$CH(OH)], 2.84 (br dd, *J* = 16.2 Hz, *J* = 7.0 Hz, 1H, CH*H*CH$_2$C(OH)H), 2.51 (m, 1H, C*H*HC(OH)H), 1.97 (m, 1H, CH*H*C(OH)H), 1.75 (br s, 1H, O*H*). MS (EI): *m*/*z* (rel intensity) 134 (M^+, 51), 133 (100), 117 (12), 116 (14), 115 (28), 105 (30), 103 (12), 91 (32), 89 (9), 79 (25), 78 (15), 77 (45), 74 (2), 66 (16), 65 (22), 63 (25), 57 (25), 55 (32), 53 (9), 52 (13), 51 (57), 50 (29).

(*R*)-1-Tetralol [(*R*)-**4**] was isolated as a colorless oil in 38% yield. $[\alpha]_D^{22} = -34.0°$ (*c* 2.12, $CHCl_3$), 92% ee. Lit. [26,27]: $[\alpha]_D = +34.4°$ (*c* 1.01, $CHCl_3$) for *S* enantiomer. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.46–7.42 (m, 1H, Ph), 7.24–7.20 (m, 2H, Ph), 7.14–7.10 (m, 1H, Ph), 4.79 (apparent t, *J* = 4.4 Hz, 1H, C*H*OH), 2.85–2.65 (m, 2H, CH_2), 2.05–1.75 (m, 5H, CH_2, CH_2, O*H*). MS (EI): *m*/*z* (rel intensity) 148 (M^+, 18), 147 (25), 131 (18), 129 (43), 128 (20), 127 (13), 121 (8), 120 (80), 119 (67), 115 (28), 105 (47), 104 (15), 92 (20), 91 (100), 90 (15), 89 (15), 79 (10), 78 (30), 77 (34), 66 (10), 65 (47), 64 (30), 63 (41), 62 (10), 60 (10), 57 (11), 55 (10), 53 (14), 52 (16), 51 (69), 50 (28), 43 (23), 41 (31), 40 (12).

2.6. Preparative biotransformations of *1–3* or *2–4* by M. ramanniana

Seventy-two hour-old stage cultures of *M. ramanniana* (30 g wet wt), cultivated in MEB media, were transferred to 500 mL Erlenmeyer flasks containing 200 mL of potassium phosphate buffer at pH 6.0 (18.4 g KH_2PO_4 and 4.025 g Na_2HPO_4 L^{-1}). A total of 100 mg of **1** or **2** (2 mL of an ethanolic solution at 50 mg mL^{-1}) was placed in the flasks, without prior sterilization. The flasks were incubated at 30 °C on an orbital shaker (150 rpm). After 48 h for flasks containing substrate **1** and 72 h for **2**, the cultures were harvested, filtered, extracted, isolated and analyzed as described above (Section 2.5), to give alcohols **3** (55 mg) or **4** (42 mg).

2.7. Preparative biotransformations of *1–3* or *2–4* by B. bassiana

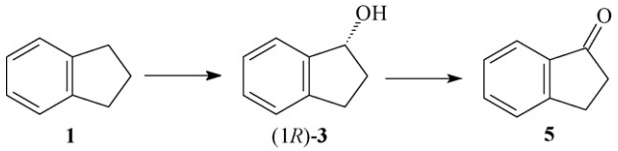
Seventy-two hour-old stage cultures of *B. basiana* (30 g wet wt), cultivated in MEB media, were transferred to 500 mL Erlenmeyer flasks containing 200 mL of potassium phosphate buffer at pH 7.0 (4.71 g KH_2PO_4 and 7.85 g Na_2HPO_4 L^{-1}). A total of 100 mg of **1** or **2** (2 ml of an ethanolic solution at 50 mg mL^{-1}) was placed in the flasks, without prior sterilization. The flasks were incubated at 28 °C on an orbital shaker (150 rpm). After 24 h, the cultures were harvested, filtered, extracted, isolated and analyzed as described above (Section 2.5), to give alcohols **3** (22 mg) or **4** (29 mg).

3. Results and discussion

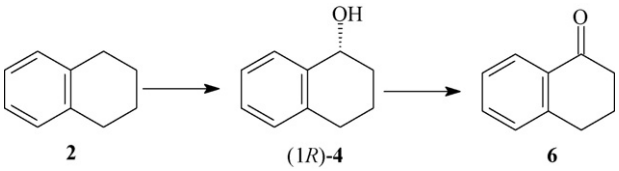
Screening experiments with 15 strains of bacteria and fungi were carried out to achieve hydroxylation of the hydrocarbons indan **1** and tetralin **2**. For optimization of the parameters necessary for obtaining maximum fungi biomass in the shaker flasks, coupled to improved conversion yields and selectivity, the microorganisms were grown in different cultivation media at 25–30 °C, followed by adding the substrates to the growth medium or buffer solution (pH 5.0–8.0).

Although some benzylic hydroxylations of **1** and **2** by *Pseudomonas* have been reported [12–14], the strains evaluated in our work did not give any hydroxylation product, probably because of the low growth microorganism rates, which was accompanied by a putrid-like odor. Satisfactory results were obtained with *M. isabellina*, *M. ramanniana* and *B. bassiana*, when the reactions were carried out in buffer solution. The fungi are relatively fast growing and more tolerant to high concentrations of substrates **1**, **2** and their derivatives, making possible the direct addition of substrate to the reaction media. A different behavior was reported with *Pseudomonas* sp. [13,14,16] in which it was necessary to add the biocatalyst in the vapor phase [13] due to their high toxicity to the bacterial cells [31–34].

Remarkably, the filamentous fungi *M. isabellina*, *M. ramanniana* and *B. bassiana* are suitable for both substrates and displayed interesting biohydroxylation potentials with similar reaction profiles. Depending on incubation conditions, variable conversion rates and enantioselection could be observed (Table 1) in favor of the (*R*)-enantiomer. Evidences indicate that those fungi contain enzymes whose active site has the characteristics of a cytochrome P-450-dependent monooxygenase [35–39]. *M. isabellina* is characterized by the presence of enzymes responsible for both benzylic hydroxylation and sulfoxidation [9], with the stereochemistries of hydrogen removal (specifically pro-*R*) and product formation (generally giving (*R*)-enantiomer) independent of each other and highly substrate dependent [5,35,37]. *M. ramanniana* [38] and *B. bassiana* [39] contain a range of hydroxylase enzymes with different substrate specificities.



Scheme 1.



Scheme 2.

The conversion rates of hydrocarbons **1** and **2** to the respective alcohols **3** and **4** decreased with the time due to over-oxidation to ketones **5** and **6** (Schemes 1 and 2). The observed over oxidation transformation to the ketones seems to be a non-enantioselective process since no significant changes in the ee during time-course

Table 1
Biohydroxylation of indan **1** or tetralin **2** mediated by *M. isabellina*, *M. ramanniana* and *Beauveria bassiana* carried out in various media, pH and temperatures

Microorganism	Growth media	Temperature (°C)	pH	Incubation of **1** (h)	1-Indanol **3** (%)[a]	ee (1*R*)-**3** (%)[b]	Incubation of **2** (h)	1-Tetralol **4** (%)[a]	ee (1*R*)-**4** (%)[b]
M. isabellina	PCDB	30	5.0	48	80	61	72	50	44
		30	6.0	48	78	86	72	70	86
		30	7.0	48	38	82	96	52	92
		28	6.0	48	65	80	72	70	36
		25	6.0	48	35	57	96	18	52
	PDB	30	7.0	48	22	36	48	32	63
		30	6.0	48	29	38	72	88	66
		28	6.0	48	38	61	24	50	55
	MEB	30	6.0	24	83	18	72	84	34
		28	6.0	48	90	35	48	60	51
		25	6.0	48	100	40	72	85	56
	MHB	30	7.0	24	23	40	24	10	16
		28	7.0	24	82	33	24	70	34
		25	7.0	24	23	40	24	15	32
M. ramanniana	MEB	30	7.0	24	35	72	24	47	66
		30	6.0	48	68	81	24	53	75
		28	6.0	48	87	60	24	10	65
B. bassiana	MEB	28	5.0	24	16	30	96	14	32
		28	6.0	24	24	52	72	25	46
		28	7.0	24	30	60	24	40	62

The experiments in triplicate were carried out in potassium buffer solutions at pH 5.0–8.0, incubated from 25 to 30 °C.
[a] Determined by GC on a HP-5 or a Supelco Simplicity 1 fused silica capillary column.
[b] Determined by Chiral GC on a Macherey 212117/91 Hydrodex-β 3P fused silica capillary column.

Table 2
Relative conversion of indan **1** and tetralin **2** (30 mg) by *M. isabellina*, *M. ramanniana* and *B. bassiana* (3 g fresh weight) to products **3**, **5** and **4**, **6**, respectively

Microorganism	Time	% Conversion of substrate **1**			% Conversion of substrate **2**		
		1	**3**	**5**	**2**	**4**	**6**
M. isabellina[a]	Day 1	75	24	1	89	10	1
	Day 2	18	78	3	65	28	7
	Day 3	18	64	18	22	52	28
	Day 4	18	59	23	14	50	36
	Day 5	13	36	51	0	40	60
M. ramanniana[b]	Day 1	29	55	6	86	14	0
	Day 2	6	70	24	70	26	4
	Day 3	1	64	35	41	53	6
	Day 4	0	42	58	12	45	43
B. bassiana[c]	Day 1	57	43	10	45	45	10
	Day 2	32	32	36	32	50	18
	Day 3	20	26	54	15	47	28

The experiments were carried out in triplicate and analyzed by GC on a HP-5 or Supelco Simplicity 1 fused silica capillary column.
[a] *M. isabellina* was grown in PCDB media at 30 °C for 72 h. The reactions were performed at 28 °C, pH 6.0 for substrate **1** and pH 7.0 for substrate **2**.
[b] *M. ramanniana* was grown in MEB media at 30 °C for 72 h. The reactions were performed at 30 °C, pH 6.0.
[c] *B. bassiana* was grown in MEB media at 28 °C for 72 h. The reactions were performed at 28 °C, pH 7.0.

analyses could be observed. The results are in agreement with those reported by Holland et al. [9], which suggest that benzylic alcohols, once produced by *M. isabellina*, are configurationally stable. That is also confirmed by results from the incubation of deuterium-labeled derivatives [10]. In order to observe the formation of the products after each period, the same reaction was repeated under the same conditions, monitoring the progress (Table 2). After 3 days, the observed over-oxidation of the initially formed **3** was more extensive with yeast *B. bassiana* than with *M. romanniana* or with *M. isabellina*. This order changes in the case of **2**, since the extent of over-oxidation of **4** to give **6** was: *B. bassiana* > *M. isabellina* > *M. romanniana*. The results also have shown that **3** undergoes over-oxidation easier than **4**.

The optimized conditions obtained with each biocatalyst are shown in Table 2 and the results of preparative hydroxylations are described in Table 3. The conversions are the percent composition of crude extracted materials obtained by GC, and the yields are those of isolated purified products. The best results where achieved with *M. isabellina* incubated in PCDB as growth media and potassium phosphate buffer (30 °C, pH 6 for substrate **1** or pH 7 for substrate **2**) as reaction conditions (Table 2) giving **3** in 64% yield and 86% ee after a 2-day-incubation and **4** in 38% yield and 92% ee after a 4-day-incubation (Table 3). As mentioned above, the conversion rates and ee obtained were highly influenced by growth and reactions conditions, but the formation of the (*R*)-alcohols as the major product was always observed.

Previous studies conducted by Holland et al. [9,10] also revealed the possibility of biohydroxylation of **1** and **2** by *M. isabellina.* As described in Table 1, under conditions similar to those used by Holland et al. (growth media MHB, 25 °C), who reported 12% conversion to (1*R*)-**3** (33% ee) and 17% conversion to (1*R*)-**4** (33% ee) with the predominant (*R*) configuration of products, similar ee and yields could be observed in the present studies (23% conversion to (1*R*)-**3**, 40% ee and 15% conversion to (1*R*)-**4**, 32% ee). Indeed, when the temperatures were changed to 28 °C the conversions was much high (82% conversion to (1*R*)-**3**, 33% ee and 70% conversion to (1*R*)-**4**, 34% ee). The results are in sharp contrast with Holland et al. [9] who suggested the absence of further oxidation products and independence of temperature over the range 18–30 °C in the ee during biohydroxylations by *M. isabellina*.

M. ramanniana and the well known fungus *B. bassiana* were highly sensitive to the growth and reaction conditions, affording **3** and **4** mainly when MEB was used as growth media and the reactions were performed in potassium buffer solutions (Table 1). *M. ramanniana*, which gave good yields (55% for **3** and 42% for **4**) and ee (81% for **3** and 70% for **4**)—Table 3,

Table 3
Preparative biohydroxylations of indan **1** or tetralin **2** mediated by *M. isabellina*, *M. ramanniana* and *B. bassiana* to give 1-indanol **3** and 1-tetralol **4**

Substrate	Microorganism	Growth media	Temperature (°C)	pH	Reaction time (h)	Alcohol yield (%)[a]	ee (% *R*)[b]
1	*M. isabellina*	PCDB	30	6.0	48	64	86
1	*M. ramanniana*	MEB	30	6.0	48	55	81
1	*B. bassiana*	MEB	28	7.0	24	22	60
2	*M. isabellina*	PCDB	30	7.0	96	38	92
2	*M. ramanniana*	MEB	30	6.0	72	42	70
2	*B. bassiana*	MEB	28	7.0	24	29	62

[a] The yields quoted are those of isolated, purified material.
[b] Determined by Chiral GC on Macherey 212117/91 Hydrodex-β 3P fused silica capillary column.

also showed good potential, but was less robust and efficient for the production of **3** and **4** when compared with *M. isabellina*. *B. bassiana* was characterized by giving relatively lower ee (60% for **3** and 62% for **4**) and yields (22% for **3** and 29% for **4**) than those found with the *Mortierella* species. It was also observed that in same reaction conditions, when **1** and **2** were added to the *B. bassiana* cultures, after few hours of reactions the substrates were completely consumed and no further products could be observed. This fact was not observed when *Mortierella* species were used as biocatalyst.

4. Conclusion

Fifteen microbial organisms were screened for bioconversion of indan **1** and tetralin **2** to their respective benzylic alcohols 1-indanol **3** and 1-tetralol **4**. Among the cultures screened, *M. isabellina* CCT3498, *M. ramanniana* CCT4428 and *B. bassiana* CCT3161 (from ATCC 7159), commercially available microorganisms, were found to mediate the production of **3** and **4**. The best results were achieved with *M. isabellina*, showing 78% conversion of **1** for (1*R*)-**3** (64% yield, 86% ee) in a 2-day-incubation, and 52% conversion of **2** to (1*R*)-**4** (38% yield, 92% ee) in a 4-day-incubation. The good yields and ee allow their use in future scaling up processes; however, to avoid the lack of efficiency, we recommend a careful control of temperature, pH and medium, since the reactions are strongly dependent on the incubation and reaction conditions.

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