

## Biotransformations of Substituted Phenylethanols and Acetophenones by Environmental Bacteria

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### Summary

Whole cells of hydrocarbon-degrading bacteria, isolated from polluted sediments in the Santos Estuary (Baixada Santista, São Paulo, Brazil), were able to catalyse oxidoreduction reactions with various substituted phenylethanols and acetophenones as substrates. A number of substituted phenylethanols were formed with high (>99 %) enantiomeric excess. The results of microbial oxidation of phenylethanols **2**, **3**, **5–7** by *Acinetobacter* sp. 6.4T and the reduction of acetophenones **1a–6a** by *Serratia marcescens* 5.4T showed that the bacteria used as biocatalysts in this study present significant potential for exploitation in biotechnological processes. The reduction of prochiral acetophenones by *Serratia marcescens* 3.5T yielded optically active alcohols with 90–99 % enantiomeric excess, and *Acinetobacter* sp. 6.4T is a potential biocatalyst for the oxidation of alcohols.

*Key words:* oxidoreduction, bacteria, chiral alcohols, biotransformations

### Introduction

The major applications of enzymatic catalysis are associated with biodegradation and biotransformation (1). Whilst enzyme-catalysed reactions are often studied using purified or partially purified enzyme preparations, the application of whole cell systems derived from living organisms offers a number of potential advantages. In particular, oxidoreduction reactions of xenobiotics mediated by whole cells derived from novel isolates of microorganisms have attracted a great deal of attention since such processes do not necessitate the isolation of enzymes, or of the associated co-enzymes and recycling systems (2). A further advantage of biocatalysis is that enzymatic reactions are often stereoselective and, as a

result, racemic, prochiral and planar substrates can be transformed into chiral compounds that can be used in the preparation of pharmaceuticals or agrochemicals (2–5).

Previously, we have explored new fungal strains from Brazilian biomes (the Atlantic rain forest and the Amazon) with regard to their abilities to reduce ketones and to deracemise alcohols, reactions that are repeatedly required in organic syntheses (6–9). In the present study, a range of environmental bacteria are screened for their capacity to oxidise racemic substituted phenylethanols **1–7** (Fig. 1) or to reduce the prochiral substituted acetophenones **1a–7a** (Fig. 2). These substrates have been chosen for inclusion in the screening program because of the importance of their respective optically active alcohols

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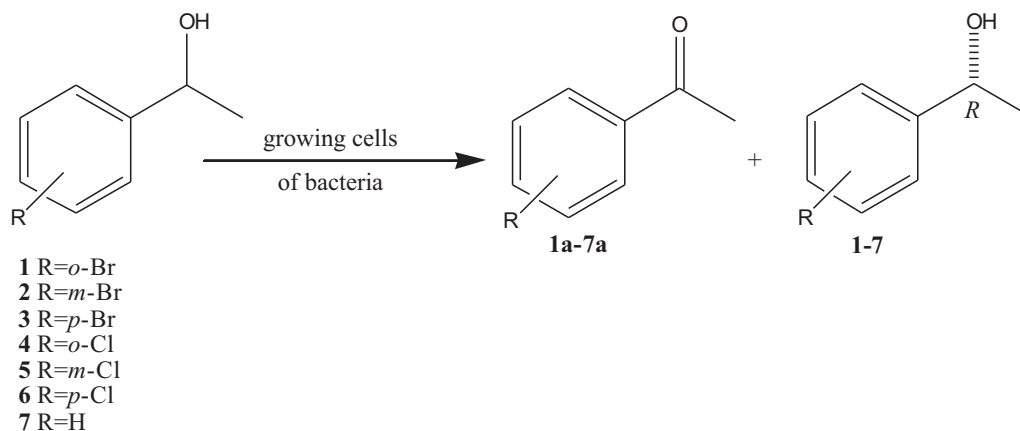


Fig. 1. Biooxidation of phenylethanols 1–7 by whole bacterial cells

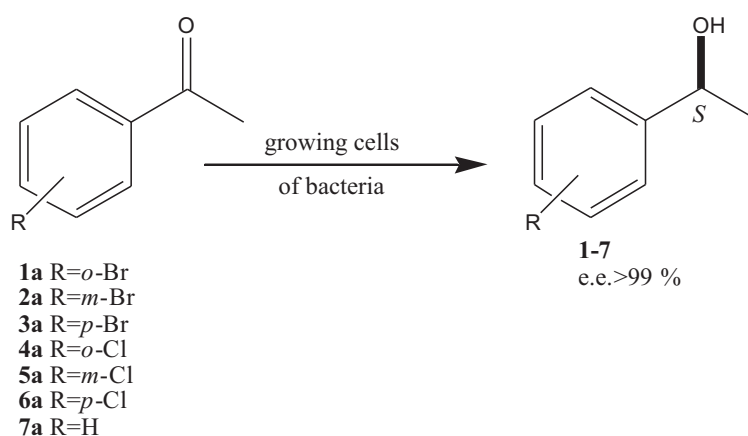


Fig. 2. Bioreduction of acetophenones 1a–7a by whole bacterial cells

(10,11). The ten isolates tested are of hydrocarbon-degrading bacteria that were previously obtained from the estuarine area of Baixada Santista, São Paulo, Brazil (12), a region that suffers from high levels of pollution with polychlorinated biphenyls and hydrocarbons.

## Materials and Methods

### General methods

Optical rotations were determined on a Jasco DIP-378 polarimeter. Biotransformations were monitored with thin layer chromatography (TLC) employing aluminium-backed silica gel 60 F<sub>254</sub> (Merck), eluted with hexane and ethyl acetate and visualised by spraying with *p*-anisaldehyde/sulphuric acid reagent or with vanillin followed by heating to approx. 120 °C for a maximum of 1 min. Gas chromatography mass spectroscopy (GC-MS) analyses were carried out on a Shimadzu GC-MS P5050A fitted with a J and W Scientific DB-5 (30 m×0.25 mm i.d.; film thickness 0.25 μm) fused silica capillary column operated under the following chromatographic conditions: oven temperature program from 50–230 °C at a rate of 10 °C/min, injector temperature 230 °C, split ratio 1:20, helium carrier gas at 100 kPa and MS interface temperature of 250 °C. Chiral analyses were carried out using a

Shimadzu GC-17A flame ionisation detector (FID) chromatograph fitted with a Chirasil-DEX CB β-cyclodextrin (25 m×0.25 mm i.d.) fused silica capillary column operated under the following chromatographic conditions: oven temperature program (Table 1), injector temperature 220 °C, split ratio 1:20, detector temperature 220 °C, and hydrogen carrier gas at 100 kPa. Absolute configurations were determined by comparison of the measured optical rotation with that reported in the literature (7–8,13) (Table 1).

### Synthesis of racemic alcohols

The racemic alcohols 1–7 were obtained by reduction of the corresponding ketones 1a–7a (purchased from Aldrich or Merck) with sodium borohydride in methanol (6,7).

### Bacterial isolates

The environmental bacteria employed in this study were collected from sediment samples obtained from the Estuário de Santos (Baixada Santista, São Paulo, Brazil), isolated in a medium enriched with diesel fuel, and identified through 16S rDNA sequencing analysis. The selected strains were: *Acinetobacter* sp. 6.4T, *Acinetobacter* sp. 7.2T, *Agrobacterium larrymoorei* 3.2B, *Calymmatobacte-*

*rium granulomatis* 4.4T, *Klebsiella ornithinolytica* 2.4T, *Pandoraea* sp. 3.3B, *Pandoraea* sp. 4.3B, *Pseudomonas* sp. 1.2B, *Serratia marcescens* 3.5T and *S. marcescens* 5.4T. All strains are deposited in the Coleção Brasileira de Microrganismos de Ambiente e Indústria (CBMAI) located at the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA) at Campinas State University (UNICAMP), Campinas, São Paulo, Brazil (12).

#### Determination of microbial biotransformation through GC analysis of the products

For each bacterial isolate, a slant culture was used to inoculate 20 mL of sterilised nutrient broth (Oxoid; 8 g/L) contained in a 125-mL Erlenmeyer flask. Under sterile conditions (Veco model VLFS-12 laminar flow cabinet), an aliquot (5  $\mu$ L) of appropriate substrate, *i.e.* phenylethanols **1–7** or acetophenones **1a–7a**, was added to the suspension of growing cells and the mixture was

incubated at 32 °C on an orbital shaker (Tecnal model TE-421 or Superohm model G-25; rotational speed 170 rpm) for 5 days. After this time, a representative sample (5 mL) of the cell suspension was removed, extracted by agitation with ethyl acetate (2.5 mL), and finally centrifuged at 6000 rpm for 5 min at room temperature. The organic phase was separated and analysed by gas chromatography and flame ionisation detector (GC-FID) (1  $\mu$ L) on a fused silica chiral capillary column (Table 2). The products of the bioreaction were compared with the racemic mixture of alcohols obtained by chemical reduction of the respective ketones **1a–7a**.

#### Determination of microbial biotransformation through isolation of the products

##### Growing cells

For each substrate, *i.e.* phenylethanols **2, 3, 5** and **6**, and acetophenones **1a–6a**, aliquots (5  $\mu$ L) were added

Table 1. Chromatographic conditions employed in the GC separation of the enantiomers of the chiral phenylethanols **1–7**, and the optical rotation values of the *S*-isomers

Compound	GC analysis oven temperature program*	GC analysis retention time/min		Optical properties of the <i>S</i> -isomer	
		<i>R</i> -isomer	<i>S</i> -isomer	$[\alpha]_D^{20}$ (concentration in CHCl <sub>3</sub> )	Enantiomeric excess/%
1-( <i>o</i> -Bromophenyl)ethanol ( <b>1</b> )	110–155 °C at 3 °C/min	12.0	13.7	–45.3 ° ( <i>c</i> =3.6)	99
1-( <i>m</i> -Bromophenyl)ethanol ( <b>2</b> )	110–155 °C at 3 °C/min	12.1	12.7	–30.7 ° ( <i>c</i> =3.1)	96
1-( <i>p</i> -Bromophenyl)ethanol ( <b>3</b> )	110–155 °C at 3 °C/min	12.5	13.2	–74.3 ° ( <i>c</i> =3.1)	90
1-( <i>o</i> -Chlorophenyl)ethanol ( <b>4</b> )	110–155 °C at 3 °C/min	9.5	10.4	–56.1 ° ( <i>c</i> =1.57)	99
1-( <i>m</i> -Chlorophenyl)ethanol ( <b>5</b> )	110–155 °C at 3 °C/min	7.5	7.9	–35.2 ° ( <i>c</i> =3.3)	96
1-( <i>p</i> -Chlorophenyl)ethanol ( <b>6</b> )	110–140 °C at 2 °C/min	11.7	12.8	–30.9 ° ( <i>c</i> =0.81)	99
1-Phenylethanol ( <b>7</b> )	110–130 °C at 3 °C/min	6.2	6.6	–35.0 ° ( <i>c</i> =2.0)	99

\*Chirasil-DEX CB  $\beta$ -cyclodextrin (25 m $\times$ 0.25 mm i.d.) fused silica capillary column; injector temperature 220 °C; split ratio 1:20; detector temperature 220 °C; hydrogen carrier gas at 100 kPa

Table 2. Microbial oxidation of phenylethanols **2, 3, 5–7** and reduction of acetophenones **1a–6a** by growing cells of *Acinetobacter* sp. 6.4T and *S. marcescens* 5.4T, respectively, incubated for 5 days at 32 °C on an orbital shaker (rotational speed 170 rpm)

Microbial oxidation	Product yield* showing absolute configuration (enantiomeric excess) of a product where appropriate					
	<b>2</b>	<b>3</b>	<b>5</b>	<b>6</b>		
<i>Acinetobacter</i> sp. 6.4T						
Resting cells	74 % <b>2a</b> 20 % <b>2-S</b> (99 %)	90 % <b>3a</b> 6 % <b>3-S</b> (99 %)	15 % <b>5a</b> 56 % <b>5-S</b> (96 %)	86 % <b>6a</b> 6 % <b>6-S</b> (99 %)		
Growing cells	48 % <b>2a</b> 34 % <b>2-S</b> (99 %)	36 % <b>3a</b> 62 % <b>3-S</b> (96 %)	25 % <b>5a</b> 53 % <b>5-S</b> (99 %)	19 % <b>6a</b> 35 % <b>6-S</b> (99 %)		
Microbial reduction	<b>1a</b>	<b>2a</b>	<b>3a</b>	<b>4a</b>	<b>5a</b>	<b>6a</b>
<i>S. marcescens</i> 5.4T						
Resting cells	44 % <b>1a</b> 44 % <b>1-S</b> (99 %)	60 % <b>2a</b> 38 % <b>2-S</b> (94 %)	55 % <b>3a</b> 44 % <b>3-S</b> (90 %)	58 % <b>4a</b> 38 % <b>4-S</b> (99 %)	47 % <b>5a</b> 43 % <b>5-S</b> (96 %)	77 % <b>6a</b> 11 % <b>6-S</b> (99 %)
Growing cells	29 % <b>1a</b> 50 % <b>1-S</b> (99 %)	59 % <b>2a</b> 35 % <b>2-S</b> (96 %)	40 % <b>3a</b> 50 % <b>3-S</b> (90 %)	40 % <b>4a</b> 32 % <b>4-S</b> (99 %)	5 % <b>5a</b> 12 % <b>5-S</b> (96 %)	19 % <b>6a</b> 14 % <b>6-S</b> (99 %)

\*Percentage as determined by isolation of the products

under sterile conditions to 20 Erlenmeyer flasks (125 mL) each containing 20 mL of a suspension of growing cells of *S. marcescens* 5.4T (for reduction) or *Acinetobacter* sp. 6.4T (for oxidation). Following incubation for 5 days at 32 °C on an orbital shaker (rotational speed 170 rpm), the reaction mixtures associated with each substrate were bulked, extracted by agitation with ethyl acetate and the organic phase was separated and fractionated by flash column chromatography over silica gel 60 (230–400 mesh; Merck), eluted with mixtures of hexane and ethyl acetate (90:10 and 80:20), to yield the alcohol products.

#### Resting cells

Slant cultures of *S. marcescens* 5.4T (for reduction) and *Acinetobacter* sp. 6.4T (for oxidation) were separately inoculated under sterile conditions, each into 2 Erlenmeyer flasks (1000 mL) containing 500 mL of sterilised nutrient broth (Oxoid; 8 g/L). The cultures were incubated for 24 h at 32 °C on an orbital shaker (rotational speed 170 rpm), and the cells were harvested by centrifugation at 20 °C on a Sorvall RC-5B Plus rotor for 15 min at 5000 rpm. The harvested cells were resuspended in water (500 mL), an aliquot (100 µL) of the appropriate substrate (phenylethanols **2**, **3**, **5**, **7** or acetophenones **1a–7a**) was added and the mixture incubated for 2 days at 32 °C on an orbital shaker (rotational speed 170 rpm). After this time, products were extracted with ethyl acetate and purified as described above.

## Results and Discussion

### Biooxidation of the substituted phenylethanols 1–7 by bacterial cells

The capacity of growing cells of each of the studied bacterial isolates to mediate the oxidation of phenylethanols 1–7 (Fig. 1) is presented in Table 3. Each isolate presented a different behaviour with respect to the biooxidation reaction, giving rise to different conversion efficiencies and different enantiomeric excesses (e.e.) of products, but typically with the *R* absolute configuration, except for *Acinetobacter* with the *S* configuration. The most efficient transformations were obtained with compounds bearing bromo or chloro groups at the *m*- and *p*-positions of the aromatic ring.

*Acinetobacter* sp. 6.4T and *Acinetobacter* sp. 7.2T were found to be the most efficient biocatalysts, although these isolates uniquely generated the (*S*)-phenylethanols **2**, **3** and **5–7**. Thus, the transformation of (*RS*)-1-(*m*-bromophenyl)ethanol (**2**) by *Acinetobacter* 6.4T occurred selectively, the (*R*)-enantiomer being oxidized to *m*-bromoacetophenone **2a**, whilst the (*S*)-enantiomer was obtained in high enantiomeric excess (e.e.>99 %) and in moderate yield (56 %). Although oxidation of (*RS*)-1-(*p*-bromophenyl)ethanol (**3**) by whole cells of all bacterial isolates studied proceeded with modest selectivity, incubation of this substrate with *Acinetobacter* 6.4T produced ketone **3a** with 44 % yield accompanied by the (*S*)-enantiomer of 1-(*p*-bromophenyl)ethanol (**3**) at 46 % e.e. Similarly, when a racemic mixture of 1-(*m*-chlorophenyl)ethanol

(**5**) was incubated with whole cells of *Acinetobacter* 6.4T, the (*S*)-enantiomer of 1-(*m*-chlorophenyl)ethanol (**5**) was formed at 85 % e.e. and with 74 % conversion. On the other hand, (*R*)-1-(*p*-chlorophenyl)ethanol (**6**) was obtained at high e.e. (72 %) with a moderate conversion of 54 % when (*RS*)-(**6**) was incubated with *Calymmatobacterium granulomatis* 4.4T. Bacterial cells were not able to promote efficient oxidation of (*RS*)-1-phenylethanol (**7**), and products were obtained with low enantioselectivity.

Although all bacterial isolates were able to oxidize alcohols **2**, **3**, and **5–7** to the corresponding acetophenones **2a**, **3a**, and **5a–7a**, the oxidation of 1-(*o*-bromophenyl)ethanol (**1**) or of 1-(*o*-chlorophenyl)ethanol (**4**) was not observed under the conditions employed in this study. Similar results, in which whole fungal cells did not mediate the oxidation of other *o*-substituted phenylethanols, have recently been reported (6,14).

The oxidation of the racemic alcohols **2**, **3**, **5** and **6** by whole bacterial cells of *Acinetobacter* sp. 6.4T was carried out on a larger scale in order to permit isolation of the product and the direct determination of yield (Table 2). As can be observed, good yields of the ketones **2a** (74 %) and **6a** (86 %) were obtained using resting cells of this isolate. Clearly, both growing and resting cells of *Acinetobacter* sp. 6.4T are efficient in catalysing the oxidation of various phenylethanols.

### Bioreduction of substituted acetophenones 1a–7a by bacterial cells

Growing cells of environmental bacteria also promoted the bioreduction of acetophenones **1a–7a** (Fig. 2), as shown in Table 4. Asymmetric reduction occurred in all cases, in accordance with Prelog's rule, and the (*S*)-enantiomers were obtained at high enantiomeric excess, typically e.e.>99 %.

Whilst *Serratia marcescens* 5.4T catalysed the reduction of *o*-bromoacetophenone (**1a**) to the corresponding (*S*)-1-(*o*-bromophenyl)ethanol (**1**) with a reasonable conversion of 49 % and high e.e. (99 %), this bacterium could only mediate the reduction of *m*-bromoacetophenone (**2a**) and *p*-bromoacetophenone (**3a**) with low yields of 19 and 15 %, respectively. In contrast, the reduction of *o*-chloroacetophenone (**4a**) was promoted by whole cells of *S. marcescens* 5.4T and of *C. granulomatis* 4.4T with good conversion yields of 61 and 56 %, respectively. All bacterial isolates were able to reduce *m*-chloroacetophenone (**5a**) and *p*-chloroacetophenone (**6a**) to the corresponding (*S*)-alcohols with low yields, although the conversion rates of 29 and 32 %, respectively, obtained with cells of *S. marcescens* 3.5T were quite acceptable. The reduction of acetophenone **7a** was mediated by all bacterial isolates tested with low yields, the highest (9 %) being achieved with whole cells of *S. marcescens* 3.5T.

The acetophenones **1a–6a** were incubated with growing and resting cells of *S. marcescens* 3.5T in a larger-scale experiment in order to isolate the products and to determine the yields directly (Table 2). In general, good conversions were obtained, but a small decrease in the enantioselectivity was observed.

Table 3. Microbial oxidation of aliquots (5  $\mu$ L) of phenylethanols 1–7 by growing bacterial cells incubated for 5 days at 32  $^{\circ}$ C on an orbital shaker (rotational speed 170 rpm)

Bacterial isolate	Product yield* showing absolute configuration (enantiomeric excess) of a product where appropriate							
	1	2	3	4	5	6	7	7**
<i>Pseudomonas</i> sp. 1.2B	100 % substrate unchanged	27 % <b>2a</b> 73 % <b>2-R</b> (35 %)	15 % <b>3a</b> 85 % <b>3-R</b> (46 %)	1 % <b>4a</b>	11 % <b>5a</b> 89 % <b>5-R</b> (39 %)	28 % <b>6a</b> 72 % <b>6-R</b> (45 %)	14 % <b>7a</b> 86 % <b>7-R</b> (18 %)	13 % <b>7a</b> 87 % <b>7-R</b> (20 %)
<i>A. larrymoorei</i> 3.2B	100 % substrate unchanged	10 % <b>2a</b> 90 % <b>2-R</b> (16 %)	8 % <b>3a</b> 92 % <b>3-R</b> (18 %)	100 % substrate unchanged	4 % <b>5a</b> 94 % <b>5-R</b> (15 %)	11 % <b>6a</b> 89 % <b>6-R</b> (26 %)	8 % <b>7a</b> 92 % <b>7-R</b> (6 %)	13 % <b>7a</b> 87 % <b>7-R</b> (15 %)
<i>Pandoraea</i> sp. 3.3B	100 % substrate unchanged	13 % <b>2a</b> 87 % <b>2-R</b> (21 %)	14 % <b>3a</b> 86 % <b>3-R</b> (31 %)	100 % substrate unchanged	6 % <b>5a</b> 94 % <b>5-R</b> (19 %)	21 % <b>6a</b> 79 % <b>6-R</b> (37 %)	8 % <b>7a</b> 92 % <b>7-R</b> (5 %)	10 % <b>7a</b> 90 % <b>7-R</b> (11 %)
<i>K. ornithinolytica</i> 2.4T	100 % substrate unchanged	6 % <b>2a</b> 94 % <b>2-R</b> (1 %)	21 % <b>3a</b> 79 % <b>3-R</b> (28 %)	100 % substrate unchanged	5 % <b>5a</b> 95 % <b>5-R</b> (3 %)	34 % <b>6a</b> 66 % <b>6-R</b> (24 %)	8 % <b>7a</b> 92 % <b>7-R</b> (1 %)	11 % <b>7a</b> 89 % <b>7-R</b> (1 %)
<i>S. marcescens</i> 3.5T	100 % substrate unchanged	17 % <b>2a</b> 83 % <b>2-R</b> (34 %)	8 % <b>3a</b> 92 % <b>3-R</b> (24 %)	2 % <b>4a</b> 98 % <b>4-R</b> (4 %)	10 % <b>5a</b> 90 % <b>5-R</b> (45 %)	22 % <b>6a</b> 78 % <b>6-R</b> (28 %)	27 % <b>7a</b> 73 % <b>7-R</b> (64 %)	14 % <b>7a</b> 86 % <b>7-R</b> (20 %)
<i>Pandoraea</i> sp. 4.3T	2 % <b>1a</b>	17 % <b>2a</b> 83 % <b>2-R</b> (15 %)	8 % <b>3a</b> 92 % <b>3-R</b> (9 %)	3 % <b>4a</b> 97 % <b>4-R</b> (5 %)	9 % <b>5a</b> 91 % <b>5-R</b> (3 %)	24 % <b>6a</b> 76 % <b>6-R</b> (27 %)	10 % <b>7a</b> 90 % <b>7-R</b> (1 %)	6 % <b>7a</b> 94 % <b>7-R</b> (2 %)
<i>C. granulomatis</i> 4.4T	1 % <b>1a</b>	15 % <b>2a</b> 85 % <b>2-R</b> (11 %)	15 % <b>3a</b> 85 % <b>3-R</b> (44 %)	1 % <b>4a</b>	7 % <b>5a</b> 93 % <b>5-R</b> (19 %)	46 % <b>6a</b> 54 % <b>6-R</b> (72 %)	15 % <b>7a</b> 85 % <b>7-R</b> (6 %)	13 % <b>7a</b> 87 % <b>7-R</b> (9 %)
<i>S. marcescens</i> 5.4T	100 % substrate unchanged	16 % <b>2a</b> 84 % <b>2-R</b> (35 %)	9 % <b>3a</b> 91 % <b>3-R</b> (22 %)	2 % <b>4a</b> 98 % <b>4-R</b> (4 %)	8 % <b>5a</b> 92 % <b>5-R</b> (37 %)	13 % <b>6a</b> 87 % <b>6-R</b> (30 %)	25 % <b>7a</b> 75 % <b>7-R</b> (51 %)	9 % <b>7a</b> 91 % <b>7-R</b> (19 %)
<i>Acinetobacter</i> sp. 6.4T	100 % substrate unchanged	44 % <b>2a</b> 56 % <b>2-S</b> (>99 %)	44 % <b>3a</b> 56 % <b>3-S</b> (46 %)	100 % substrate unchanged	26 % <b>5a</b> 74 % <b>5-S</b> (85 %)	63 % <b>6a</b> 37 % <b>6-S</b> (64 %)	12 % <b>7a</b> 88 % <b>7-S</b> (10 %)	12 % <b>7a</b> 88 % <b>7-S</b> (9 %)
<i>Acinetobacter</i> sp. 7.2T	1 % <b>1a</b>	24 % <b>2a</b> 76 % <b>2-S</b> (25 %)	29 % <b>3a</b> 71 % <b>3-S</b> (19 %)	100 % substrate unchanged	17 % <b>5a</b> 83 % <b>5-S</b> (27 %)	34 % <b>6a</b> 66 % <b>6-S</b> (32 %)	7 % <b>7a</b> 93 % <b>7-S</b> (6 %)	4 % <b>7a</b> 96 % <b>7-S</b> (4 %)

\*Percentage as determined by GC; unless shown otherwise, unchanged substrate accounted for the balance of products in the reaction mixture

\*\*Substrate consisted of 20  $\mu$ L of 1-phenylethanol (7)

Table 4. Microbial reduction of aliquots (5  $\mu$ L) of acetophenones **1a–7a** by growing bacterial cells incubated for 5 days at 32 °C on an orbital shaker (rotational speed 170 rpm)

Bacterial isolate	Product yield* showing absolute configuration (enantiomeric excess) of a product where appropriate							
	<b>1a</b>	<b>2a</b>	<b>3a</b>	<b>4a</b>	<b>5a</b>	<b>6a</b>	<b>7a</b>	<b>7a**</b>
<i>Pseudomonas</i> sp. 1.2B	3 % 1-S (>99 %)	3 % 2-S (>99 %)	2 % 3-S (>99 %)	22 % 4-S (>99 %)	7 % 5-S (>99 %)	2 % 6-S (>99 %)	5 % 7-S (>99 %)	1 % 7-S (>99 %)
<i>A. larrymoorei</i> 3.2B	5 % 1-S (>99 %)	2 % 2-S (>99 %)	3 % 3-S (>99 %)	12 % 4-S (>99 %)	8 % 5-S (>99 %)	3 % 6-S (>99 %)	5 % 7-S (>99 %)	1 % 7-S (>99 %)
<i>Pandoraea</i> sp. 3.3B	2 % 1-S (>99 %)	99 % substrate unchanged	1 % 3-S (>99 %)	7 % 4-S (>99 %)	6 % 5-S (>99 %)	3 % 6-S (>99 %)	1 % 7-S (>99 %)	1 % 7-S (>99 %)
<i>K. ornithinolytica</i> 2.4T	30 % 1-S (>99 %)	2 % 2-S (>99 %)	8 % 3-S (>99 %)	27 % 4-S (>99 %)	100 % substrate unchanged	12 % 6-S (>99 %)	1 % 7-S (>99 %)	100 % substrate unchanged
<i>S. marcescens</i> 3.5T	19 % 1-S (>99 %)	10 % 2-S (>99 %)	15 % 3-S (>99 %)	25 % 4-S (>99 %)	29 % 5-S (>99 %)	32 % 6-S (>99 %)	9 % 7-S (>99 %)	6 % 7-S (>99 %)
<i>Pandoraea</i> sp. 4.3T	99 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	3 % 4-S (>99 %)	100 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	1 % 7-S (50 %)
<i>C. granulomatis</i> 4.4T	42 % 1-S (>99 %)	2 % 2-S (>99 %)	4 % 3-S (>99 %)	61 % 4-S (>99 %)	4 % 5-S (>99 %)	2 % 6-S (>99 %) 97 % substrate unchanged	1 % 7-S (>99 %)	1 % 7-S (>99 %)
<i>S. marcescens</i> 5.4T	49 % 1-S (>99 %)	19 % 2-S (>99 %)	15 % 3-S (>99 %)	56 % 4-S (>99 %)	6 % 5-S (>99 %)	8 % 6-S (>99 %) 91 % substrate unchanged	7 % 7-S (>99 %)	5 % 7-S (>99 %)
<i>Acinetobacter</i> sp. 6.4T	99 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	100 % substrate unchanged	1 % 7-S (>99 %)	100 % substrate unchanged
<i>Acinetobacter</i> sp. 7.2T	6 % 1-S (>99 %)	5 % 2-S (>99 %)	100 % substrate unchanged	8 % 4-S (>99 %)	7 % 5-S (>99 %)	7 % 6-S (50 %)	100 % substrate unchanged	4 % 7-R (67 %)

\*Percentage as determined by GC; unless shown otherwise, unchanged substrate accounted for the balance of products in the reaction mixture

\*\*Substrate consisted of 20  $\mu$ L of acetophenone (**7a**)



## Conclusions

The results of the screening of environmental strains of hydrocarbon-degrading bacteria with respect to their capacity to mediate the oxidoreduction reactions of phenylethanol and acetophenone derivatives indicate that these microorganisms exhibit the potential to perform bioenzymatic reactions. The preference of oxidation reactions in relation to reductions can be attributed to the selective isolation of these microorganisms. In this context, bacterial strains in which catabolic dioxygenase genes have been up-regulated through genetic manipulation have significant potential as sources of stereoselective enzymes for biotransformations.

The bacterial isolates described in this study present significant potential for exploitation in biotechnological processes. Thus the reduction of prochiral acetophenones by *Serratia marcescens* 3.5T yielded optically active alcohols with 90–99 % enantiomeric excess, and *Acinetobacter* sp. 6.4T is a potential biocatalyst for the oxidation of alcohols.

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