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Enantioselective reduction of *ortho*-substituted acetophenones by bacterial strains isolated from medium enriched with biphenyl or diesel fuel

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Abstract

Application of 21 new bacterial strains from natural environments (coastal plain of Santos and Atlantic Rain Forest, São Paulo, Brazil) in the asymmetric reduction of acetophenone derivatives is described. The bioreduction was carried out with whole bacterial cells leading to (*S*)-chiral alcohols in up to ≥99% e.e. The (*S*)-(−)-1-(2-bromo-phenyl)-ethanol was employed in the preparation of chiral tellurium derivatives.

Keywords: Bioreduction; Bacteria; Ketone; Tellurium; Alcohol; Selenium

1. Introduction

Biocatalysis is one of the most important scientific topics in Green Chemistry in which materials are expected to be used efficiently, emissions and waste production reduced and inherently safe process sought [1]. In addition to this concern, the growing interest in the synthesis of chiral molecules in enantiomerically pure form has also promoted a great development in biocatalysis [2,3]. Among the compounds of interest, chiral alcohols have been prepared by reduction of aromatic ketones by fungi, bacteria and isolated enzymes as biocatalyst. The biocatalytic reactions can be carried out using whole microbial cells or isolated enzymes. In the case of isolated dehydrogenases the external addition of expensive coenzymes and a recycling system are required what confer to this protocol some economical disadvantages. In this way screening for new microorganisms strains isolated from different natural environments are used in the search for efficient enzymatic systems, which can promote the biotransformation with no need of external additives [4]. In view of the vast diversity of the Brazilian biomes we initiated a study aiming to explore microorganisms isolated from them in ketone reductions and alcohol deracemizations. Initially new fungi strains were studied for this purpose [5]. In this communication we report a study on the potential of new bacterial strains isolated from coastal plain and forest areas of São Paulo state (Brazil) as enantioselective reducing agents for acetophenone derivatives.

2. Experimental

2.1. General methods

2′-Bromoacetophenone, 3′-bromoacetophenone, 4′-bromoacetophenone, 2′-chloroacetophenone, 3′-chloroacetophenone, 4′-chloroacetophenone, 2′-fluoroacetophenone and

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2′-nitroacetophenone are commercially available and were used without further purification. 2′-Methylselenoacetophenone and 2′-phenylselenoacetophenone were prepared according to previous report [5b]. Thin-layer chromatography (TLC) was performed using precoated plates (Aluminum foil, silica gel 60 F_{254} Merck, 0.25 mm). Merck 60 silica gel (230–400 mesh) was used for flash chromatography. GC analyses were performed in a Shimadzu GC-17A instrument with a FID detector, using hydrogen as a carrier gas (100 kPa). Mass spectra were recorded on a Shimadzu GCMS P5050A (70 eV) spectrometer. The fused silica capillary columns used were a J&W Scientific DB-5 (30 m × 0.25 mm) and a chiral column Chirasil-Dex CB β-cyclodextrin (25 m × 0.25 mm) for determination of the enantiomeric excesses. Optical rotations were determined on a JASCO DIP-378 polarimeter. NMR spectra were recorded on Bruker DPX 500 instrument. For ^{1}H (instrument operating at 500.13 MHz) δ values are referenced to Me_4Si (0 ppm) and for ^{13}C (instrument operating at 125.77 MHz) δ values are referenced to $CDCl_3$ (77.0 ppm). Chemical shifts are given in ppm and coupling constants are given in Hertz.

2.2. Microorganisms library and preservation

The bacterial strains were stored in a freezer at −70 °C. They were preserved as suspensions in glycerol solution (20%). Composition of the culture media: Nutrient Broth—5 g/L beef extract; 3 g/L peptone.

2.3. General procedure for the bioreduction reaction

2.3.1. Growth conditions for the bacteria cultures

The isolated bacteria were grown in Erlenmeyer flasks (250 mL) containing 100 mL of culture medium (Nutrient Broth—5 g/L beef extract; 3 g/L peptone) at 30 °C (48 h) in an orbital shaker (170 rpm). After this stage, the cells were harvested by centrifugation (5000 rpm, 20 min, 10 °C) and were used for bioreductions.

2.3.2. Small scale reactions

The cells produced from 100 mL of culture medium were resuspended in 30 mL of a phosphate buffer solution (pH 7.0, 0.1 M) in an Erlenmeyer flask (125 mL) followed by addition of the *ortho*-bromoacetophenone (**1a**) (5 μL). The reactional mixture was stirred in an orbital shaker (30 °C, 170 rpm) for the time indicated in Table 1. The progress of the bioreduction was monitored by GC analysis.

2.3.3. Preparative-scale reaction

The bacteria *Serratia marcescens* (1.2T) was grown in a set of five 250 mL Erlenmeyer flasks containing 100 mL of culture medium each (Nutrient Broth—beef extract 5 g/L; peptone 3 g/L) at 30 °C (48 h) in an orbital shaker (170 rpm). After this time, 25 mg of the *ortho*-bromoacetophenone (**1a**) was added into each Erlenmeyer flask. After the appropriate conversion time (7 days), the content of all flasks were combined and then saturated with sodium chloride. The aqueous phase was extracted with ethyl acetate (4 mL × 200 mL). The organic phases were combined and dried over $MgSO_4$. The

Table 1
Screening for bacterial strains in the bioreduction of *ortho*-bromoacetophenone (**1a**)

Entry	Bacterial isolates	*t*	*c* (%)	e.e. (%)	abs. conf.
1	ICB-04	6	61	≥99	*S*
2	*Bacillus* sp. (ICB7)	6	95	≥99	*S*
3	ICB-09	6	74	≥99	*S*
4	ICB-19	6	16	≥99	*S*
5	ICB-28	5	99	≥99	*S*
6	ICB-29	6	69	≥99	*S*
7	*Pseudomonas* sp. (1.5B)	6	87	≥99	*S*
8	*Pseudomonas* sp. (1.6B)	6	20	≥99	*S*
9	*Pseudomonas* sp. (1.7B)	6	21	≥99	*S*
10	*Comamonas* (4.1B)	7	45	≥99	*S*
11	*Rhodococcus* sp. (8.1B)	7	48	≥99	*S*
12	*Rhodococcus* sp. (8.5B)	7	48	≥99	*S*
13	*Serratia marcescens* (1.2T)	7	91	≥99	*S*
14	*S. marcescens* (3.5T)	7	63	≥99	*S*
15	*S. marcescens* (5.4T)	6	83	≥99	*S*
16	*Acinetobacter* sp. (6.4T)	7	25	≥99	*S*
17	*Pandorea* sp. (4.3T)	7	10	≥99	*S*
18	*Klebsiella* sp. (2.1T)	7	9	≥99	*S*
19	*Acinetobacter baumannii* (7.1T)	7	32	≥99	*S*
20	*Agrobacterium larrymoorei* (3.2B)	6	21	≥99	*S*
21	*Calymmatobacterium granulomatis* (4.4T)	7	28	≥99	*S*

t: time (days); *c*: conversion determined by GC using the chiral column Chiral-Dex-CB (beta-cyclodextrin, 25 m × 0.25 mm); e.e.: enantiomeric excess; abs. conf.: absolute configuration determined by comparison with literature data [12].

solvent was removed in vacuum and the residue was purified by column chromatography on silica gel using a mixture of hexane and ethyl acetate (4:1) as eluent to afford (*S*)-(−)-1-(2-bromo-phenyl)-ethanol (**2a**): Yield: 52 %. e.e.: 99%. $[\alpha]_D^{20}$: −62.5° (*c* 2.33, $CHCl_3$).

2.3.4. Determination of the enzymatic activity of the bacteria

The reaction progress was monitored every 3 and 7 days by collecting 2 mL samples (Table 1). These samples were extracted by stirring with ethyl acetate (0.5 mL) followed by centrifugation (6000 rpm, 5 min). The organic phase was analyzed by GC (1 μL) in a chiral capillary column (Chirasil-Dex CB β-cyclodextrin −25 m × 0.25 mm) for determination of the conversion and enantiomeric excesses. The products of the biocatalyzed reactions were compared with a racemic mixture. The preparation of the alcohols (*RS*)-**2a**–**j** was carried out by reduction of the corresponding acetophenones with sodium borohydride in ethanol [5].

Racemic compounds **2a**–**g**: GC conditions (carrier gas H_2, 100 kPa, injector 220 °C; detector 220 °C; 110 °C, 3 °C/min up to 180 °C). t_R (min): retention time of (*RS*)-1-(2-bromo-phenyl)-ethanol (**2a**) (*R*-enantiomer 12.38 min; *S*-enantiomer 14.01 min), (*RS*)-1-(3-bromo-phenyl)-ethanol (**2b**) (*R*-enantiomer 11.90 min; *S*-enantiomer 12.38 min), (*RS*)-1-(4-bromo-phenyl)-ethanol (**2c**) (*R*-enantiomer 12.12 min; *S*-enantiomer 12.77 min), (*RS*)-1-(2-chloro-phenyl)-ethanol (**2d**) (*R*-enantiomer 8.85 min; *S*-enantiomer 10.08 min), (*RS*)-1-(3-chloro-phenyl)-ethanol (**2e**) (*R*-enantiomer 9.38 min; *S*-enantiomer 9.89 min), (*RS*)-1-(4-chloro-phenyl)-ethanol (**2f**) (*R*-enantiomer 9.36 min; *S*-enantiomer 10.04 min), (*RS*)-1-(2-fluoro-phenyl)-ethanol (**2g**) (*R*-enantiomer 4.88 min; *S*-enantiomer 5.27 min). *Racemic compound* **2h**: GC conditions (carrier gas H_2, 100 kPa, injector 220 °C; detector 220 °C; 150 °C, hold 20 min, 30 °C/min up to 180 °C). t_R (min): retention time of (*RS*)-1-(2-methylseleno-phenyl)-ethanol (**2h**) (*R*-enantiomer 9.07 min; *S*-enantiomer 10.22 min). *Racemic compound* **2i**: GC conditions (carrier gas H_2, 100 kPa, injector 220 °C; detector 220 °C; 150 °C, 3 °C/min up to 180 °C). t_R (min): retention time of (*RS*)-1-(2-phenylseleno-phenyl)-ethanol (**2i**) (*R*-enantiomer 21.55 min; *S*-enantiomer 20.13 min). *Racemic compound* **2j**: GC conditions (carrier gas H_2, 100 kPa, injector 220 °C; detector 220 °C; 150 °C, 1 °C/min up to 180 °C). t_R (min): retention time of (*RS*)-1-(2-nitro-phenyl)-ethanol (**2j**) (*R*-enantiomer 7.54 min; *S*-enantiomer 8.56 min).

2.3.5. Application of Pseudomonas sp. (1.5B) and Bacillus sp. (ICB7) in the bioreduction of acetophenone derivatives

The bacteria were grown in Erlenmeyer flasks (4 × 2 L) containing 1 L of culture medium (Nutrient Broth—5 g/L beef extract; 3 g/L peptone) at 30 °C (48 h) in an orbital shaker (170 rpm). After this stage, the cells were harvested by centrifugation (5000 rpm, 20 min, 10 °C). The cells produced from 400 mL of culture medium were resuspended in 30 mL of a phosphate buffer solution (pH 7.0, 0.1 M) in an Erlenmeyer flask (125 mL) followed by addition of the acetophenone derivatives (**1**) (5 μL). The reactional mixture was stirred in an orbital shaker (30 °C, 170 rpm) for the time indicated in Table 2. The progress of the bioreduction was monitored by GC analysis.

2.3.6. Assignment of the absolute configurations for the alcohols *2a–d*

The absolute configurations were attributed by chiral GC correlation with standards (*S*)-1-(2-bromo-phenyl)-ethanol (**2a**), (*S*)-1-(3-bromo-phenyl)-ethanol (**2b**), (*S*)-1-(4-bromo-phenyl)-ethanol (**2c**), (*S*)-1-(2-chloro-phenyl)-ethanol (**2d**), (*S*)-1-(3-chloro-phenyl)-ethanol (**2e**), (*S*)-1-(4-chloro-phenyl)-ethanol (**2f**), (*S*)-1-(2-fluoro-phenyl)-ethanol (**2g**), (*S*)-1-(2-methylseleno-phenyl)-ethanol (**2h**), (*S*)-1-(2-phenylseleno-phenyl)-ethanol (**2i**) and (*S*)-1-(2-nitro-phenyl)-ethanol (**2j**) prepared by us as previously reported [5].

2.4. Synthesis of (S)-(−)-1-(2-n-butyltelluro-phenyl)-ethanol (3)

To an oven-dried two-necked round-bottomed flask (50 mL), (*S*)-1-(2-bromo-phenyl)-ethanol (**2a**) (1 mmol; 0.202 g) was dissolved in tetrahydrofuran (5 mL) and cooled to −76 °C. To this cooled solution, *n*-BuLi (1.77 mL, from a 1.30 N solution in hexane, 2.30 mmol) was added dropwise. The mixture was maintained at 0 °C for 30 min. After this time, dibutyl ditelluride (2.3 mmol) was added dropwise and the mixture was stirred for 18 h at room temperature. The reaction was quenched with brine (5 mL). The aqueous layer was extracted with ethyl acetate (3 mL × 20 mL). The organic phases were combined and dried over $MgSO_4$. The solvent was removed in vacuum and the residue was purified by column chromatography on silica gel eluting with a mixture of hexane and ethyl acetate (4:1) to afford compound (*S*)-**3**. Yield: 50%. e.e.: 99%. $[\alpha]_D^{20}$: −10.3° (*c* 1.94, $CHCl_3$).

H^1 NMR (500 MHz, $CDCl_3$, ppm) δ 7.72 (dd, ^3J = 7.7 Hz, ^4J = 1.2 Hz, 1H), 7.47 (dd, ^3J = 7.8 Hz, ^4J = 1.5 Hz, 1H), 7.26 (ddd, ^3J = 7.6, 7.4 Hz, ^4J = 1.2 Hz, 1H), 7.07 (ddd, ^3J = 7.5 Hz, ^4J = 1.5 Hz, 1H), 5.10 (q, ^3J = 6.1 Hz, 1H), 2.86 (td, ^3J = 7.7 Hz, ^4J = 2.2 Hz, 2H), 2.35 (br, 1H), 1.76 (qn, ^3J = 7.6 Hz, 2H), 1.46 (d, ^3J = 6.5 Hz, 3H), 1.40 (sext, ^3J = 7.35 Hz, 2H), 0.90 (t, ^3J = 7.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$, ppm) δ 149.1, 137.6, 128.1, 127.9, 125.3, 113.9, 73.0, 33.6, 25.1, 24.2, 13.4, 8.60 [6].

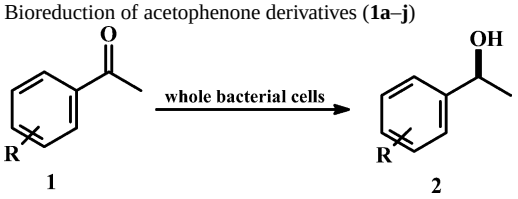
3. Results and discussion

3.1. Isolation of the bacterial strains

The isolates were obtained after an enrichment procedure, carried out through the inoculation of sampled sedi-

ment (1.0 g) in flasks containing a mineral salt medium and diesel fuel (0.05%) or biphenyl crystals (100 mg) as carbon source [7,8]. The cultures were incubated in an orbital shaker (160 rpm; 28 °C) until growth was observed, followed by three serial transfers and isolation in solid medium. The identification of part of the isolates was performed through 16S rDNA gene sequencing and comparison with GenBank top hits after a BLAST search [9]. The strains named ICB (04, 07-*Bacillus* sp., 09, 19, 28 and 29), *Pseudomonas* sp. (1.5B), *Pseudomonas* sp. (1.6B), *Pseudomonas* sp. (1.7B), *Comamonas* (4.1B), *Rhodococcus* sp. (8.1B), *Rhodococcus* sp. (8.5B) and *Agrobacterium larrymoorei* (3.2B) were isolated from medium enriched with biphenyl. *Serratia marcescens* (1.2T), *S. marcescens* (3.5T), *S. marcescens* (5.4T), *Acinetobacter* sp. (6.4T), *Pandorea* sp. (4.3T), *Klebsiella* sp. (2.1T), *Acinetobacter baumannii* (7.1T) and *Calymmatobacterium granulomatis* (4.4T) were isolated from medium enriched with diesel fuel. The microorganisms were conserved as suspensions in a 20% glycerol solution. All the strains were stored at −80 °C in cryotubes in our laboratory. These strains

Table 2
Bioreduction of acetophenone derivatives (**1a**–**j**)



Entry	R-(**1**)	Bacterial isolates	*t*	*c* (%)	e.e. (%)	abs. conf.
1	*ortho*-Br-**1a**	*Bacillus* sp. (ICB7)	3	45	99	(*S*)
			6	90	99	(*S*)
2	*ortho*-Br-**1a**	*Pseudomonas* sp. (1.5.B)	3	37	99	(*S*)
			6	80	99	(*S*)
3	*meta*-Br-**1b**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	–	–	–
4	*meta*-Br-**1b**	*Pseudomonas* sp. (1.5.B)	3	5	90	(*S*)
			6	7	90	(*S*)
5	*para*-Br-**1c**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	–	–	–
6	*para*-Br-**1c**	*Pseudomonas* sp. (1.5.B)	3	1	82	(*S*)
			6	4	82	(*S*)
7	*ortho*-Cl-**1d**	*Bacillus* sp. (ICB7)	3	44	99	(*S*)
			6	65	99	(*S*)
8	*ortho*-Cl-**1d**	*Pseudomonas* sp. (1.5.B)	3	19	99	(*S*)
			6	45	99	(*S*)
9	*meta*-Cl-**1e**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	–	–	–
10	*meta*-Cl-**1e**	*Pseudomonas* sp. (1.5.B)	3	6	85	(*S*)
			6	10	85	(*S*)
11	*para*-Cl-**1f**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	3	99	(*S*)
12	*para*-Cl-**1f**	*Pseudomonas* sp. (1.5.B)	3	3	89	(*S*)
			6	5	89	(*S*)
13	*ortho*-F-**1g**	*Bacillus* sp. (ICB7)	3	13	99	(*S*)
			6	40	99	(*S*)
14	*ortho*-F-**1g**	*Pseudomonas* sp. (1.5.B)	3	44	99	(*S*)
			6	80	99	(*S*)
15	*ortho*-MeSe-**1h**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	3	99	(*S*)
16	*ortho*-MeSe-**1h**	*Pseudomonas* sp. (1.5.B)	3	–	–	–
			6	–	–	–
17	*ortho*-PhSe-**1i**	*Bacillus* sp. (ICB7)	3	–	–	–
			6	–	–	–
18	*ortho*-PhSe-**1i**	*Pseudomonas* sp. (1.5.B)	3	–	–	–
			6	–	–	–
19	*ortho*-NO_2-**1j**	*Bacillus* sp. (ICB7)	3	(17)[a]	–	–
			6	2 (19)[a]	40	(*S*)
20	*ortho*-NO_2-**1j**	*Pseudomonas* sp. (1.5.B)	3	–	–	–
			6	1 (2)[a]	75	(*S*)

t: time (days); *c*: conversion determined by GC using the chiral column Chiral-Dex-CB (beta-cyclodextrin, 25 m × 0.25 mm); e.e.: enantiomeric excess; abs. conf.: absolute configuration determined by comparison with literature data [12,13a,16].

[a] *ortho*-aminoacetophenone.

were deposited in the culture collection of CBMAI (Coleção Brasileira de Microrganismos de Ambiente e Indústria) from CPQBA/UNICAMP.

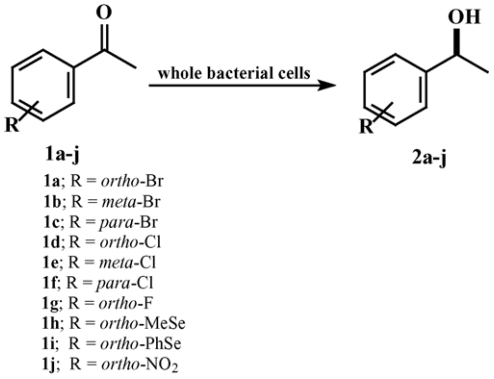
3.2. Biotransformation of (*1a*)

We initiated the study with acetophenone itself, but the results were unsatisfactory, most of the substrate was recovered after a long contact with the microorganisms. A possible explanation for this fact is that besides the ketone reduction, an alcohol oxidation is also occurring along the biotransformation process. This assumption is supported by the observed enantioselective oxidation of (*RS*)-phenylethanol by the strains *Agrobacterium larrymoorei* (3.2B), *Pandorea* sp. (4.3T), *Calymmatobacterium granulomatis* (4.4T), *S. marcescens* (5.4T) and *Acinetobacter* sp. (6.4T) [10]. The alcohol dehydrogenases present in the bacterial isolates should be more active towards the substituted acetophenones reduction than with acetophenone itself. This feature was also observed by using *para*- and *meta*-substituted acetophenones with the bacterial strain *Bacillus* sp. (ICB7) (See details in the Section 3.3). On the other hand, *ortho*-bromoacetophenone (**1a**) showed to be reactive towards all the microorganisms tested (Scheme 1). In view of this fact we performed a detailed study with the microorganisms shown in Table 1 using **1a** as substrate.

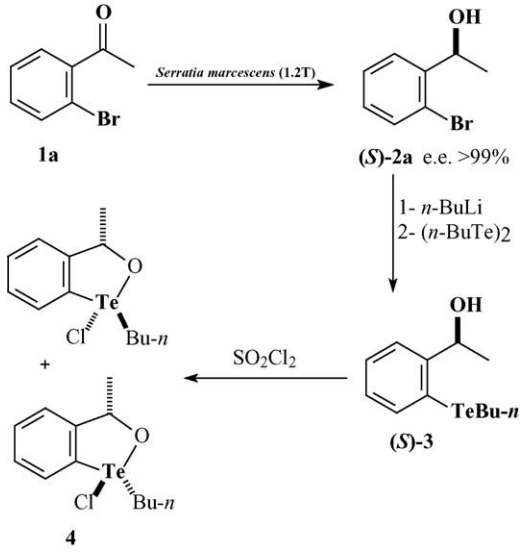
The microbial reduction was performed by resuspending the wet bacterial cells in a phosphate buffer solution, **1a** was then added and the mixture was incubated in an orbital shaker at 32 °C. The progress of the reaction was monitored by GC analysis after 3 and 7 days after the addition of the ketone. As can be observed in Table 1, all the bacterial strains under study produced (*S*)-1-(2-bromo-phenyl)-ethanol (**2a**) with high stereoselectivity (≥99% e.e.). Some bacterial strains, namely *Bacillus* sp. (ICB7), ICB-28, *Pseudomonas* sp. (1.5B), *S. marcescens* (1.2T), *S. marcescens* (5.4T) (Entries 2, 5, 7, 9, 13 and 15, respectively), afforded (*S*)-(−)-**2a** with high conversion (83–99%). The other bacterial strains gave low conversions (9–74%), although with high enantioselectivity (≥99% e.e.). Chiral chromatography comparison of the product **2a** with an authentic sample showed that all the reductions proceeded according to Prelog's rule [11]. In spite of the different compounds as carbon source used in the enrichment step, the enantioselectivity and enantiopreference were the same for all the strains studied.

As mentioned before, the small-scale reactions were performed with resuspended cells in a buffer solution. In order to have enough material for further transformations, a preparative scale experiment using *S. marcescens* (1.2T) was performed. In this case the biotransformation was promoted in the presence of growing bacterial cells. A set of five 250 mL Erlenmeyer flasks was used, with 25 mg of **1a** in each one. After workup of the five combined aqueous phases 65 mg of **2a** (52% yield) were obtained with 99% e.e., what shows that the enantioselectivity of the transformation in the presence of isolated cells is similar to the one in the presence of growing bacteria. The possibility of performing the transformation in preparative scale is interesting, since we are using chiral aryl-ethanols to prepare chiral chalcogen compounds [5b], which can be used as chiral building blocks, chiral derivatizing agents or chiral ligands in the reaction of organometallics with carbonyl compounds [5b,13]. To demonstrate this potential use we transformed (*S*)-**2a** into (*S*)-**3**, by treating (*S*)-**2a** with *n*-butyllithium and dibutyl ditelluride (Scheme 2). Compound (*S*)-**3** was obtained in good yield. Organotelluro-α-methylbenzyl alcohols like (*S*)-**3** can be transformed into diastereiomeric mixture of 1-chloro-1butyl-3H-benzo-2,1-oxatellurole (**4**) (Scheme 2) [14].

Recently we found that some classes of Te(IV) compounds are specific serine protease inhibitors [15]. The easy access to chiral compounds of the type **3** allowed the synthesis of chiral compounds of the type **4**, which are being used by us to investigate the influence of the geometry of the Te(IV) compounds in their protease inhibitory activity [14]. In view of this fact we decided to extend the study of the substrate specificity toward the bacterial isolates to other substituted



Scheme 1.



Scheme 2.

acetophenones (**1b**–**j**, Scheme 1), which could be chiral precursors of compounds of the type **4**.

3.3. Biotransformation of acetophenone derivatives (1a–j)

Among the 21 bacteria tested for the reduction of **1a**, we selected the bacteria *Bacillus* sp. (ICB7) and *Pseudomonas* sp. (1.5B) as biocatalyst for the bioreduction reaction of the additional ketones in view of the good results obtained in the exploratory study with **1a**. Acetophenone derivatives with different substituents (bromine, fluorine, chlorine atoms and nitro, phenylseleno and methylseleno groups) in the benzene ring were chosen to test the efficiency and stereoselectivity of the ketone group bioreduction by the alcohol dehydrogenases present in the enzymatic system of the selected bacteria.

As can be seen in Table 2, the bacteria *Bacillus* sp. (ICB7) only performed the bioreduction of the *ortho*-substituted acetophenones, except the *ortho*-phenylselenoacetophenone (**1i**) (Entries 16–18). All ketones were transformed into the (*S*)-alcohol in high enantiomeric excess (99%). It seems that the bacteria *Bacillus* sp. (ICB7) is specific for *ortho*-substituted acetophenones.

In the bioreduction of *ortho*-nitroacetophenone (**1j**) by *Bacillus* sp. (ICB7), besides the ketone group reduction, the nitro group was also reduced leading to *ortho*-aminoacetophenone (19%, Table 2, Entry 19). The same was observed when *Pseudomonas* sp. (1.5B) was reacted with **1j** (2%, Table 2, Entry 20). The bromine or chlorine atom at the *meta*- and *para*-position in the aromatic ring reduce the ketones reactivity towards the alcohol dehydrogenase present in the bacteria *Bacillus* sp. (ICB7), except for the *para*-chloroacetophenone (**1f**), which was reduced to the corresponding chiral alcohol in low conversion (Table 2, Entry 11).

The bioreduction of **1a**–**j** was also performed with *Pseudomonas* sp. (1.5B), which gave good results in the exploratory experiments with **1a** (Table 1). In several cases the chiral alcohols were obtained with enantiomeric excesses up to 99% (Table 2). *Pseudomonas* sp. (1.5B) also promotes the bioreduction of the *meta*- and *para*-substituted ketones leading to the (*S*)-alcohols. Enantioselectivity for the reduction of the *ortho*-substituted alcohols was 99%, except for the *ortho*-nitroacetophenone (**1j**) (75% e.e., Table 2, Entry 20). This fact can be occurring due to electronic and hindrance effects caused by the nitro group to the ketone group. The phenylseleno group *ortho* to the ketone group also hinders its reduction. The same behaviour was observed in the reduction of **1i** using *Daucus carrota* root [13a] and fungal cells [5b].

4. Conclusion

In summary, several bacterial strains isolated from medium enriched with biphenyl or diesel fuel can be used to perform efficient enantioselective reduction of *ortho*-substituted acetophenones to their corresponding chiral (*S*)-alcohols. The chiral products can be employed in the preparation of chiral tellurium derivatives.

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