Bioreduction of Acetophenone Derivatives by Red Marine Algae *Bostrychia radicans* and *B. tenella*, and Marine Bacteria Associated

by **Ana M. Mouad**[a]), **Mariana P. Martins**[a]), **Hosana M. Debonsi**[b]), **Ana L. L. de Oliveira**[b]), **Rafael de Felicio**[b]), **Nair S. Yokoya**[c]), **Mutue T. Fujii**[c]), **Claúdia B. A. de Menezes**[d]), **Fabiana Fantinatti-Garboggini**[d]), and **André L. M. Porto***[a])

[a]) Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-carlense, 400, CEP 13560-970, CP 780, São Carlos-SP, Brazil (phone: +55 16 33738103; fax: +55 16 33739952; e-mail: almporto@iqsc.usp.br)

[b]) Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Departamento de Física e Química, Universidade de São Paulo, Via do Café s/n, CEP 14040-903, Ribeirão Preto-SP, Brazil

[c]) Instituto de Botânica de São Paulo, Av. Miguel Estéfano, 3687, CEP 04301-902, CP 4005, Água Funda, São Paulo-SP, Brazil

[d]) Recursos de Divisão Microbianos, Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas, CPQBA, Universidade Estadual de Campinas, Rua Alexandre Casellato, 999, CEP 13140-000, Paulínia-SP, Brazil

The biocatalytic reduction of acetophenone derivatives was exploited by using algal biomass from *Bostrychia radicans* and *B. tenella* producing exclusively (*S*)-2-phenylethanols with high enantiomeric excess (>99% ee). Bacterial populations associated with algal biomass were identified as the *Bacillus* genus. This report deals with the first investigations involving the use of marine bacteria associated with *B. radicans* and *B. tenella* marine algae for the biocatalytic reduction of acetophenone derivatives.

1. Introduction. – Reduction reactions of prochiral ketones promote the production of enantiomerically pure alcohols which are valuable intermediates in asymmetric organic synthesis. Microorganisms from different natural sources may carry out these biotransformations. Marine biodiversity has an enormous potential for biotechnological processes. Marine metagenomics offers strategies for the discovery of new enzymes with biotechnological applications [1][2].

The biocatalytic reduction of prochiral ketones has been extensively studied by several methods [3]. However, the use of algae for the reduction of ketones is rare in comparison to cells of microorganisms, such as fungi, yeast, and bacteria [4][5]. *Yoshizako et al.* [6–9] described the asymmetric reduction of the CO groups using the *Chorella pyrenoidosa* alga. Biotransformation of aliphatic and aromatic ketones, and aldehydes were reported from five cultures of photosynthetic marine microalgae [10][11]. *Dunaliella tertiolecta* (green microalga) catalyzed the biotransformation of terpenoids [12], and several keto esters were converted to the corresponding hydroxy esters by marine microalgae such *Chaetoceros* and *Nannochloropsis* [13]. The *Spirulina platensis* alga catalyzed alkanone debromation with good yields [14]. There are excellent studies involving the reduction of various ketones by red algae yielding enantiomerically pure alcohols [4].

Recently, we reported the bioreduction of α-chloroacetophenone and the biotransformation of α-bromoacetophenones using marine fungi *Trichoderma* sp., *Penicillium mickyzinskii*, and *Bionectria* sp. [15][16]. These fungi catalyzed these biotransformations only when they were growing in artificial seawater.

Here, we present the use of marine algae *Bostrychia tenella* and *B. radicans*, and their associated microorganisms for the reduction of acetophenone derivatives. In addition, we isolated strains of marine bacteria of the *Bacillus* genus from *B. radicans* which catalyzed the enantioselective reduction of acetophenone derivatives with high enantiomeric excess (> 99% ee).

2. Experimental. – 2.1. *General.* All manipulations involving algae and marine bacteria were carried out under sterile conditions in a *Veco* laminar flow cabinet. *Technal TE-421* or *Superohm G-25* orbital shakers were employed in the biocatalytic transformation experiments. Reagents *ortho*-iodoacetophenone (**1**), *meta*-iodoacetophenone (**2**), *para*-iodoacetophenone (**3**), *ortho*-fluoroacetophenone (**4**), *ortho*-chloroacetophenone (**5**), *ortho*-bromoacetophenone (**6**) and *ortho*-nitroacetophenone (**7**), and $NaBH_4$ were purchased from *Sigma–Aldrich*. Products derived synthetically or from enzymatic processes were purified by column chromatography (CC) on silica gel (SiO_2; 230–400 mesh) eluted with hexane/AcOEt mixtures. The column eluent was monitored by TLC on pre-coated silica gel *60* F_{254} layers (aluminum-backed: *Sorbent*) eluted with hexane/AcOEt 9:1 and 8:2. Reaction products were analyzed using a *Shimadzu* model *2010* GC/FID equipped with an auto-injector *AOC20i* and *Varian CP-Chiralsil-DEX* β-*Cyclodextrin* column (25 m × 0.25 mm i.d.; 0.39 μm). The programs used for GC analyses of alcohols are described in *Table 1*. The injector and detector were maintained at 200°, the split ratio of the injector was 1:20, and the carrier gas was N_2 at 60 kPa. The ee values of alcohols were determined by GC analyses.

Table 1. *Programs Used for Identification of the Alcohols* **8**–**14** *by GC Analyses*[a])

Alcohols	T_i [°]	t_i [min]	T_f [°]	t_f [min]	r [°/min]	t_R [min]
8	115	2	175	8	3	(*R*) 18.0, (*S*) 20.0
9	120	2	190	8	2	(*R*) 21.0, (*S*) 22.5
10	120	2	165	8	2	(*R*) 21.0, (*S*) 22.0
11	100	2	180	5	2	(*R*) 21.0, (*S*) 22.0
12	60	2	180	5	2	(*R*) 23.0, (*S*) 25.0
13	90	2	140	6	2	(*R*) 24.0, (*S*) 26.0
14	100	2	150	6	2	(*R*) 26.0, (*S*) 27.0

[a]) Chiral column: *CP-Chiralsil-DEX* β-*Cyclodextrin* (25 m × 0.25 mm i.d.; 0.39 μm); T_i: initial temperature; T_f: final temperature; t_i: initial time; t_f: final time; r: rate; t_R: retention time.

2.2. *Synthesis of Racemic Alcohols* **8**–**14**. Racemic alcohols **8**–**14** were obtained by reduction of the corresponding ketones **1**–**7** with $NaBH_4$ and MeOH [17]. The spectroscopic data (IR, ^{1}H-NMR, and MS) of **8**–**14** were in agreement with those reported in the literature [18–22].

2.3. *Collection and Identification of Algae.* The algae *Bostrychia tenella* and *Bostrychia radicans* were collected in Ubatuba, in the South Atlantic Ocean off the northern coast of the State of São Paulo, Brazil, in September 2007. Algae were identified by using conventional taxonomic methods by *N. S. Yokoya* from the Instituto de Botanica de São Paulo, Brazil [23]. Algal material (2 kg) was stored in a freezer (−14°). Voucher specimens were deposited with the Herbarium of the Instituto de Botânica (São Paulo, SP, Brazil) under the accession Nos. SP 365678 (*B. radicans*) and SP 400217 (*B. tenella*).

2.4. *Isolation and Identification of Marine Bacteria from* B. tenella *and* B. radicans. Algae *B. tenella* and *B. radicans* stored in a freezer at −14° were transferred to a refrigerator at a temp. of 4°, and then

immersed in dist. H_2O. The algae were washed with artificial seawater (pH 8), immersed for 30 s in HClO (0.01%), and washed with dist. H_2O. The algal biomass (5.0 g) was transferred to 250-ml *Erlenmeyer* flasks, containing 100 ml of H_2O (pH 8), and incubated at 32° for 5 d on a rotary shaker (150 rpm). Then, the algal culture was inoculated in *Petri* dishes containing nutrient broth. Triplicate plates were incubated in an oven at 32° for 8 d. The microorganisms grown on the plates containing agar nutrient broth were isolated and identified by 16S rRNA gene-sequence analysis.

Genomic DNA was obtained according to the method of *Pospiech* and *Neumann* [24]. 16S rRNA gene was amplified by PCR (= polymerase chain reaction) using specific primers, 27f and 1401r for universal *Bacteria* Domain [25].

Fifty-µl reaction mixtures contained 100 ng of total DNA, 2 U of *Taq* polymerase (*Invitrogen*), 0.2 mM of deoxynucleoside triphosphates, and 0.4 µM of each primer. The PCR amplifications were performed using an initial denaturation step of 2 min at 95°, followed by 30 cycles of 1 min at 94°, 1 min at 55° and 3 min at 72°, and the extension of 3 min at 72°, in an *Eppendorf* thermal cycler.

The PCR product was purified on *GFX™ PCR DNA* and a *Gel Band Purification* kit (*GE HealthCare*) for automated sequencing in the *MegaBace DNA Analysis System 1000*. The sequencing was carried out using the 10f (5′GAG TTT GAT CCT GGC TCA G3′), 765f (5′ATT AGA TAC CCT GGT AG3′), 782r (5′ACC AGG GTA TCT AAT CCT GT3′), and 1100r (5′AGG GTT GGG GTG GTT G 3′) primers, and the *DYEnamic ET Dye Terminator Cycle Sequencing Kit* for the automated *MegaBace 500* system (*GE HealthCare*), according to the manufacturer's recommendations.

Partial 16S rRNA sequences obtained from isolates were assembled in a contig using the phred/Phrap/CONSED program [26][27]. Identification was achieved by comparing the contiguous 16S rRNA sequences obtained with 16S rRNA sequence data from reference and type strains available in the public databases GenBank and RDP (Ribosomal Database Project, Michigan State University, USA) using the BLASTn and Seqmatch, resp. The sequences were aligned using the CLUSTAL X program and analyzed with MEGA v.4 software [28][29]. Evolutionary distances were derived from sequence-pair dissimilarities calculated as implemented in MEGA, using *Kimura*'s DNA substitution model [30]. The phylogenetic reconstruction was achieved using the neighbor-joining (NJ) algorithm, with bootstrap values calculated from 1000 replicate runs, using the routines included in the MEGA software [29][31]. Phylogenetic analysis of partial 16S rRNA gene sequences of bacterial associated from *B. tenella* and *B. radicans* algae is shown in the *Figure*.

2.5. *Biocatalytic Reduction of Iodoacetophenones* **1**–**3** *using* B. tenella *and* B. radicans. The *B. tenella* and *B. radicans* algae stored in the freezer at −14° were transferred to a refrigerator at a temp. of 4°, and

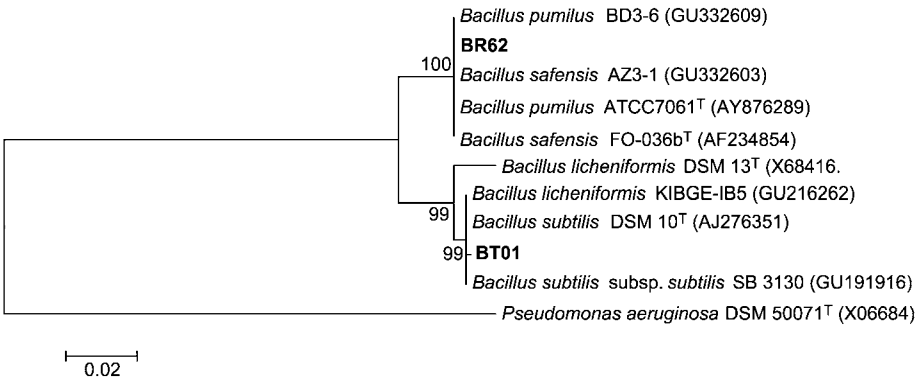


Figure. *Phylogenetic analysis of partial 16S rRNA gene sequences of bacteria associated with marine algae* B. tenella *and* B. radicans. Evolutionary distances were based on *Kimura* 2p model and tree reconstruction on the neighbor joining method. Bootstrap values (1000 replicate runs, shown as %) > 70% are listed. *Pseudomnas aeruginosa* PAO1 (NC_002516.2) was used as outgroup.

then immersed in dist. H_2O. The algae were washed with artificial seawater (pH 8), immersed for 30 s in HClO (0.01%), and washed with dist. H_2O. Algae (5.0 g) were transferred to 250-ml *Erlenmeyer* flasks containing dist. H_2O (100 ml), and the ketones **1**–**3** (50.0 mg; 0.20 mmol) dissolved in 300 µl of DMSO were added immediately. The *Erlenmeyer* flasks were shaken at 32° for 8 d on an orbital shaker (150 rpm). Afterwards, the algal biomass was harvested by filtration, and the aq. phases were extracted with AcOEt (3 × 30 ml). The org. phases were separated, dried (Na_2SO_4), and evaporated until dryness under vacuum. The reactions products were analyzed by GC-FID, and the results are compiled in *Table 2*.

2.6. *Biocatalytic Reduction of* ortho-*Acetophenone Derivatives with Bacteria Associated from* B. tenella *and* B. radicans. The bacteria associated from *B. tenella* and *B. radicans* algae were used for the reduction of *ortho*-acetophenones **4**–**7**. The strains were preserved in agar culture containing nutrient broth at 4°. In addition, the strains were grown in liquid culture medium and incubated in 250-ml *Erlenmeyer* flasks with 100 ml of nutrient broth at 32° for 48 h on an orbital shaker (150 rmp). Then, the *ortho*-acetophenones (50.0 mg) dissolved in 300 µl of DMSO were added immediately. The *Erlenmeyer* flasks were shaken at 32° for 4–8 d on an orbital shaker (150 rmp). The mixtures were extracted with AcOEt (3 × 30 ml), and org. phases were separated, dried (Na_2SO_4), and evaporated until dryness under vacuum. The results are collected in *Tables 3–5*.

2.7. *Assignment of the Absolute Configurations.* The optical rotations of compounds obtained from the biocatalytic reactions were determined in 1-dm cuvette using a *Perkin–Elmer* model *241* polarimeter and were referenced to the Na-D line. The abs. configurations of alcohols **8**–**14** were determined by comparing the specific signs of rotation measured for the products with those reported in the literature (*Table 6*).

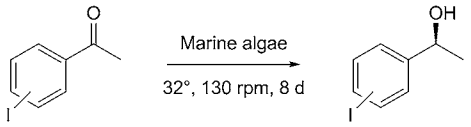
3. Results and Discussion. – Initially, the biocatalytic reductions of the iodoacetophenones **1**–**3** were carried out using red algae *Bostrychia tenella* and *Bostrychia radicans*. The algal biomass *B. tenella* and *B. radicans* stored in a freezer at −14° were transferred to a refrigerator at a temperature of 4°, and then immersed in distilled H_2O. The algae were washed with artificial seawater (pH 8), immersed for 30 s in HClO (0.01%), and washed with distilled H_2O. The algae were used for reduction of ketones **1**–**3**, and the results are summarized in *Table 2*.

One of the prime requirements of a biocatalytic reduction is the optimization of the reaction parameters, *i.e.*, amount of cell biomass, type of substrates, the effects of reaction temperature, and time. In this study, we used *ortho*-, *meta*- and *para*-iodoacetophenones **1**–**3**. With the optimizations of the experiments, the reactions were carried out for 8 d and then analyzed by GC. *B. tenella* and *B. radicans* algae yielded enantiomerically pure (iodophenyl)ethanols **8**–**10** with high enantiomeric excesses (> 99% ee). The absolute configurations of the alcohols **8**–**10** were in accordance with *Prelog*'s rule, *i.e.*, (*S*)-alcohols (*Table 2*).

In this study, we observed that the *ortho*-iodoacetophenone (**1**) afforded the best results for the bioreduction by *B. tenella* and *B. radicans* algae. Concentrations of (*S*)-alcohol **8** were obtained with 40–42% after 8 d of incubation (*Table 2*). However, the reduction of *meta*-iodoacetophenone (**2**) and *para*-iodoacetophenone (**3**) with the two algae produced lower amounts of the alcohols **9** and **10**, respectively. Ketone **1** was more reactive than compounds **2** and **3** under these conditions.

To test the capacity of the new biocatalyst, the biomass of *B. tenella* was used for the reduction of the ketones **4**–**7** with different groups in the *ortho*-position. The different groups strongly influenced the results (*Table 3*). The reduction of *ortho*-fluoroacetophenone **4** yielded alcohol **11** with high enantiomeric excesses (> 99% ee) and 33% concentration after 4 d of incubation (*Table 3*, *Entry 1*). Extending the reaction time to

Table 2. *Bioreduction of the Iodoacetophenones* **1**–**3** *by Marine Algae* Bostrychia tenella *and* B. radicans[a])



1 – 3 **8 – 10**

Entry	Algae	Ketones	*c* [%][b])		ee [%][c])[d])
			Ketones	Alcohols	Alcohols (abs. conf.)
1	*B. radicans*	2-I **1**	58	42[e])	99 (*S*)
2		3-I **2**	92	8	99 (*S*)
3		4-I **3**	83	17	99 (*S*)
4	*B. tenella*	2-I **1**	60	40	99 (*S*)
5		3-I **2**	95	5	99 (*S*)
6		4-I **3**	84	16	99 (*S*)

[a]) Reaction conditions: biomass (5.0 g) was transferred to 250-ml *Erlenmeyer* flasks containing distilled H_2O (100 ml) with the ketones **1**–**3** (50.0 mg, 0.20 mmol) dissolved in 300 µl of DMSO. *Erlenmeyer* flasks were shaken at 32° for 8 d on an orbital shaker (150 rmp). [b]) *c* [%]: Concentration determined by GC. [c]) ee [%]: Enantiomeric excess. [d]) Chiral column: *CP-Chiralsil-DEX β-Cyclodextrin* (25 m × 0.25 mm i.d.; 0.39 µm). [e]) Yield isolated: 25%.

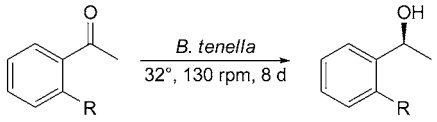
more than 8 d, (*S*)-fluoroalcohol **11** was obtained with 52% concentration (*Table 3*, *Entry 2*).

Surprisingly, the bulky groups in the *ortho*-position (Cl, Br, and NO_2) led to a decrease in the concentration of the alcohols **12**–**14** (*Table 3*), but high enantiomeric excesses were obtained. Longer incubation times in these experiments led to higher yields of alcohols **11**–**14** (*Table 3*). For example, the reduction of nitroacetophenone **7** did not occur after 4 d. The increase of the time to 8 d afforded alcohol **14** with 13% concentration (*Table 3*, *Entries 7* and *8*).

During these studies employing biomass algae, we promoted the isolation of associated microorganisms of the red algae, given that bacteria are opportunists and capable of growth in algal culture [32]. The mild sterilization of algae (artificial seawater and HClO) allowed the growth of the associated microorganisms. The *B. tenella* and *B. radicans* algal cultures were cultivated on an orbital shaker containing the distilled H_2O (see *Experimental*). In addition, the algal solutions were inoculated in *Petri* dishes containing agar medium. The bacteria associated grown in agar medium was transferred to liquid medium and the acetophenones dissolved in 300 µl of DMSO were added (see *Experimental*).

Bacteria associated with algae promoted bioreduction of iodoacetophenones **1**–**3** with high enantiomeric excess (> 99% ee) with different concentrations (*Table 4*). Strain BR62 from *B. radicans* catalyzed reductions of iodoacetophenones **1** and **3** with 46 and 48% concentrations, respectively, within 8 d (*Table 4*, *Entries 2* and *6*). Strain BT01 from *B. tenella* under the same conditions afforded the alcohols **8** and **9** in good

Table 3. *Bioreduction of the* ortho-*Acetophenones* **4**–**7** *by Marine Algae* Bostrychia tenella[a])



4 R = F
5 R = Cl
6 R = Br
7 R = NO_2

11 – **14**

Entry	Ketones	Time [d]	*c* [%][b])		ee [%][d])[e])
			Ketones	Alcohols (Yield [%])[c])	Alcohols (abs. conf.)
1	2-I **4**	4	67	33 (24)	99 (*S*)
2		8	48	52 (39)	95 (*S*)
3	2-Cl **5**	4	93	7	99 (*S*)
4		8	80	20	98 (*S*)
5	2-Br **6**	4	95	5	99 (S)
6		8	90	10	99 (*S*)
7	2-NO_2 **7**	4	100	0	0
8		8	87	13	99 (*S*)

[a]) Reaction conditions: biomass (5.0 g) was transferred to 250-ml *Erlenmeyer* flasks containing distilled H_2O (100 ml) with the ketones **4**–**7** (50.0 mg) dissolved in 300 µl of DMSO. *Erlenmeyer* flasks were shaken at 32° for 8 d on an orbital shaker (150 rmp). [b]) *c* [%]: Concentration determined by GC. [c]) Yield isolated. [d]) ee (%): Enantiomeric excess. [e]) Chiral column: *CP-Chiralsil-DEX β-Cyclodextrin* (25 m × 0.25 mm i.d.; 0.39 µm).

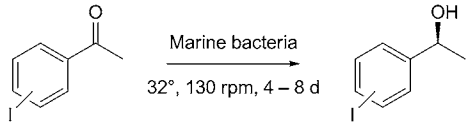
enantiomeric excess (> 99% ee), and low concentrations (*Table 4*, *Entries 8* and *12*). *meta*-Iodoacetophenone **2** was reduced in poor concentrations and high enantiomeric excess (*Table 4*).

Similarly, strain BR62 from *B. tenella* catalyzed the reduction of *ortho*-acetophenones **4**–**7** (*Table 5*). Strain BR62 was also efficient in providing alcohols **11**–**13** with high enantiomeric excesses and good concentrations (*Table 5*, *Entries 1–3*). In these investigations, only the *ortho*-nitroacetophenone was obtained in poor optical purity and concentration (*Table 5*, *Entry 4*).

The biocatalytic experiments demonstrated that marine algae *B. tenella*, *B. radicans*, and associated bacteria catalyzed the bioreduction of acetophenone derivatives with high optical purities. Strain BR62 associated from *B. radicans* was identified as *Bacillus* sp., supporting values of 100% on the boot strap, similar to strain types *B. pumilus* and *B. safensis*. The nucleotide alignment of strain BT01 supported bootstrap values of 99%, showing similarity to different *Bacillus* strains including the sequences of the strains *B. subtilis* and *B. licheniformis*. In addition, the strains were identified as *Bacillus* sp. through their morphology and by 16S rRNA gene sequence analysis (*Fig.*).

The bacterial cultures may be contaminants present in algal biomass [32]. The associated bacteria with marine algae may be opportunists, utilizing algal extracellular products. In the literature, the *Bacillus* strains were isolated from the brown marine alga *Sargassum* sp. This report represents the first article to deal with *Bacillus* sp.

Table 4. *Bioreduction of the Iodoacetophenones* **1**–**3** *by Bacteria Associated with Marine Algae* Bostrychia tenella *and* B. radicans[a])



1 – 3 **8 – 10**

Entry	Ketones	Time [d]	*c* [%][b])		ee [%][d])[e])
			Ketones	Alcohols (Yield [%])[c])	Alcohols (abs. conf.)
Bacillus sp. strain BR62 associated with the marine alga *B. radicans*					
1	2-I **1**	4	97	3	99 (*S*)
2		8	54	46 (30)	99 (*S*)
3	3-I **2**	4	98	2	99 (*S*)
4		8	83	17	99 (*S*)
5	4-I **3**	4	96	4	99 (*S*)
6		8	52	48 (34)	99 (*S*)
Bacillus sp. strain BT01 associated with the marine alga *B. tenella*					
7	2-I **1**	4	94	6	99 (*S*)
8		8	70	30 (23)	99 (*S*)
9	3-I **2**	4	92	8	99 (*S*)
10		8	87	13	99 (*S*)
11	4-I **3**	4	91	9	99 (*S*)
12		8	80	20	99 (*S*)

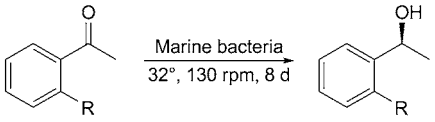
[a]) Reaction conditions: bacterial consortiums of the *Bacillus* sp., isolated from marine algae *B. radicans* and *B. tenella* were grown in *Erlenmeyer* flasks containing nutrient broth for 3 d, and then the ketones **1**–**3** (50.0 mg; 0.20 mmol) dissolved in 300 µl of DMSO were added. *Erlenmeyer* flasks were shaken at 32° for 8 d on an orbital shaker (150 rmp). [b]) *c* [%]: Concentration determined by GC. [c]) Yield isolated. [d]) ee [%]: Enantiomeric excess. [e]) Chiral column: *CP-Chiralsil-DEX β-Cyclodextrin* (25 m × 0.25 mm i.d.; 0.39 µm).

associated with interdital marine alga [33]. Recently, red algae species yielded marine bacteria of the *Bacillus* genus [34].

4. Conclusions. – In conclusion, the biocatalytic reduction of acetophenone derivatives was accomplished using *B. tenella* and *B. radicans* algal biomass yielding enantiomerically pure alcohols (>99% ee). In addition, we isolated bacteria strains associated with red marine algae *B. tenella* and *B. radicans*. Bacteria were identified as *Bacillus* sp., and they catalyzed the bioreduction of the acetophenone derivatives with high enantiomeric excess (>99% ee). The use of the new marine organisms is important for the discovery of new enzymes for biotechnological applications.

The authors wish to thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq; grant No. 472663/2004-6 to *A. L. M. P.*) and the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP; grant No. 2006/54401-2 to *A. L. M. P.*) for their financial support. Thanks are also

Table 5. *Bioreduction of the* ortho-*Acetophenones* **4**–**7** *by* Bacillus *sp. strain BR62 Isolated from Marine Algae* B. radicans[a])



4 R = F
5 R = Cl
6 R = Br
7 R = NO_2

11 – 14

Entry	Ketones	*c* [%][b]) Ketones	Alcohols (Yield [%])[c])	ee [%][d])[e]) Alcohols (abs. conf.)
1	2-F **4**	27	73 (59)	99 (*S*)
2	2-Cl **5**	51	49 (35)	99 (*S*)
3	2-Br **6**	5	95 (77)	99 (*S*)
4	2-NO_2 **7**	93	7	10 (*S*)

[a]) Reaction conditions: bacteria isolated from marine alga *B. radicans* was grown in *Erlenmeyer* flasks containing nutrient broth for 3 d, and then the ketones **4**–**7** (50.0 mg) dissolved in 300 µl of DMSO were added. *Erlenmeyer* flasks were shaken at 32° for 8 d on an orbital shaker (150 rmp). [b]) *c* [%]: Concentration determined by GC. [c]) Yield isolated. [d]) ee [%]: Enantiomeric excess. [e]) Chiral column: *CP-Chiralsil-DEX β-Cyclodextrin* (25 m × 0.25 mm i.d.; 0.39 µm).

Table 6. *Optical Rotations of the Alcohols* **8**–**14**

Alcohols	$[\alpha]_D^{25}$ (exper.)	$[\alpha]_D^{25}$ (literature)
1-(2-Iodophenyl)ethanol (**8**)	(*S*)-**8** −17.6 (*c* = 1.7, $CHCl_3$)	(*S*)-**8** −41.3 (*c* = 0.20, $CHCl_3$) [17]
1-(3-Iodophenyl)ethanol (**9**)	[a])	(*S*)-**9** −12.9 (*c* = 2.4, $CHCl_3$) [18]
1-(4-Iodophenyl)ethanol (**10**)	[a])	(*S*)-**10** −32.7 (*c* = 5.99, $CHCl_3$) [18]
1-(2-Fluorophenyl)ethanol (**11**)	(*S*)-**11** −45.5 (*c* = 2.0, $CHCl_3$)	(*S*)-**11** −34.7 (*c* = 3.63, CH_2Cl_2) [19]
1-(2-Chlorophenyl)ethanol (**12**)	[a])	(*S*)-**12** −32.9 (*c* = 0.22, $CHCl_3$) [17]
1-(2-Bromophenyl)ethanol (**13**)	(*R*)-**13** +26 (*c* = 2.5, $CHCl_3$)	(*S*)-**13** −54.5 (*c* = 0.20, $CHCl_3$) [17]
1-(2-Nitrophenyl)ethanol (**14**)	(*S*)-**14** +17 (*c* = 1.8, $CHCl_3$)	(*S*)-**14** +30.6 (*c* = 3.6, $CHCl_3$) [20]

[a]) Absolute configurations of **9**, **10**, and **12** were attributed by retention times on GC-FID chiral (see *Table 1*) [19].

due to *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) and *CNPq* for scholarships awarded to *M. P.M.* and *A. M.M.*, respectively.

REFERENCES

[1] J.-T. Kim, S. G. Kang, J.-H. Woo, J.-H. Lee, B. C. Jeong, S.-J. Kim, *Appl. Microbiol. Biotechnol.* **2007**, *74*, 820.
[2] J. Kennedy, J. R. Marchesi, A. D. W. Dobson, *Microb. Cell Factories* **2008**, *7*, 27.
[3] K. Nakamura, R. Yamanaka, T. Matsuda, T. Harada, *Tetrahedron: Asymmetry* **2003**, *14*, 2659.
[4] T. Utsukihara, O. Misumi, N. Kato, T. Kuroiwa, C. A. Horiuchi, *Tetrahedron: Asymmetry* **2006**, *17*, 1179.

[5] H. Arabi, M. T. Yazdi, M. A. Faramarzi, *J. Mol. Catal. B: Enzym.* **2010**, *62*, 213.
[6] F. Yoshizako, A. Nishimura, M. Chubachi, *J. Ferment. Bioeng.* **1994**, *77*, 144.
[7] F. Yoshizako, M. Ogino, A. Nishimura, M. Chubachi, T. Horii, *J. Ferment. Bioeng.* **1995**, *79*, 141.
[8] F. Yoshizako, A. Nishimura, M. Chubachi, M. Kirihata, *J. Ferment. Bioeng.* **1996**, *82*, 601.
[9] F. Yoshizako, T. Kuramoto, A. Nishimura, M. Chubachi, *J. Ferment. Bioeng.* **1998**, *85*, 439.
[10] I. L. Hook, S. Ryan, H. Sheridan, *Phytochemistry* **1999**, *51*, 621.
[11] I. L. Hook, S. Ryan, H. Sheridan, *Phytochemistry* **2003**, *63*, 31.
[12] Y. Noma, Y. Asakawa, 'Medical and Aromatic Plants VII', Vol. 28 of 'Biotechnol. Agric. Forestry', Springer-Verlag, Berlin, 1994, p. 185.
[13] K. Ishihara, N. Nakajima, H. Yamaguchi, H. Hamada, Y.-S. Uchimura, *J. Mol. Catal. B: Enzym.* **2001**, *15*, 101.
[14] T. Utsukihara, S. Okada, N. Kato, C. A. Horiuchi, *J. Mol. Catal. B: Enzym.* **2007**, *45*, 68.
[15] L. C. Rocha, H. V. Ferreira, E. F. Pimenta, R. G. S. Berlinck, M. H. R. Seleghim, D. C. D. Javaroti, L. D. Sette, R. C. Bonugli, A. L. M. Porto, *Biotechnol. Lett.* **2009**, *31*, 1559.
[16] L. C. Rocha, H. V. Ferreira, E. F. Pimenta, R. G. S. Berlinck, M. O. O. Rezende, M. D. Landgraf, M. H. R. Seleghim, L. D. Sette, A. L. M. Porto, *Marine Biotechnol.* **2010**, *5*, 552.
[17] D. L. Pavia, G. M. Lampman, G. S. Kriz, R. G. Engel, 'Introduction to organic laboratory techniques', 3rd edn., Sunders College Publishing, Orlando, 1999.
[18] Y. A. Xu, G. F. Docherty, G. Woodward, M. Wills, *Tetrahedron: Asymmetry* **2006**, *17*, 2925.
[19] L. C. Rocha, I. G. Rosset, R. F. Luiz, C. Raminelli, A. L. M. Porto, *Tetrahedron: Asymmetry* **2010**, *21*, 926.
[20] J. V. Comasseto, A. T. Omori, L. H. Andrade, A. L. M. Porto, *Tetrahedron: Asymmetry* **2003**, *14*, 711.
[21] J. V. Comasseto, L. F. Assis, L. H. Andrade, I. H. Schoenlein-Crusius, A. L. M. Porto, *J. Mol. Catal. B: Enzym.* **2006**, *39*, 24.
[22] F. Hammerschmidt, B. P. Simov, S. Schmidt, S. Schneider, I. Zolle, *Monatsh. Chem.* **2005**, *136*, 229.
[23] http://www.ibot.sp.gov.br/.
[24] A. Pospiech, B. Neumann, *Trends Genetics* **1995**, *11*, 217.
[25] D. J. Lane, '16S/23S rRNA sequencing', in: 'Nucleic acid techniques in bacterial systematics', Eds. E. Stackebrandt, M. Goodfellow, Academic, Chichester, UK, 1991, pp. 115–175.
[26] B. Ewing, L. Hillier, M. Wendl, P. Green, *Genome Res.* **1998**, *8*, 175.
[27] J. J. Godon, E. Zumstein, P. Dabert, F. Habouzit, R. Moletta, *Appl. Environ. Microbiol.* **1997**, *63*, 2802.
[28] J. D. Thompson, D. G. Higgins, T. J. Gibson, *Nucleic Acids Res.* **1994**, *22*, 4673.
[29] K. Tamura, J. Dudley, M. Nei, S. Kumar, *Mol. Biol. Evol.* **2007**, *24*, 1596.
[30] M. Kimura, *J. Mol. Evol.* **1980**, *16*, 111.
[31] N. Saitou, M. Nei, *Mol. Biol. Evol.* **1987**, *4*, 406.
[32] W. H. Bell, J. M. Lang, R. Mitchell, *Limnol. Oceanogr.* **1974**, *19*, 833.
[33] B. R. Mohapatra, R. K. Sad, U. C. Banerjee, *Lett. Appl. Microbiol.* **1995**, *21*, 380.
[34] M. Kanagasabhapathy, H. Sasaki, S. Negata, *Word J. Microbiol. Biotechnol.* **2008**, *24*, 2315.

Received November 28, 2010