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Bioreduction of prochiral ketones by growing cells of *Lasiodiplodia theobromae*: Discovery of a versatile biocatalyst for asymmetric synthesis

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ABSTRACT

Growing cells of the phytopathogen fungus *Lasiodiplodia theobromae* in potato dextrose broth have shown their potential for the stereoselective bioreduction of different prochiral aromatic and aliphatic ketones. Optically active alcohols were obtained under mild reaction conditions in high conversions (up to 90%) and moderate to excellent enantioselectivity (35–≥99% *ee*) depending on the ketone structure. Prelog alcohols were isolated, except in the bioreduction of cyclohexylmethylketone and octan-2-one where anti-Prelog alcohols were obtained.

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1. Introduction

In the last years, biocatalysis has been established as a very efficient and environmentally friendly methodology for the production of a varied set of enantiopure compounds with application to industrial sectors [1–7]. Among the vast number of enzymes, lipases, lyases, oxidoreductases and transferases have demonstrated their outstanding potential in the production of optically active alcohols under generally benign reaction conditions, catalyzing clean bioprocesses with remarkable levels of chemo-, regio- and stereospecificity [8].

Chiral alcohols and their derivatives are of special interest in organic chemistry due to their presence in a variety of biologically active compounds such as agrochemicals and pharmaceuticals. Besides, enantiomerically pure alcohols are essential and versatile chiral building-blocks for the preparation of more complex structures. Probably, the bioreduction of prochiral ketones is the most common biotransformation for the production of a wide range of optically active alcohols in high enantiomeric excesses and under very mild reaction conditions [9]. For that aim, whole cells systems or isolated enzymes are used as ideal catalysts. Unfortunately, for isolated dehydrogenases, the addition of an external coenzyme and the corresponding regeneration system are generally required for the correct working of the enzyme. In contrast, the use of whole cells seems adequate for carrying out this type of processes because the presence of cofactors is not required.

Microorganisms and vegetables have been established as efficient biocatalyst for the introduction of chirality in prochiral compounds. The use of whole cells systems has led to the preparation of the corresponding chiral alcohols in high yields and selectivities, being achieved in most cases the compounds of (*S*)-configuration [10–15], the so-called Prelog product.

In our ongoing project focused on the identification, analysis and application of novel biocatalysts from the Brazilian biodiversity, vegetables and microorganisms are being investigated in reactions such as the bioreduction of carbonyl compounds and the kinetic resolution of racemic alcohols and esters through acetylation or hydrolysis procedures, respectively [16–19]. Recently, we have focused our attention in *Lasiodiplodia theobromae* (Deuteromycete), a phytopathogen fungus responsible for the infection of several cultures in tropical areas and associated to more than 500 species of plants. In Brazil, this phytopathogen is considered a serious problem in agriculture sector [20]. The literature reports the use of growing cells of *L. theobromae* in the biotransformation of natural products, such as steroids [21], terpenes [22,23], ionones [22], and a sesquiterpene lactone [24]. The kinetic resolution of a racemic epoxide by this microorganism has also been reported [25]. In all the reactions tested, this microorganism has shown remarkable activities in the catalysis of redox processes. Herein, we describe the

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use of *L. theobromae* as a novel source of a stereoselective enzyme for the reduction of prochiral aromatic and aliphatic ketones to the corresponding enantiomerically enriched alcohols.

2. Experimental

2.1. Materials and methods

L. theobromae (strain #009) was obtained from EMBRAPA Agroindústria Tropical (Fortaleza, CE, Brazil) where the microorganism was isolated in the Laboratory of Phytopathology from infected guava. Flash chromatography columns were performed using silica gel 60 (230–240 mesh). High performance liquid chromatography (HPLC) analyses were carried out in a Hewlett Packard 1100 chromatograph, UV detector using a Tracer Spherisorb (25 cm × 4.6 mm), Daicel Chiralcel OB-H (25 cm × 4.6 mm) or Chiralpak AS (25 cm × 4.6 mm) columns, varying the conditions according to the specific substrate. Gas chromatography (GC/FID) analyses were performed on a Hewlett Packard 6890 Series II chromatograph equipped with a Hewlett Packard HP-1 (crosslinked methyl siloxane, 30 m × 0.25 mm × 0.25 μm), a Varian CP-Chiralsil DEX CB or a Restek RT-βDEXse (30 m × 0.25 mm × 0.25 μm). In all the experiments, the injector temperature was 225 °C and the detector temperature was heated at 250 °C. ^{1}H, ^{13}C NMR and DEPT experiments were obtained using a DPX-300 (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz). The chemical shifts are given in delta (δ) values and the coupling constants (J) in Hertz (Hz). Measurements of the optical rotation values were done in a Perkin-Elmer 241 polarimeter.

Starting ketones **1**–**9a** and alcohols (±)-**1b** and (±)-**7**–**9b** were purchased from Sigma–Aldrich or Fluka. Dry solvents were distilled over an appropriate desiccant under nitrogen. All other reagents and solvents were of the highest quality grade available, and purchased from Sigma–Aldrich, Fluka or Acros Organics.

2.2. Typical procedure for the preparation of racemic alcohols 2–6b

To a solution of the corresponding ketone **2**–**6a** (200 mg) in dry MeOH (4.0 mL) was slowly added sodium borohydride (4 equiv.) at 0 °C under nitrogen atmosphere. The reaction was stirred at room temperature during 3 h and its progress monitored by TLC analysis (20% EtOAc/hexane) until complete disappearance of the starting ketone. Solvent was evaporated under reduced pressure and the resulting suspension was redissolved in H_2O and extracted with EtOAc (3 × 10 mL). Organic phases were combined and dried over anhydride Na_2SO_4. After solvent distillation under reduced pressure, the resulting crude product was purified by flash chromatography [10% EtOAc/hexane for **2b** (74% yield), **4b** (74% yield), and 20% EtOAc/hexane for **3b** (97% yield), **5b** (95% yield) and **6b** (95% yield)] [26].

2.3. General method for the biocatalyzed reduction of ketones 1–9a using L. theobromae

Erlenmeyers containing 50 mL of commercial potato dextrose (PD) culture medium were previously autoclaved at 121 °C for 15 min and inoculated under aseptic condition with two 7 mm disk of the microorganism (7 days old in PDA). After 7 days in static conditions at 28 °C, 10 mg of the corresponding ketone **1**–**9a** was added to the Erlenmeyer and shaken (200 rpm) at 28 °C. Aliquots of 1 mL were collected after 1, 3 and 6 days reaction from each flask and extracted with ethyl acetate (3 × 2 mL). The organic solvent was evaporated under reduced pressure. The crude products were analyzed by HPLC or GC in order to determine the conversion and the optical purity of alcohols **1**–**9b**. Experiments were performed in triplicate.

*(S)-1-Phenylethanol (**1b**).* Determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (**1a**) 5.8 min, t_R (**1b**) 8.3 min; determination of the *ee* by HPLC: Chiralcel OB-H, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 10.2 min, t_R (*R*) 15.4 min. $[\alpha]_D^{25} = -22.5$ ($c = 1.0$, CH_2Cl_2) for the (*S*)-enantiomer in >99% *ee*. Absolute configuration was assigned after comparing their retention times with the ones from commercially available enantiopure compounds.

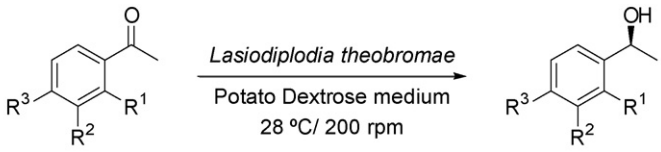
*(S)-1-(2-Methoxyphenyl)ethanol (**2b**).* ^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.52 (3H, d, $J = 6.6$ Hz, H_2), 3.88 (3H, s, OCH_3), 5.11 (1H, q, $J = 6.6$ Hz, H_1), 6.90 (1H, dd, $J = 7.5$ and 1.5 Hz, $H_{3'}$), 6.98 (1H, dt, $J = 8.5$ and 1.5 Hz, $H_{5'}$), 7.29 (1H, dt, $J = 8.5$ and 1.5 Hz, $H_{6'}$), 7.36 (1H, dd, $J = 7.5$ and 1.5 Hz, $H_{4'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 22.8 (C_2), 55.2 (OCH_3), 66.3 (C_1), 110.3 ($C_{3'}$), 120.7 ($C_{5'}$), 126.0 ($C_{6'}$), 128.2 ($C_{4'}$), 133.4 ($C_{1'}$), 156.4 ($C_{2'}$); determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (**2a**) 6.3 min, t_R (**2b**) 7.1 min; determination of the *ee* by HPLC: Chiralcel OB-H, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 10.3 min, t_R (*R*) 16.9 min. $[\alpha]_D^{25} = -17.3$ ($c = 0.75$, $CHCl_3$) for the (*S*)-enantiomer in >99% *ee*. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,27].

*(S)-1-(2-Nitrophenyl)ethanol (**3b**).* ^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.55 (3H, d, $J = 6.3$ Hz, H_2), 5.40 (1H, q, $J = 6.3$ Hz, H_1), 7.41 (1H, dt, $J = 8.1$ and 1.2 Hz, $H_{4'}$), 7.63 (1H, dt, $J = 8.1$ and 1.5 Hz, $H_{5'}$), 7.82 (1H, dd, $J = 8.1$ and 1.2 Hz, $H_{6'}$), 7.88 (1H, dd, $J = 8.1$ and 1.5 Hz, $H_{3'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 24.2 (C_2), 65.2 (C_1), 124.2 ($C_{3'}$), 127.5 ($C_{6'}$), 128.0 ($C_{4'}$), 133.5 ($C_{5'}$), 140.9 ($C_{1'} + C_{2'}$); determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (**3a**) 6.3 min, t_R (**3b**) 8.9 min; determination of the *ee* by HPLC: Chiralpak AS, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (*S*) 24.0 min, t_R (*R*) 22.1 min. $[\alpha]_D^{25} = +18.1$ ($c = 0.23$, MeOH) for the (*S*)-enantiomer in 92% *ee*. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,28].

*(S)-1-(3-Methoxyphenyl)ethanol (**4b**).* ^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.47 (3H, d, $J = 6.6$ Hz, H_2), 3.81 (3H, s, OCH_3), 4.83 (1H, q, $J = 6.6$ Hz, H_1), 6.81 (1H, dd, $J = 8.5$ and 1.2 Hz, $H_{6'}$), 6.93 (2H, m, $H_{2'}$ and $H_{4'}$), 7.26 (1H, t, $J = 8.5$ Hz, $H_{5'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.0 (C_2), 55.1 (OCH_3), 70.0 (C_1), 110.8 ($C_{2'}$), 112.7 ($C_{4'}$), 117.6 ($C_{6'}$), 129.3 ($C_{5'}$), 147.6 ($C_{1'}$), 159.6 ($C_{3'}$); determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (**4a**) 5.8 min, t_R (**4b**) 8.4 min; determination of the *ee* by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 13.7 min, t_R (*R*) 18.8 min. $[\alpha]_D^{25} = -25.1$ ($c = 0.85$, MeOH) for the (*S*)-enantiomer in 87% *ee*. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,29].

*(S)-1-(3-Nitrophenyl)ethanol (**5b**).* ^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.47 (3H, d, $J = 6.6$ Hz, H_2), 4.96 (1H, q, $J = 6.6$ Hz, H_1), 7.47 (1H, t, $J = 7.8$ Hz, $H_{5'}$), 7.67 (1H, d, $J = 7.8$ Hz, $H_{6'}$), 8.04 (1H, ddd, $J = 8.3$, 2.1 and 0.9 Hz, $H_{4'}$), 8.18 (1H, t, $J = 1.8$ Hz, $H_{2'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.2 (C_2), 69.1 (C_1), 120.2 ($C_{4'}$), 122.1 ($C_{2'}$), 129.3 ($C_{5'}$), 131.6 ($C_{6'}$), 147.8 ($C_{1'}$), 148.1 ($C_{3'}$); determination of the conversion by HPLC: Spherisorb, 0.8 mL/min; 5% 2-propanol/hexane; 20 °C; t_R (**5a**) 9.2 min, t_R (**5b**) 12.0 min; determination of the *ee* by HPLC: Chiralcel OB-H, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (*S*) 13.2 min, t_R (*R*) 14.8 min. $[\alpha]_D^{25} = -14.5$ ($c = 1.0$, $CHCl_3$) for the (*S*)-enantiomer in 45% *ee*. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,30].

*(S)-1-(4-Nitrophenyl)ethanol (**6b**).* ^{1}H NMR ($CDCl_3$, 300.13 MHz): 1.50 (3H, d, $J = 6.6$ Hz, H_2), 5.01 (1H, q, $J = 6.6$ Hz, H_1), 7.53 (2H, d, $J = 8.7$ Hz, $H_{2'}$ and $H_{6'}$), 8.17 (2H, d, $J = 8.7$ Hz, $H_{3'}$ and $H_{5'}$); ^{13}C NMR ($CDCl_3$, 75.5 MHz): 25.3 (C_2), 69.5 (C_1), 123.6 ($C_{2'}$ and $C_{6'}$), 126.0 ($C_{3'}$ and $C_{5'}$), 147.0 ($C_{1'}$), 153.1 ($C_{4'}$); determination of the conversion



1-6a (*S*)-**1-6b**

1 R^1=H; R^2=H; R^3=H
2 R^1=OMe; R^2=H; R^3=H
3 R^1=NO_2; R^2=H; R^3=H
4 R^1=H; R^2=OMe; R^3=H
5 R^1=H; R^2=NO_2; R^3=H
6 R^1=H; R^2=H; R^3=NO_2

Scheme 1. Bioreduction of prochiral aromatic ketones **1**–**6a** by *Lasiodiplodia theobromae*.

by HPLC: Spherisorb, 0.8 mL/min; 10% 2-propanol/hexane; 20 °C; t_R (**6a**) 6.5 min, t_R (**6b**) 7.7 min; determination of the *ee* by HPLC: Chiralpak AS, 0.8 mL/min; 20% 2-propanol/hexane; 20 °C; t_R (*S*) 13.7 min, t_R (*R*) 15.8 min. $[\alpha]_D^{25} = -10.5$ (c = 1.2, $CHCl_3$) for the (*S*)-enantiomer in 42% *ee*. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,31].

*(R)-1-Cyclohexylethanol (**7b**).* Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 150 °C; t_R (**7a**) 3.7 min, t_R (**7b**) 4.2 min. Determination of the *ee* by GC: Chiralsil DEX CB, 90 °C (5 min), 2.5 °C/min, 105 °C, 5 °C/min, 120 °C, 20 °C/min, 180 °C; t_R (*S*) 13.0 min, t_R (*R*) 13.7 min. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,32].

*(R)-Octan-2-ol (**8b**).* Determination of the conversion by GC analysis: HP-1, 70 °C (4 min) 20 °C/min, 150 °C; t_R (**8a**) 3.0 min, t_R (**8b**) 3.3 min. Determination of the *ee* by GC: RTβDEXse, 90 °C (5 min), 2.5 °C/min, 105 °C, 5 °C/min, 130 °C (2 min), 20 °C/min, 180 °C; t_R (*S*) 14.7 min, t_R (*R*) 16.4 min. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,33].

*(R)-Methyl 3-hydroxy-4-chloroacetoacetate (**9b**).* Determination of the conversion by GC analysis: HP-1, 70 °C (4 min), 20 °C/min, 200 °C; t_R (**9a**) 3.6 min, t_R (**9b**) 4.2 min. Determination of the *ee* by GC: RTβDEXse, 90 °C (5 min), 2.5 °C/min, 105 °C, 5 °C/min, 130 °C (2 min), 20 °C/min, 180 °C; t_R (*S*) 20.3 min, t_R (*R*) 20.6 min. Absolute configuration was assigned by comparing the retention times with the one previously reported in the literature [16,34].

3. Results and discussion

In order to explore the substrate specificity and selectivity of this new enzymatic system, growing cells of *L. theobromae* were employed in the bioreduction of both aromatic and aliphatic ketones, and the results have been compared with the ones previously obtained in our research groups using the basidiomycete *Lentinus strigellus* as biocatalyst [16]. Initially, the bioreductions of acetophenone (**1a**) and five different derivatives substituted in the aromatic ring (**2**–**6a**) were analyzed employing a PD medium (Scheme 1). Aliquots were regularly taken and analyzed after 1, 3 and 6 days, being the most representative results summarized in Table 1. All the aromatic ketones were stereoselectively reduced by *L. theobromae*, and the hydride attack occurred in the *Re* face of the corresponding aromatic ketones **1**–**6a**, showing a Prelog selectivity as occurs with *L. strigellus*.

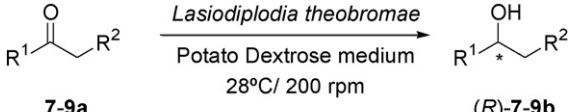
Acetophenone (**1a**) was stereoselectively reduced to almost enantiopure (*S*)-1-phenylethanol (**1b**) achieving a complete conversion after 1 day of reaction (entry 1). Higher reaction times (entry 2) increased the *ee* of (*S*)-**1b**, being possible to obtain this alcohol in enantiopure form after 3 days. These excellent results encouraged us to study the biocatalyzed reduction of different aromatic ketones, selecting monosubstituted ketones such as 2- or 3-methoxyacetophenone (**2a** and **4a**) and 2-, 3- or 4-nitro acetophenone (**3a**, **5a** and **6a**) as candidates. For all the substrates very high activities were found, reaching conversions over 90%. Respecting the growing cells selectivity, very different enantiomeric excess values were reached in the preparation of the alcohols depending on the substrate structure (*ee* values from 35% until 99%). *Ortho*-substituted ketones **2a** and **3a** were completely reduced to the (*S*)-alcohols in high enantiomeric excesses (entries 3–6). A loss in the enantiomeric excess of (*S*)-2-methoxy-1-phenylethanol (**2b**) was observed at longer reaction times, maybe caused by the existence of concurrent oxidation-reduction processes (>99–93% *ee*, entries 3 and 4).

Table 1
Lasiodiplodia theobromae biocatalyzed reduction of aromatic ketones **1**–**6a** in potato dextrose medium at 28 °C and 200 rpm[a].

Entry	Ketone	Time (days)	c (%)[b]	ee_P (%)[b]	Config. alcohol
1	**1a**	1	≥99	97	*S*
2	**1a**	3	≥99	≥99	*S*
3	**2a**	1	≥99	≥99	*S*
4	**2a**	3	≥99	93	*S*
5	**3a**	3	≥99	92	*S*
6	**3a**	6	≥99	92	*S*
7	**4a**	1	91	87	*S*
8	**4a**	3	94	84	*S*
9	**5a**	1	≥99	45	*S*
10	**5a**	3	≥99	43	*S*
11	**5a**	6	≥99	38	*S*
12	**6a**	1	≥99	42	*S*
13	**6a**	3	≥99	35	*S*

[a] For more detailed reaction conditions see Section 2.
[b] Conversion (c) and enantiomeric excesses (ee) were determined by HPLC.

The presence of an electrondonating group (OMe) in the *meta* position of the aromatic ring (**4a**) led to higher enantiomeric excesses (up to 80% *ee*, entries 7 and 8) in comparison with the existence of an electron-withdrawing functionality (NO_2) in the same position. Thus, for ketone **5a** moderate enantiomeric excesses values were attained (38–45% *ee*, entries 9–11), being observed for both substrates a slight decrease of the stereopreference at longer reaction times. Finally, the bioreduction of *para*-nitro acetophenone allowed the recovery of (*S*)-**6b** with similar optical purity as obtained with the *meta* derivative (entries 12 and 13). The



7-9a (*R*)-**7-9b**

7 R^1=Cy; R^2=H
8 R^1=$CH_2(CH_2)_4CH_3$; R^2=H
9 R^1=CH_2COOCH_3; R^2=Cl

Scheme 2. Bioreduction of prochiral aliphatic ketones **7a** and **8a**, and ketoester **9a** by *Lasiodiplodia theobromae*.

Table 2
Lasiodiplodia theobromae biocatalyzed reduction of compounds **7**–**9a** in potato dextrose medium at 28 °C and 200 rpm[a].

Entry	Compound	Time (days)	c (%)[b]	ee_P (%)[b]	Config. alcohol
1	**7a**	1	96	35	*R*
2	**7a**	3	99	37	*R*
3	**7a**	6	98	40	*R*
4	**8a**	1	90	60	*R*
5	**8a**	3	97	72	*R*
6	**8a**	6	96	78	*R*
7	**9a**	1	≥99	40	*R*
8	**9a**	3	96	40	*R*
9	**9a**	6	97	40	*R*

[a] For more detailed reaction conditions see Section 2.
[b] Conversion (c) and enantiomeric excesses (ee) were determined by GC.

reduction and oxidation of the ketones were the unique activities observed during the processes, without observing any reactivity of the growing cells towards the nitro functionality for compounds **3a**, **5a** and **6a**.

Comparing these results with the ones obtained in the investigation using *L. strigellus* as biocatalyst [16] *L. theobromae* catalyzes the bioreduction of the same aromatic ketones with higher reaction rates (91–99% conversion) in the same reaction times. However, the best enantioselectivities were obtained when using *L. strigellus*. In this case, the chiral alcohols **1**–**6b** were produced in optical purities higher than 90% while the optical activities achieved with *L. theobromae* stayed in the interval of 42–99% *ee*.

At this point, we decided to study the bioreduction of aliphatic ketones, considering different structural motifs such as cyclohexyl methyl ketone (**7a**) and 2-octanone (**8a**), and the biocatalyzed reduction of a β-ketoester such as methyl 4-chloroacetoacetate (**9a**) in order to also study the chemoselectivity properties of our enzyme (Scheme 2). Surprisingly, anti-Prelog products (*R*-alcohols) were recovered from the bioreductions of **7a** and **8a**, while the expected Prelog alcohol (*R*)-**9b** was observed when ketone **9a** was reduced in presence of this enzymatic system.

Table 2 summarized the results obtained for these three compounds. As occurred in the reduction of aromatic ketones, conversion values were in all cases very high (90–99%), however in these reactions (*R*)-alcohols were achieved, all of them in low to moderate enantiomeric excesses. Cyclic ketone **7a** was almost completely reduced after 1 day yielding (*R*)-1-cyclohexylethanol (**7b**) in nearly 40% *ee* (entries 1–3). 2-Octanone (**8a**) led to (*R*)-**8b** in moderate enantiomeric excess (60–78% *ee*, entries 4–6), with an increase in the alcohol optical purity at longer reaction times. Finally the β-ketoester **9a** was analyzed in the biocatalyzed process from a chemo- and stereoselective point of view. The reaction was completely chemoselective towards the bioreduction of the ketone, maintaining the ester functionality unchanged, and yielding the hydroxyester (*R*)-**9b** in 40% *ee* and excellent conversion (96–99%, entries 7–9). In these processes, the reaction rates showed by *L. strigellus* [16] and *L. theobromae* were very similar, with both biocatalysts leading to the same conversion values after 1 or 3 days of reaction. In the other hand, while *L. theobromae* produced alcohols **7**–**8b** with anti-Prelog selectivity, *L. strigellus* showed Prelog selectivity in the reduction of compounds **7**–**8a**. In the case of the later biocatalyst, the chiral alcohols were produced with much higher enantiomeric excesses (91–99% *ee*) than those produced by *L. theobromae* (37–72% *ee*).

4. Conclusions

In summary, we have presented *L. theobromae* as a promising biocatalyst for the production of optically active alcohols. Thus, the bioreduction of a set of aromatic and aliphatic ketones has been illustrated finding an excellent activity after 1 day of reaction. Growing cells of *L. theobromae* have nicely worked in aqueous medium under very mild experimental conditions allowing the production of (*S*)-aromatic and (*R*)-aliphatic alcohols in moderate to excellent enantiomeric excesses. Further investigations are currently ongoing, trying to expand the specificity and exploring new catalytic activities of this biocatalytic agent.

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