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Stereoselective biotransformation of α-alkyl-β-keto esters by endophytic bacteria and yeast

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ABSTRACT

Several strains of endophytic bacteria and yeast were isolated from *Daucus carota*, *Erythrina crista-galli*, *Curcubita maxima* and *Fortunella margarita*, and tested for the reduction of two model α-alkyl-β-ketoesters. The yeast strains resulted better biocatalysts for the reduction of the tested substrates with the exception of *Enterobacter agglomerans* C8 strain, isolated from *Erythrina crista-galli*, that provided the best results for the preparation of *syn* (2*R*,3*S*) α-alkyl β-hydroxyesters. Among the isolated yeast strains, the *Pichia* sp. strain isolated from *D. carota*, was the best biocatalyst for production of *anti* (2*S*,3*S*) α-alkyl β-hydroxyesters. Our results show that the microbial endophytic community provides an interesting niche on the search for novel biocatalysts.

1. Introduction

The increasing demand for environmentally benign industrial processes, as well as the rising need for stereoselective synthetic routes, has turned the attention of many organic chemists to biocatalysis. Enzymes are highly regio- and stereoselective biodegradable catalysts, thus providing an environmentally friendly alternative to classical synthetic methodologies [1,2]. Several microorganisms isolated from very different sources have provided useful biocatalysts for a manifold of reactions [3,4]. With the increased interest in biocatalysis, the search for novel enzymes and microorganisms suitable for particular biotransformations has received a great deal of attention. Recent estimates indicate that less than 1% of the microbial diversity has been cultured in the laboratory [5]. This represents a major challenge as well as a foremost opportunity for those working in the area. The diversity of microbial life is enormous and the niches in which microbes live are really amazing; among them, an underexplored source of biocatalytic diversity is probably represented by the endophytic community. Endophytes are bacterial or fungal microorganisms which colonize the healthy plant tissue in a symbiotic manner [6]. They have been isolated from several plants, and many of them have shown to be a source of highly bioactive secondary metabolites [7,8]. It has been proposed that endophytic microorganisms have acquired a diversity of biosynthetic capabilities through years of coevolution and genetic recombination with their host plant [9,10]. In addition, the symbiotic relation with the plant force them to deal with several toxic compounds, and thus to develop biodegradation pathways to cope with them [9,11,12]. As a whole, these precedents favour the hypothesis that there is an important reservoir of novel biocatalysts among the endophytic community.

Up to now only scarce research has been done in this field and few experimental data are available [9,13–15]. The first report was the biotransformation of 2-benzoxazolinone and 2-hydroxy-1,4-benzoxazin-3-one by endophytic fungi isolated from the roots of *Aphelandra tetragona* [12]. More recently, endophytic fungi isolated from *Eugenia hallii*, *Schinus molle*, *Crataegus monogyna*, *Juniperus communis* and *Sambucus nigra* were screened for the reduction of a cocktail of ketones of wide structural diversity. Among them, strains of *Phomopsis*, *Pestalotia* and *Epicoccum* showed the best results [16]. These previous reports focused on the biotransformation with endophytic fungi; however, from our perspective the biocatalytic potential of endophytic yeast or bacteria has been overlooked.

Recently, the use of plant fragments in biocatalysis has been described. Since the first report on the use of *Daucus carota* for

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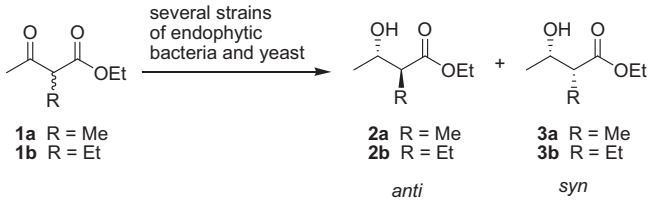

Scheme 1. Reduction of ketoesteres **1a** and **1b** by endophytic microorganisms.

the stereoselective reduction of many ketones [17], several reports on the use of this and other plants have appeared in the literature [18–21]. Most of these studies have focused on the reduction of carbonyl compounds to yield the corresponding chiral alcohols. Inspired by these reports, and the foreseen biocatalytic potential of endophytes, we isolated several microorganisms from *D. carota* and compared their biocatalytic activity with that of the plant fragments [22]. Our results support the hypothesis that endophytic microorganisms might play a role when using plant fragments for biocatalysis. Following this idea, we isolated several bacteria and yeast strains from *D. carota*, *Erythrina crista-galli*, *Curcubita maxima* and *Fortunella margarita*, and tested their biocatalytic activity for the reduction of α-alkyl-β-keto esters **1a** and **1b** (Scheme 1).

α-Substituted β-keto esters are common substrates to evaluate potential bioreducing agents. The reduction of these compounds not only renders optically active hydroxyesters, but also the presence of two contiguous stereocenters in the products allows to test for enantio and diastereoselectivity in a single experiment. Unselective reduction of α-alkyl-β-keto esters yields four stereoisomers. However, by choosing the appropriate biocatalyst, complete conversion of the starting material to one out of the four isomers can be achieved due to facile racemisation of the starting material in the reaction conditions [23–28]. Several purified dehydrogenases from baker's yeast have been systematically screened for the reduction of acyclic and cyclic α-substituted-β-keto esters with very interesting results [24,29,30]. Some of these enzymes have been used in biocatalytic routes for the preparation of important chiral synthons such as (1*R*,2*S*)-2-methyl-1-cyclohexanol, or intermediates for the synthesis of the taxol side chain [30,31]. Accessing all possible isomers of these chiral alcohols continues to be an interesting topic since anti Prelog reductases are scarce [24,32].

2. Experimental

2.1. Plant material

Carrot (*D. carota*) and pumpkin (*C. maxima*) were purchased in a local vegetable market in Montevideo, Uruguay. Kumquats (*F. margarita*) were collected from a local farm. Cockspur coral tree leaves (ceibo – *E. crista-galli*) were collected from Prado City Park in Montevideo, Uruguay.

2.2. Isolation of endophytic microorganisms

The endophytic strains used in this study were isolated from carrot roots, kumquat and pumpkin fruits and cockspur coral tree leaves (Table 1). Carrot roots, kumquat and pumpkin were thoroughly washed with running tap water. The external layer was removed with a sterile knife. Then, the plant tissues were washed with sterile distilled water, disinfected with 10% sodium hypochlorite for 3 min, and rinsed with sterile distilled water. Next, the plant parts were transferred to 70% aq. ethanol for 3 min and rinsed three times with sterile distilled water. Cockspur coral tree leaves were treated in a similar way, but the external layer was not removed. In order to confirm that the surface disinfection process was successful, tissue surface impressions and water from the final rinsing steps were used to inoculate Petri dishes of tryptic soy agar (TSA, Sigma) and potate dextrose agar (PDA, Difco) [33]. No contamination was detected after incubation at 25 °C for 72 h.

Table 1
Endophytic microorganisms tested in this study and source of isolation.

Source of isolation	Isolated endophytic strain
Daucus carota (carrot root)	*Pseudomonas* sp. EB
Erythrina crista-galli (cockspur coral tree leaves)	*Enterobacter agglomerans* C8
Curcubita maxima (pumpkin fruit)	*Leuconostoc* sp.
Daucus carota (carrot root)	*Pichia* sp.
Daucus carota (carrot root)	*Rhodotorula* sp.
Fortunella margarita (kumquats fruit)	*Aureobasidium pullulans* CQA
Curcubita maxima (squash fruit)	*Wickerhamomyces anomalus* Z1

Disinfected plant tissues were sliced with a sterile knife and the plant fragments were used to inoculate a customized medium composed of a sterile suspension of each triturated plant (see supplementary material for details). The culture was incubated at 25 °C and 150 rpm in an orbital shaker Sanyo IOXX400.XX2.C for 48 h. Serial dilutions of each culture were performed on physiologic serum, and 100 μL of the 10^{-2}, 10^{-3} and 10^{-4} dilutions were spread onto agar plates (same composition as the broth but supplemented with 2% agar) and incubated at 25 °C for 24–72 h.

Isolated colonies were characterized as bacteria, yeast or filamentous fungi by macro and microscopic observation. Yeast and bacterial strains were transferred to PDA and TSA respectively. Isolated microorganisms were stored as frozen cultures in 15% glycerol at −20 and −70 °C.

2.3. Identification of isolated microorganisms

Bacterial strains were identified based on classical phenotypic analysis, and phylogenetic analysis of the 16S RNA gene. Extraction of genomic DNAs were carried out with PureLink™ Genomic DNA Mini Kit (Invitrogen) and amplification of almost full-length 16S rRNA gene fragments were performed using primers 27F (5′-AGAGTTTGATC(A/C)TGGCTCAG-3′) and 1492R (5′-ACGG(C/T)TACCTTG TTACGACTT-3′) as described previously [34]. Extraction of yeasts genomic DNAs were done by standard procedures [35]. The ITS1–5.8S–ITS2 region was amplified using primer pair ITS1–ITS4 [36]. Amplification of D1/D2 domain of the LSU rRNA gene was performed with primers ITS1-F (TCCGTAGGTGAACCTGCGG) and NL-4 (5′-TCC TCC GCT TAT TGA TAT GC-3′). The resulting PCR products were sequenced with an Applied Biosystems automatic sequencer ABI 3730XL at Macrogen Corp., Seoul, Korea. Sequences were compared with other released sequences in the GenBank database using BLAST program (National Center for Biotechnology Information) [37].

Based on these analyses the isolated strains were identified as *Pseudomonas* sp. EB, *Enterobacter agglomerans* C8, *Leuconostoc* sp. Z2, *Pichia* sp. CR, *Rhodotorula* sp. CR, *Aureobasidium pullulans* CQA, *Wickerhamomyces anomalus* Z1 (formerly *Pichia anomala*).

2.4. Chemicals and substrates

Ethyl 2-methylacetoacetate (96%, GC) and ethyl 2-ethylacetoacetate (>99%, GC) were purchased from Aldrich. The mixture of all possible α-alkyl-β-hydroxyesters was synthesized by reduction of the corresponding substrate with $NaBH_4$. Optically enriched mixtures with known diasteromeric

Table 2
Reduction of ethyl α-methyl acetoacetate (**1a**) and ethyl α-ethyl acetoacetate (**1b**) with endophytic yeast strains.

Isolated strain	Substrate	% Conversion	*t* (h)	*Anti* (2*S*,3*S*) (% ee)	*Syn* (2*R*,3*S*) (% ee)	% de[a]
Daucus carota	**1a**	95	48	62 (>98)	33 (>98)	31
Daucus carota	**1b**	90	48	54 (>98)	36 (>98)	20
S. cerevisiae	**1a**	>99	Ref. [42]	17 (>98)	83 (>98)	66
S. cerevisiae	**1b**	>99	Ref. [43]	34 (>98)	66 (>98)	32
Pichia sp.	**1a**	>99	4	86 (>98)	14 (>98)	72
Pichia sp.	**1b**	>99	4	90 (>98)	10 (>98)	80
Rhodotorula sp.	**1a**	>99	4	23 (>98)	77 (>98)	54
Rhodotorula sp.	**1b**	>99	4	21 (>98)	79 (>98)	58
A. pullulans CQA	**1a**	>99	4	94 (>98)	6 (>98)	78
A. pullulans CQA	**1b**	>99	4	73 (>98)	27 (>98)	46
Wickerhamomyces anomalus Z1	**1a**	86	4	57 (>98)	29 (>98)	32
Wickerhamomyces anomalus Z1	**1b**	90	4	68 (>98)	22 (>98)	51

[a] The shading indicates the major diasteromer.

composition were obtained by biotransformation of substrates **1a** and **1b** with *Escherichia coli* JM105 (pKTS3) and *E. coli* BL21 (DE3) Δ*yqhE* (pPP4) as previously reported [26,38,39].

2.5. General procedure for biotransformations with yeast strains

Fresh plates of each yeast strain were streaked from the frozen stock in PDA. A single colony was used to inoculate 100 mL of YM Broth. The culture was incubated at 28 °C and 150 rpm for 48 h. The cells were collected by centrifugation at 4000 rpm and 4 °C, for 15 min. The pellet was washed three times with 50 mL physiological serum. 0.5 g of yeast cells (wet weight) were suspended in 5 mL of 10% dextrose solution and the appropriate substrate was added to a final concentration of 10 mM. The reaction mixture was incubated at 28 °C and 150 rpm. Samples were aseptically withdrawn from the reaction mixture at different times for GC analysis.

2.6. General procedure for biotransformations with bacterial strains

Fresh plates of each strain were streaked from the frozen stock in TSA. A single colony was used to inoculate 5 mL of TSB. Each culture was incubated at 28 °C in a rotary shaker at 150 rpm overnight, and 500 μL of this culture were used to inoculate 5 mL of fresh TSB, containing the appropriate substrate in a concentration of 10 mM, in a 25 mL Erlenmeyer flask. The fresh culture was grown under the same conditions and sampled periodically for GC analysis.

2.7. Analysis of the biotransformation products

Analytical samples were prepared by mixing 200 μL of the biotransformation broth with 400 μL of ethyl acetate. After mixing in a vortex for 10 s, the mixture was centrifuged in a microcentrifuge for 2 min, the organic layer was removed and dried over anhydrous Na_2SO_4; 1 μL was used for GC analysis. The conversion was monitored by GC on a GC2014 Shimadzu chromatograph equipped with a HP Carbowax (25 m, 0.25 mm) column and a FID detector. Temperature program: 60 °C (5 min)/8 °C/min/140 °C/25 °C/min/240 °C (5 min). TSPLIT: 220 °C, TFID: 250 °C. Enantiomeric and diasteromeric excess values were determined by chiral GC on Shimadzu 2010 chromatograph equipped with a Megadex DET-TBS (25 m, 0.25 mm) column (MEGA, Italy) and a FID detector. The operating conditions were 60 °C (6 min)/15 °C/min/90 °C(20 min)/45 °C/min/180 °C (5 min). Analytical conditions for separation of all possible optical isomers were established by analysis of the diasteromeric mixture obtained by $NaBH_4$ reduction of substrates **1a** and **1b**. Authentic samples for **2a and b** or **3a and b** were obtained respectively by reduction of **1a** and **1b** with *E. coli* BL21 (DE3) Δ*yqhE* (pPP4) and *E. coli* JM105 (pKTS3) as previously reported [26,38]. Retention times are **1a** (t_R = 14.3), **1b** (t_R = 16.3, 16.9), **2a** (t_R = 23.9), **2b** (t_R = 22.1), **3a** (t_R = 16.6) and **3b** (t_R = 25.0). Assignment of the absolute configuration was based on comparison of the retention times and co-injection with the abovementioned samples of **2a and b** or **3a and b** (see supplementary material for details).

3. Results and discussion

In the course of our studies related to the potential role of endophytic microorganisms in the bioreduction with whole plant fragments, we isolated several endophytes from a variety of plants by enrichment and subsequent isolation in a customized medium made of the triturated plant tissue. This strategy was designed to mimic the availability of nutrients that could be present in the original plant. A total of 30 bacterial and 4 yeast strains were isolated. All the yeast strain and some bacterial strain were selected to assess their effectiveness on the bioreduction of α-alkyl β-keto esters **1a** and **1b** (Scheme 1). The selected strains were identified following a polyphasic approach, including classical biochemical test and phylogenetic studies. The plant sources, as well as the isolated strains selected for this study are described in Table 1.

All the tested strains presented reductase activity and followed the Prelog's rule for the reduction of substrates **1a** and **1b**. The

Table 3
Reduction of ethyl α–methyl acetoacetate (**1a**) and ethyl α–ethyl acetoacetate (**1b**) with selected endophytic bacterial strains.

Isolated strain	Substrate	% Conversion	*t* (h)	*Anti* (2*S*,3*S*) (% ee)	*Syn* (2*R*,3*S*) (% ee)	% de[a]
Daucus carota	**1a**	95	48	62 (>98)	33 (>98)	31
Daucus carota	**1b**	90	48	54 (>98)	36 (>98)	20
Pseudomonas sp. EB	**1a**	11	48	6 (>98)	5 (>98)	0
Pseudomonas sp. EB	**1b**	25	48	9 (>98)	16 (>98)	28
E. agglomerans C8	**1a**	50	20	5 (>98)	45 (>98)	80
E. agglomerans C8	**1b**	89	20	9 (>98)	80 (>98)	79
Leuconostoc sp.	**1a**	11	48	9 (>98)	2 (>98)	63
Leuconostoc sp.	**1b**	11	48	7 (>98)	4 (>98)	27

[a] The shading indicates the major diasteromer.

yeast strains achieved very high conversions in 4 h, with excellent enantioselectivity and good diasteroselectivity (Table 2).

The isolated *Rhodotorula* strain yielded the *syn* (2*R*,3*S*) alcohol as the major product, in a similar manner than *Saccharomyces cerevisiae* but with better diasteromeric excess for substrate **1b**. All the other yeast strains yielded the less common *anti* (2*S*,3*S*) alcohol with better diastereoselectivity than *D. carota*. Among those, *Pichia* presented the most promising results since it yielded the *anti* (2*S*,3*S*) alcohol with very nice diastereoselectivity and excellent enantioselectivity for both tested substrates. The results obtained with this strain are comparable with the best results described in the literature for the reduction of **1a** with yeast strains [40]. In a recent screening Ramos et al. reported the bioreduction of **1a** with *Candida* sp. yielding the *anti* diasteromer with 76% *de* and excellent enantioselectivity. However, the *Pichia* sp. strain included in that report presented the opposite behavior yielding the *syn* (2*R*,3*S*) alcohol as the major product with 78% ee and 27% de [40]. Other *Pichia* strains have already exhibited different diastereoselectivity on the reduction of α-alkyl β-hydroxyesters [27]. This is not surprising, since reductase expression profiles can vary among different strains.

The *A. pullulans* strain isolated from kumquats also provided a very good biocatalyst for reduction of **1a**, yielding the *anti* (2*S*,3*S*) alcohol **2a** as the major product with 78% de and >98% ee. To the best of our knowledge there is only one report on bioreduction using a strain of *A. pullulans*, that was assayed for the reduction of **1a** yielding the same diasteromer [28].

The reported results for *Rhodotorula minuta* and *Rhodotorula rubra* on the bioreduction of **1a** are in agreement with the results obtained with the endophytic *Rhodotorula* sp. strain isolated by our group [40]. They all yielded the *syn* (2*R*,3*S*) diasteromer with fair to good diasteromeric excess; however, the endophytic strain isolated from carrot's root presented excellent enantioselectivity while the others exhibited only an 80% *ee*.

Among the bacterial strains, *E. agglomerans* yielded the *syn* (2*R*,3*S*) alcohol as the major product with excellent enantioselectivity and fairly good diastereoselectivity (Table 3). Even more, the *E. agglomerans* strain isolated from *E. crista-galli* presented better diastereoselectivity for the reduction of **1a** and **1b** than most reported yeast strains. On the contrary, *Pseudomonas* and *Leuconostoc* showed very low reductase activity towards these substrates.

From our results, the isolated yeasts resulted better biocatalysts for the reduction of the tested substrates than the bacterial strains. This is in agreement with previous reports, since yeasts have usually been used for the reduction of carbonyl compounds. Yeasts are also easy to handle, biotransformation times are short, and the reaction setup is extremely simple what makes them very attractive reagents for organic synthesis. However, the best results for the synthesis of the *syn* (2*R*,3*S*) α-alkyl β-hydroxyesters were obtained by reduction of **1a** and **1b** with *E. agglomerans* C8. This is an interesting finding since most bacterial strains do not show remarkable reductase activity [41].

4. Conclusions

The microbial community has provided most of the biocatalysts used up to date; however, the demonstrated scarce knowledge of the microbial diversity indicates that there is a huge reservoir of biocatalysts awaiting discovery. Moreover, the endophytic diversity has received little attention, and most report on endophytes focus on endophytic fungi and their role in the production of natural products [7].

Herein, we have isolated endophytic bacterial and yeast strains from four different plants, and we have tested their biocatalytic activity on the reduction of α-alkyl-β-ketoesters. The isolation procedure, using a plant tissue based medium, provided an effective strategy for the recovery of endophytic yeast and bacteria. One of the selected bacterial strains and all the yeast strains presented good to excellent reductase activity when tested for the bioreduction of two model α-alkyl-β-ketoesters. The *E. agglomerans* C8 strain, isolated from *E. crista-galli*, provided the best results for the preparation of *syn* (2*R*,3*S*) α-alkyl β-hydroxyesters. Among the isolated yeast strains, the *Pichia* sp. strain isolated from *D. carota* and the *A. pullulans* strain isolated from *F. margarita*, provided the best biocatalysts for production of *anti* (2*S*,3*S*) α-alkyl β-hydroxyesters. Our results show that the microbial endophytic community can provide an interesting niche on the search for novel biocatalysts.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.04.003.

References

[1] R.N. Patel, Curr. Org. Chem. 10 (2006) 1289–1321.
[2] R.N. Patel, Chem. Rev. 252 (2008) 659–701.
[3] E. Santaniello, P. Ferraboschi, P. Grisenti, A. Manzocchi, Chem. Rev. 92 (1992) 1071–1140.
[4] J. Tao, J.H. Xu, Curr. Opin. Chem. Biol. 13 (2009) 43–50.
[5] R. Sharma, R. Ranjan, R.K. Kapardar, A. Grover, Curr. Sci. 89 (2005) 72–77.
[6] D. Wilson, Oikos 73 (1995) 274–276.
[7] G. Strobel, Curr. Opin. Microbiol. 9 (2006) 240–244.
[8] G. Strobel, B. Daisy, U. Castillo, J. Harper, J. Nat. Prod. 67 (2004) 257–268.
[9] W.D.S. Borges, K.B. Borges, P.S. Bonato, S. Said, M.T. Pupo, Curr. Org. Chem. 13 (2009) 1137–1163.
[10] A. Stierle, G. Strobel, D. Stierle, P. Grothaus, G. Bignami, J. Nat. Prod. 58 (1995) 1315–1324.
[11] C. Werner, O. Petrini, M. Hesse, FEMS Microbiol. Lett. 155 (1997) 147–153.
[12] M. Zikmundova, K. Drandarov, L. Bigler, M. Hesse, C. Werner, Appl. Environ. Microbiol. 68 (2002) 4863–4870.
[13] A. Agusta, S. Maehara, K. Ohashi, P. Simanjuntak, H. Shibuya, Chem. Pharm. Bull. 53 (2005) 1565–1569.
[14] K.B. Borges, W.D.S. Borges, R. Durán-Patrón, M.T. Pupo, P.S. Bonato, I.G. Collado, Tetrahedron: Asymmetry 20 (2009) 385–397.
[15] K.B. Borges, W.D.S. Borges, M.T. Pupo, P.S. Bonato, Appl. Microbiol. Biotechnol. 77 (2007) 669–674.
[16] P. Pedrini, P.P. Giovannini, M. Mantovani, E. Andreotti, C. Colalongo, J. Mol. Catal. B: Enzym. 60 (2009) 145–150.
[17] F. Baldassarre, G. Bertoni, C. Chiappe, F. Marioni, J. Mol. Catal. B: Enzym. 11 (2000) 55–58.
[18] L.H. Andrade, R.S. Utsunomiya, A.T. Omori, A.L.M. Porto, J.V. Comasseto, J. Mol. Catal. B: Enzym. 38 (2006) 84–90.
[19] J.V. Comasseto, A.T. Omori, A.L.M. Porto, L.H. Andrade, Tetrahedron Lett. 45 (2004) 473–476.
[20] J.S. Yadav, S. Nanda, P.T. Reddy, A.B. Rao, J. Org. Chem. 67 (2002) 3900–3903.
[21] N. Blanchard, P. Van De Weghe, Org. Biomol. Chem. 4 (2006) 2348–2353.
[22] P. Rodríguez, M. Barton, V. Aldabalde, S. Onetto, P. Panizza, P. Menéndez, D. Gonzalez, S. Rodríguez, J. Mol. Catal. B: Enzym. 49 (2007) 8–11.
[23] T. Matsuda, R. Yamanaka, K. Nakamura, Tetrahedron: Asymmetry 20 (2009) 513–557.
[24] I.A. Kaluzna, T. Matsuda, A.K. Sewell, J.D. Stewart, J. Am. Chem. Soc. 126 (2004) 12827–12832.
[25] S. Rodriguez, M. Kayser, J.D. Stewart, J. Am. Chem. Soc. 123 (2001) 1547–1555.
[26] S. Rodríguez, K.T. Schroeder, M. Kayser, J.D. Stewart, J. Org. Chem. 65 (2000) 2586–2587.
[27] R. Gandolfi, E. Cesarotti, F. Molinari, D. Romano, I. Rimoldi, Tetrahedron: Asymmetry 20 (2009) 411–414.
[28] S.P. Ravía, I. Carrera, G.A. Seoane, S. Vero, D. Gamenara, Tetrahedron: Asymmetry 20 (2009) 1393–1397.
[29] I.A. Kaluzna, B.D. Feske, W. Wittayanan, I. Ghiviriga, J.D. Stewart, J. Org. Chem. 70 (2005) 342–345.
[30] S.K. Padhi, I.A. Kaluzna, D. Buisson, R. Azerad, J.D. Stewart, Tetrahedron: Asymmetry 18 (2007) 2133–2138.
[31] B.D. Feske, I.A. Kaluzna, J.D. Stewart, J. Org. Chem. 70 (2005) 9654–9657.

[32] I.A. Kaluzna, J.D. Rozzell, S. Kambourakis, Tetrahedron: Asymmetry 16 (2005) 3682–3689.
[33] M. Surette, A. Sturz, R. Lada, J. Nowak, Plant Soil 253 (2003) 381–390.
[34] W.G. Weisburg, S.M. Barns, D.A. Pelletier, D.J. Lane, J. Bacteriol. 173 (1991) 697–703.
[35] A. Wach, H. Pick, P. Philippsen, in: J.R. Johnston (Ed.), Genetics of Yeast. The Practical Approach, IRL Press, New York, 1994.
[36] T.J. White, T. Bruns, S. Lee, J. Taylor, in: M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White (Eds.), PCR Protocol: A Guide to Methods and Applications, Academic Press, San Diego, CA, 1990, pp. 315–322.
[37] S.F. Altschul, W. Gish, W. Miller, E.W. Myers, D.J. Lipman, J. Mol. Biol. 215 (1990) 403–410.
[38] P. Panizza, S. Onetto, S. Rodriguez, Biocatal. Biotransform. 25 (2007) 414–417.
[39] P. Panizza, α-alquil-β-hidroxiésteres quirales: ampliando el repertorio de biocatalizadores hacia las síntesis enantiodivergentes, Universidad de la República, Montevideo, 2005.
[40] A.D.S. Ramos, J.B. Ribeiro, L. Vazquez, S.B. Fiaux, S.G.F. Leite, R.D.A. Cruz, M.D.C.K.V. Ramos, F.R.D.A. Neto, O.A.C. Antunes, Tetrahedron: Asymmetry 20 (2009) 559–561.
[41] E. Maconi, F. Aragozzini, Appl. Microbiol. Biotechnol. 31 (1989) 29–31.
[42] K. Faber, Biotransformations in Organic Chemistry, 5th ed., Springer-Verlag, Berlin, 2004.
[43] Y. Kawai, M. Tsujimoto, S. Kondo, K. Takanobe, A. Ohno, K. Nakamura, Bull. Chem. Soc. Jpn. 67 (1994) 524–528.