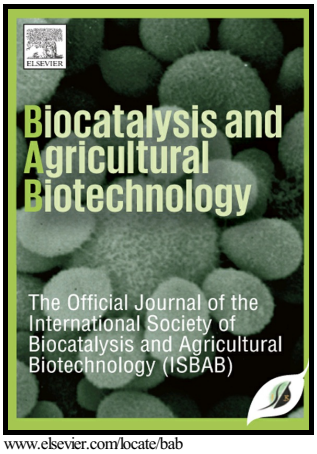
Synthesis of α-chloroacetophenones with NH_4Cl/Oxone® *in situ* followed by bioreduction with whole cells of marine-derived fungi

Aline T.do B. Morais, Irlon M. Ferreira, David E.Q. Jimenez, André L.M. Porto



www.elsevier.com/locate/bab

PII: S1878-8181(17)30489-9
DOI: https://doi.org/10.1016/j.bcab.2018.08.003
Reference: BCAB836

To appear in: *Biocatalysis and Agricultural Biotechnology*

Received date: 22 September 2017
Revised date: 19 March 2018
Accepted date: 9 August 2018

Cite this article as: Aline T.do B. Morais, Irlon M. Ferreira, David E.Q. Jimenez and André L.M. Porto, Synthesis of α-chloroacetophenones with NH_4Cl/Oxone® *in situ* followed by bioreduction with whole cells of marine-derived fungi, *Biocatalysis and Agricultural Biotechnology,* https://doi.org/10.1016/j.bcab.2018.08.003

Synthesis of α-chloroacetophenones with NH_4Cl/Oxone® *in situ* followed by bioreduction with whole cells of marine-derived fungi

Aline T. do B. Morais,[a] Irlon M. Ferreira,[b,*] David E. Q. Jimenez, [a,] André L. M. Porto [a,*]

[a]Laboratório de Química Orgânica e Biocatálise, Instituto de Química de São Carlos, Universidade de São Paulo, Av. João Dagnone, 1100, Ed. Química Ambiental, Santa Angelina, 13563-120, São Carlos, SP, Brazil.

[b]Grupo de Biocatálise e Biotransformação em Química Orgânica, Colegiado de Química, Universidade Federal do Amapá, Rod. Juscelino Kubitschek, Km 2, J. Marco Zero, 68902-280, Macapá, AP, Brazil.

E-mail address: almporto@iqsc.usp.br (A.L.M. Porto)

irlon.ferreira@gmail.com (I.M. Ferreira)

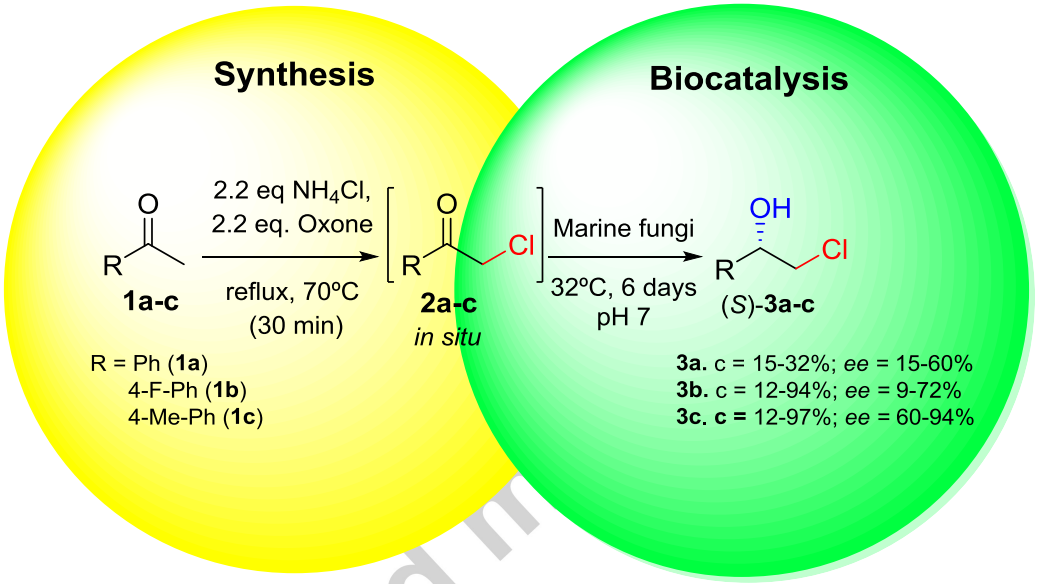
* Corresponding author. Tel.: +55 16 3373 8103. Fax.: +55 16 33739952.

Abstract

Chiral chlorohydrins are used as intermediates in the synthesis of various compounds with biological activities. This paper reports the synthesis of α-chloroketones **2a-c** with oxone® and NH_4Cl at reflux via 30 min of exposure to microwave irradiation and conventional heating, *in situ*, followed by reduction with whole cells of marine-derived fungi (*Penicillium citrinum* CBMAI 1186, *Mucor racemosus* CBMAI 847, *Aspergillus sydowii* CBMAI 935, *Penicillium raistrickii* CBMAI 931, and *Penicillium oxalicum*

CBMAI 1185), yielding the respective chlorohydrins **3a-c** with good conversion (32-97%) and enantioselectivities (60-94%). This is the first study involving the synthesis of α-chlorophenones *in situ* followed by biocatalytic reduction from whole cells of marine-derived fungi to obtain enantio-enriched chlorohydrins **3a-c**.

Graphical abstract



Keywords: Biocatalysis; Marine-derived fungi; Chlorohydrins; Microwave irradiation**.**

1. Introduction

Marine-derived fungi have garnered biotechnological interest due to their ability to produce secondary metabolites and intra and/or extracellular enzymes that are different from those produced by their terrestrial homologous counterparts. Through millions of years of evolution in the marine environment, the marine fungi have been adapted to the different ecosystem conditions, such as high salt concentration, different pH ranges, low

temperature, high pressure, and in the presence of ocean currents (Trincone 2011).

Marine-derived fungi are currently used in several biocatalytic applications, such as the reduction of acetophenone derivatives (Rocha et al. 2015; Rocha et al. 2012), chemeoselective and regioselective biohydrogenation (Ferreira et al. 2015; Ferreira et al. 2015a; Ferreira et al. 2014; Walton et al. 2011), ene-reduction of Knoevenagel adducts (Jimenez et al. 2016), and in various reactions involving biodegradation of pesticides (Alvarenga et al. 2014; Rodrigues et al. 2016; Vacondio et al. 2015), bio-oxidation of saturated carbons in natural products, and biotransformation of organic compounds (Martins et al. 2015; Birolli et al. 2015).

The chiral halohydrins can be used as intermediates in the preparation of a range of organic compounds, including some with interesting pharmacological activities (Barbieri et al. 2001; Ferreira et al. 2014a). Methodologies for the preparation of chiral halohydrins usually include reducing borane or organometallic catalysts with rhodium or ruthenium (Lu et al. 2011).

Some microorganisms such as *Rhodotorula* sp. AS2.2241 (Aguirre-Pranzoni et al. 2015; Yang et al. 2006), *Escherichia coli* pET28-KtCR (Aguirre-Pranzoni et al. 2015), *Geotrichum candidum* CCT 1205 (Zampieri et al. 2013), and *Rhodotorula glutinis* CCT 2182 (Fardelone et al. 2011) were used for the preparation of asymmetric halohydrins. However, few reports are available for directly obtaining asymmetric halohydrins from the reduction of haloketone in marine-derived fungi. For example, our group reported the reduction of α-chloroacetophenone and α-bromoacetophenone by whole cells of marine-derived fungi, resulting in a yield of 99% and an enantiopurity of 17-54% *e.e*, respectively (Rocha et al. 2010; Rocha et al. 2009).

In order to obtain chiral halohydrins, herein we report the first example of a synthesis of α-chloroketones *in situ* followed by biocatalytic reduction with whole cells of marine-derived fungi to obtain halohydrins **3a-c**.

2. Materials and methods

2.1. General

Acetophenone **1a** (99%), 1-(4-fluorophenyl)ethan-1-one **1b** (99%) and 1-(*p*-tolyl)ethan-1-one **1c** (95%) were purchased from Sigma-Aldrich (São Paulo, Brazil). The NH_4Cl (99.99%) and Oxone® ($KHSO_5 \cdot 0.5KHSO_4 \cdot 0.5K_2SO_4$) were purchased from Sigma-Aldrich. Sodium borohydride (98%) was purchased from Synth. The solvents hexane and methanol were purchased from Panreac (Brazil), and the solvents ethyl acetate, ethanol, DMSO, and acetonitrile were purchased from Synth. The salts used for the preparation of the artificial seawater were purchased from Vetec and Synth (São Paulo, Brazil).

^{1}H NMR and ^{13}C NMR spectra were recorded on Agilent Technologies 500/54 Premium Shielded operating at 400 MHz for NMR ^{1}H and 100 MHz for NMR ^{13}C. The spectra were obtained using chloroform-*d* ($CDCl_3$), a solvent, and TMS as the internal standard the chemical shifts were given in parts per million (ppm), and the coupling constant (*J*) was given in Hz.

2.2. Gas chromatography analyses

To obtain gas chromatography mass spectrometry (GC-MS) measurements, a Shimadzu GC-MS-QP-2010 Plus gas chromatography system equipped with an auto injector AOC-20i coupled to a mass selective detector (Shimadzu MS2010 Plus) and an

Agilent Technologies column DB-5MS (5% phenyl e 95% dimethylpolysiloxane) (30 m x 0.25 mm x 0.25 mm) in electron ionization (70 eV) mode were used. The following conditions were employed in the gas chromatography mass spectrometry analyses: ratio split 5; carrier gas helium, 62.8 kPa; injection volume, 1.0 µL; injector temperature, 250 °C; column temperature, 80 °C; initial column temperature, 80 °C for 2 min; heating rate, 2 °C/minute to 200 °C lasting for 4 minutes. The total analysis time was 30 minutes. The mass spectrometer was set to scan from *m*/*z* 50–500.

For the gas chromatography analyses using a flame ionization detector (GC-FID), enzymatic reactions were analyzed using a Shimadzu GC 2010 gas chromatograph equipped with an AOC 20i auto injector, a flame ionization detector (FID), and a Varian chiral column CP-Chiralsil-DEX (β-cyclodextrin) (25 m x 0.25 mm x 0.39 mm). The following conditions were employed in the gas chromatography analyses: ratio Split 1:10; carrier gas nitrogen, 69 kPa; injection volume, 1.0 µL; injector temperature, 250 °C, detector temperature: 250 °C, initial column temperature, 120 °C, remaining for 2 min; heating rate, 2 °C/minute to 165 °C, lasting for 15 minutes. The total analysis time was 32.5 minutes. The following retention time of enantiomers (*R*,*S*)-chlorohydrins **3a-c** were obtained: (*R*)-**3a** = 20.5 min, (*S*)-**3a** = 20.0 min; (*R*)-**3b** = 22.4 min, (*S*)-**3b** = 21.7 min; (*R*)-**3c** = 23.4 min, (*S*)-**3c** = 22.5 min.

2.3. Absolute configuration

The optical rotations of (*S*)-chlorohydrins **3a-c** were determined in a JASCO P2000 polarimeter equipped with a Na-lamp (λ = 589 nm) and Spectra Manager software; a cell of diameter 0.98 dm was used, and concentrations were expressed in g/100 mL. Ethyl acetate

was used as the solvent, and the analyses were performed in triplicate. The absolute configuration of chlorohydrins **3a-c** was determined by the comparison of specific rotations described in the literature (see Table 5).

2.4. Synthesis of 2-chloro-1-phenylethanone under microwave irradiation (Microwave) and conventional heating (CH)

Microwave radiation: Acetophenone **1a** (1.0 mmol, 120 mg), NH_4Cl (2.2 eq.; 58.84 mg), oxone® (2.2 eq.; 338.12 mg), and methanol (5 mL) were added to a 25 mL flask. The reactional mixture was maintained at reflux in the microwave reactor for 15 min at 70 °C and 150-200 W. At the end of the reaction, the solution was filtered, and the solvents were removed by evaporation under vacuum. The reaction was monitored by thin layer chromatography (TLC) using hexane and ethy acetate (9:1) as eluents. The obtained mixture was purified using flash column chromatography (CC) over silica gel using hexane and ethy acetate (9:1) as eluent to yield the 2-chloro-1-acetophenone **2a**. The synthesis 2-chloro-1-acetophenone **2a** was conducted using others solvents (i.e., water, acetonitrile, ethanol, and dimethylsulfoxide). To obtain the best condition in the formation of 2-chloro-1-phenylethanone **2a**, different conditions were used under a microwave reactor, including different reaction times (i.e., 15, 30, and 60 min) and different equivalents of NH_4Cl and Oxone®. The detailed data are described in Table 1.

Conventional heating: The same procedure was performed using 1.0 mmol of acetophenone **1a,** 2.2 eq. NH_4Cl and Oxone® in 5 mL of methanol under reflux via conventional heating at 70 °C at 30 min. At the end of the reaction (30 min), the solution was filtered, and the solvent was removed by evaporation under vacuum. The reaction was

monitored by TLC using hexane and ethyl acetate (8:2) as eluents to observe the formation of compound **2a**. The product **2a** was purified by column chromatography (silica gel 60) with a mixture of hexane and ethyl acetate (9:1) as eluents to yield the 2-chloro-1-phenylethanone **2a** (97%) and characterized by ^{1}H NMR and ^{13}C NMR and MS (Supplementary Material). Under conventional heating, the synthesis of α-chloroketones **1b-c** in good yields (**2b**, 93% and **2c**, 90%) was also carried out. The compounds **2a-c** were characterized by NMR and MS analysis and compared with the literature data (Ferreira et al. 2014a).

2.5. Preparation of (±)*-chlorohydrins* ***3a-c***

Methanol (10 mL), chloroketone **2a-c** (2.5 mmol), and $NaBH_4$ (3 mmol) were added to a round-bottomed flask (50 mL). The mixture was stirred for 30 min at 0 °C and for 1.5 h at room temperature. The methanol was evaporated under reduced pressure, and HCl (1.0 mL, 10%) was added to the reaction. The mixture was then extracted with ethyl acetate (3 × 20 mL), and the organic phase was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by gel silica column chromatography with a mixture of *n*-hexane and ethyl acetate (8:2) as eluents, leading to the corresponding products: **3a** (82%), **3b** (81%) and **3c** (86%). The compounds **3a-c** were characterized by NMR and MS analysis and compared with the literature data (Ferreira et al. 2014a).

2.6. *Marine-derived fungi used for biocatalytic reduction of α-chloroacetophenones* ***2a-c***

The marine-derived fungal strains *Penicillium raistrickii* CBMAI 931 and *Aspergillus sydowii* CBMAI 935 were isolated from marine sponge *Chelonaplysilla erecta*, the fungus *Penicillium citrinum* CBMAI 1186 was isolated from the marine alga *Caulerpa* sp., and the fungus *Mucor racemosus* CBMAI 847 was isolated from marine alga *Mussismilia hispida*. The algae and sponges were collected by Prof. R.G.S. Berlinck (Chemistry Institute of São Carlos, University of São Paulo, IQSC-USP, Brazil) in the town of São Sebastião, on the coast of the State of São Paulo, Brazil (Rocha et al. 2009).

The marine-derived fungi used in this work were identified by both conventional and molecular methods at the Chemical, Biological, and Agricultural Pluridisciplinary Research Center (CPQBA) at the State University of Campinas (UNICAMP), SP, Brazil, deposited in the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI, http://webdrm.cpqba.unicamp.br/cbmai/). The marine-derived fungi employed in this work can be acquired in CBMAI.

2.7. Growth of marine-derived fungi on a solid medium

The marine-derived fungi were cultivated on Petri plates containing the malt extract (Acumedia) (20.0 g L^{-1}), Agar (Acumedia) (20.0 g L^{-1}), and artificial seawater (ASW). The pH was adjusted to 7 (pH of the sea coast) with a KOH solution (0.1 mol L^{-1}) (Kossuga et al. 2012). The composition of ASW included $CaCl_2 \cdot 2H_2O$ (1.36 g L^{-1}), $MgCl_2 \cdot 6H_2O$ (9.68 g L^{-1}), KCl (0.61 g L^{-1}), NaCl (30.0 g L^{-1}), Na_2HPO_4 (0.014 mg L^{-1}), Na_2SO_4 (3.47 g L^{-1}), $NaHCO_3$ (0.17 g L^{-1}), KBr (0.10 g L^{-1}), $SrCl_2.6H_2$ (0.04 g L^{-1}), and H_3BO_3 (0.03 g L^{-1}) (Menezes et al. 2010). The solution containing the solid medium was sterilized in an autoclave (AV-50, Phoenix, Brazil) at 20 min at 120 ºC to subsequently perform the inoculation of the marine-derived fungi.

2.8. Growth of strains in a liquid medium

A liquid medium (100 mL, 2% malt extract in artificial seawater, pH 7) contained in Erlenmeyer flasks (250 mL) was inoculated in with disks (0.5 cm diameter) from the stock solid culture. The mycelia were incubated at 32 °C and 130 rpm for 7 days in a rotary shaker (Nova Ética, Brazil).

*2.9. Synthesis of **2a** in situ followed by biocatlytic reduction with marine-derived fungus P. citrinum CBMAI 1186*

The one-pot reactions were performed at three different experimental conditions:

i) Experiment A: after synthesis of **2a** by conventional heating, the reactional mixture was fully transferred to an Erlenmeyer flask (250 mL) containing 5.0 g (wet weight) of mycelia of *P. citrinum* CBMAI 1186 and 100 mL of a phosphate buffer (Na_2HPO_4/KH_2PO_4, pH 7 / 0.1 mol L^{-1}).

ii) Experiment B: after synthesis of **2a** by conventional heating, the excess Oxone salt was filtered and the reactional mixture was fully transferred to an Erlenmeyer flask (250 mL) containing 5.0 g (wet weight) of mycelia of *P. citrinum* CBMAI 1186 and 100 mL of a phosphate buffer (Na_2HPO_4/KH_2PO_4, pH 7 / 0.1 mol L^{-1}).

iii) Experiment C: after synthesis of **2a** by CH, the excess Oxone salt was filtered, and the excess solvent (methanol) was removed after the reactional mixture was fully transferred to an Erlenmeyer flask (250 ml) containing 5.0 g (wet weight) of mycelia of *P. citrinum* CBMAI 1186 and 100 mL of a phosphate buffer (Na_2HPO_4/KH_2PO_4, pH 7 / 0.1 mol L^{-1}).

In all cases, the reactional mixtures were incubated for 6 days on an orbital shaker (Nova Ética, Brazil) (130 rpm at 32 °C). The reactions were monitored by TLC every 48 h. After 6 days of incubation, the cells were filtered through apparatus Buchner funnels. The mycelial pellet was transferred to an Erlenmeyer flask with 50 mL of ethyl acetate and water (1:1) and maintained under magnetic stirring for 30 min. This process was used to break the cells. The mixture was filtered to remove the cells, and the liquid phase was submitted to liquid extraction with EtOAc (3 x 50 mL).

The organic phase was dried with anhydrous Na_2SO_4, filtered, and the excess solvent was removed in a rotevaporator. The obtained extracts were purified by column chromatography on silica gel, and the enantiomeric excess of **3a** was measured by GC-FID / chiral column.

3. Results and discussion

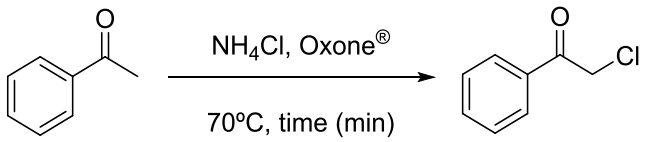
According to the method reported by Zhou et al. (2012) for the synthesis of ketone **2a**, we decided to study this reaction using microwave irradiation as an innovative methodology. Initially, a screening of different reactional conditions was conducted, including using different types of solvents, temperatures, times, and amounts of NH_4Cl and Oxone® (Table 1).

For producing 2-chloro-1-acetophenone **2a,** ketone **1a** (1.0 mmol, 120 mg), NH_4Cl (1.1 eq.), and Oxone® (1.1 eq.) were used in the presence of ethanol under microwave irradiation (70 °C, 15 minutes). In these conditions, the ketone **1a** was produced with a 16% yield (entry 1, Table 1).

Under similar conditions, the use of methanol, as solvente, was crucial for the success of the reaction under microwave irradiation, yielding 55% of ketone **2a** (entry 3, Table 1). On the other hand, we obtained traces in the formation of ketone **2a** when acetonitrile and water were used as solvents in the reactions under microwave irradiation, 2 and 3%, respectively (entries 4 and 5, Table 1). Ketone **2a** was not obtained when dimethylsufoxide (DMSO) was used in the reaction (entry 6, Table 1). In addition, the reaction in the absence of solvent produced the ketone **2a** only with a 14% yield (entry 11, Table 1).

Next, the NH_4Cl and Oxone® equivalent was investigated in methanol under microwave irradiation (entries 7-9, Table 1), and it was found that 2.2 eq. of NH_4Cl and 2.2 eq. of Oxone® was appropriate to obtain the product **2a** with a good yield (80%). This condition was applied for obtaining ketone **2a** under microwave irradiation. When the reaction was performed under conventional heating at 70 ºC in the same conditions of entry 9 in Table 1, the compound **2a** was isolated with a 70% yield.

Table 1. Optimization of reactional conditions for the production of 2-chloro-1-phenylethanone **2a** using NH_4Cl and Oxone® under microwave irradiation.



1a **2a**

Entry	Solvent	Time (min)	NH_4Cl (mmol)	Oxone® (mmol)	Yield (%)[a]
1	Ethanol	15	1.1	1.1	16
2	Ethanol	30	1.1	1.1	29
3	Methanol	30	1.1	1.1	55

4	Acetonitrile	30	1.1	1.1	2
5	Water	30	1.1	1.1	3
6	DMSO	30	1.1	1.1	0
7	Methanol	30	2.2	1.1	69
8	Methanol	30	1.1	2.2	59
9	Methanol	30	2.2	2.2	80 (70)[b]
10	Methanol	60	2.2	2.2	86 (74)[b]
11	Solvent-free	30	2.2	22	14

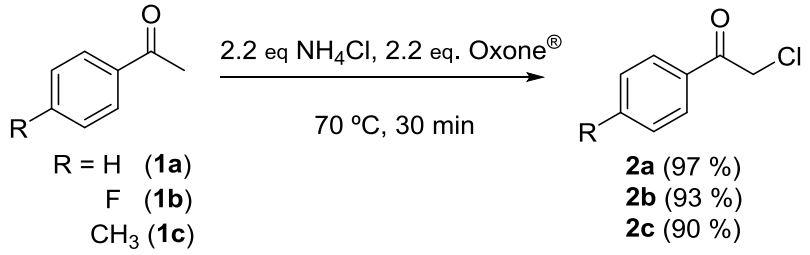
[a]Conversion determined by GC-FID.
[b]Isolated yield.

The mechanism for the chlorination of ketones by NH_4Cl and Oxone® was proposed by Zhou et al. (2012). In this reaction, $HO^{-}Cl^{+}$ is produced, which is an unstable species that readily decomposes. Therefore, the lower yield of the product **2a** was obtained when the reaction was performed under microwave irradiation when compared to conventional heating.

The reaction under microwave irradiation provided by-products (i.e., 2,2-dichloro-1-phenylethanone and 2-chloro-2-hydroxy-1-phenylethanone) that were identified by GC-MS (Supplementary Material) due the reaction reactivity under microwave irradiation. These by-products were identified by their mass spectra, and their similarities with the NIST-05 library "database" (Mass Spectral Library NIST/EPA/NIH) were more than 95%.

After the optimized conditions under microwave irradiation, we explored the applicability of the reaction using other acetophenone derivatives (**1a-c**) to explore the scope of this methodology in the optimized reactional condition under conventional heating (Table 2).

Table 2. Synthesis of 2-chloro-1-phenylethanone derivatives **2a-c** under conventional heating.



The treatment of ketones **1a-c** with 2.2 eq. NH_4Cl and 2.2 eq. Oxone® in reflux for 30 minutes led to the formation of chloro-ketones **2a-c** with good yields (90-97%, Table 2).

One-pot reactions are greatly desired by chemists because it optimizes time and resources by eliminating the separation purification processes of the chemical intermediates and improves the overall yield (Kour et al.2016).

After optimizing the production of α-chloroacetophenones **2a-c** with NH_4Cl and Oxone® by conventional heating, a biocatalytic reaction was performed in order to reduce the carbonyl group of ketones **1a-c** using mycelia of the marine-derived fungus *P. citrinum* CBMAI 1186 and yielding the chlorohydrins **3a-c**. The marine fungus *P. citrinum* CBMAI 1186 was used because in previous studies it was shown to be effective in the reduction of ketones (Rocha et al. 2015; Rocha et al. 2012).

As shown in Table 3, the reactions were significantly dependent on the medium conditions. In *Experiment A* (entries 1-3, Table 3) and *Experiment B* (entries 3-6, Table 3), after 144 h of reaction (130 rpm, 32 ºC), did not occur bioreduction of α-chloroacetophenone **2a**. It is possible that the excess of Oxone® salt used was harmful to

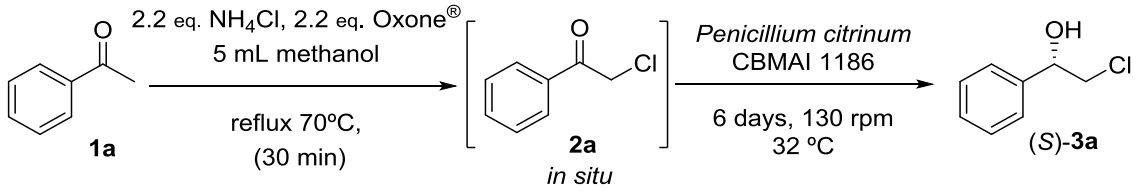
the fungal metabolism maintenance and therefore prevented the reaction from occurring under these conditions.

However, *Experiment C* showed that ketone **2a** was obtained under conventional heating by a one-pot reduction using whole cells of *P. citrinum* CBMAI 1186 and yielding (*S*)-chlorohydrin **3a** with 20% conversion and 10% enantiomeric excess (entry 7, Table 3). In addition, the enantiomeric excess of (*S*)-**3a** increased when the reaction time was 144 hours (entry 9, Table 3).

According to previously obtained results, other marine-derived fungi (e.g., *Mucor racemosus* CBMAI 847, *Aspergillus sydowii* CBMAI 935, *Penicillium raistrickii* CBMAI 931, and *P. oxalicum* CBMAI 1185) can also be used to obtain chlorohydrin **3a** using the same methodology that was performed with *P. citrinum* CBMAI 1186, as previously described in Table 3 under conventional heating.

Analyzing the reduction of ketone **2a** using marine-derived fungi, different yields and enantiomeric excesses were obtained for chlorohydrin **3a** (Table 4). All marine fungi catalyzed the reduction to (*S*)-**3a** in accordance with Prelog's rule (Cahn 1966). The (*S*)-**3a** product was obtained with moderate enantiomeric excess using *A. sydowii* CBMAI 935 (50 % *e.e.*) and *M. racemosus* CBMAI 847 (60% *e.e.*). The *P. raistrickii* CBMAI 931 and *P. oxalicum* CBMAI 1185 catalyzed the bioreduction of chloroketone **2a** to (*S*)-**3a** in lower selectivities (12% and 21% *e.e.*) and conversions (18% and 15%), respectively (Table 4).

Table 3. Cascade reaction in synthesis of **2a** and followed by reduction one pot by fungus *P. citrinum* CBMAI 1186 under conventional heating.



Entry	Reaction condition	Time (h)	Conversion (%) **3a**[a]	*e.e.* (%) **3a**[b]
1	*Experiment A*	48	-	-
2		96	-	-
3		144	-	-
4	*Experiment B*	48	-	-
5		96	-	-
6		144	-	-
7	*Experiment C*	48	20	10
8		96	24	12
9		144	32	15

[a] Conversion obtained by CG-FID analysis.
[b] *e.e.* = enantiomeric excess.

Based on these results, we decided to perform the reduction of different chloroketones **2b-c**, synthetized *in situ*, to obtain the chlorohydrins **3b-c** using whole cells of marine-derived fungi *P. citrinum* CBMAI 1186, *M. racemosus* CBMAI 847, *A. sydowii* CBMAI 935, *P. raistrickii* CBMAI 931, and *P. oxalicum* CBMAI 1185 under conventional heating (Table 4).

The best results for the conversion of chloroketones **2a** and **2b** were obtained by whole cells of the *P. oxalicum* CBMAI 1185. The product (*S*)-**3a** showed low conversions for all of the marine fungi and the moderate enantiomeric excesses was obtained using *A. sydowii* CBMAI 935 and *M. racemosus* CBMAI 847 for (*S*)-**3a** (50-60% *e.e.*) (Table 4).

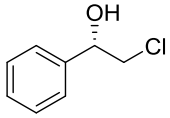
Table 4. Synthesis of **2a-c** *in situ* followed of the bioreduction by marine-derived fungi for obtaining of chlorohydrins of (*S*)-**3a-c** under conventional heating.

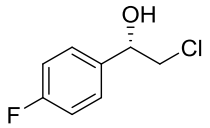
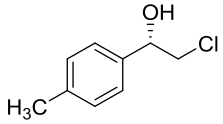
Marine-derived fungi	c **3a**	*e.e.* **3a**	c **3b**	*e.e.* **3b**	c **3c**	*e.e.* **3c**
P. citrinum CBMAI 1186	32	15	45	48	40	60
P. oxalicum CBMAI 1185	18	21	94	25	97	94
A. sydowii CBMAI 935	21	50	68	9	90	89
P. raistrickii CBMAI 931	15	12	47	72	86	82
Mucor racemosus CBMAI 847	16	60	12	66	12	66

c (%) = conversion determined by GC-FID analysis;
e.e.(%) = enantiomeric excess.

However, the chlorohydrin (*S*)-**3b** was obtained with high conversion (c = 94%) in biotransformation reaction using whole cells of *P. oxalicum* CBMAI 1185 (Table 4). At last, the product (*S*)-**3c** was obtained with high conversion of 97% and high enantiomeric excess (94% *e.e.*) with *P. oxalicum* CBMAI 1185 (Table 4).

Table 5. Optical rotations of the chlorohydrins **3a-d** obtained by the reduction using marine-derived fungi.

Chlorohydrins	$[\alpha]^{T}_{D}$ Experimental data (mg/100 mL)	$[\alpha]^{T}_{D}$ Literature data (mg/100 mL)
 (*S*)-**3a**	$[\alpha]_D^{20}$ = + 14.6 (*c* 0.19, EtOAc) (60 % *e.e.*)*	$[\alpha]_D^{26}$ = + 43.1 (*c* 0.76, $CHCl_3$) (88 % *e.e.*) (Soni et al. 2015)

 (*S*)-**3b**	$[\alpha]_D^{20}$ = + 27.2 (*c* 0.5, EtOAc) (66 % *e.e.*)*	$[\alpha]_D^{20}$ = + 47 (*c* 0.21, $CHCl_3$) (90 % *e.e.*) (Wu et al. 2011)
 (*S*)-**3c**	$[\alpha]_D^{20}$ = + 32.1 (*c* 0.14, EtOAc) (89 % *e.e.*)*	$[\alpha]_D^{25}$ = + 37.5 (*c* 1.0, $CHCl_3$) (83 % *e.e.*) (Basavaiah et al. 2009)

*Optical rotations of the chlorohydrins **3a-c:** (*S*)-**3a** from *Mucor racemosus* CBMAI 931; (*S*)-**3b** from *Mucor racemosus* CBMAI 931; (*S*)-**3c** from *A. sydowii* CBMAI 935.

In addition, the reactions using chloroketones **2a-c** were performed with the culture broth of marine-derived fungi to produce chlorohydrins **3a-c,** followed the principles of Green Chemistry. These studies showed that the enzymes responsible for the bioreduction of chloroketones were intracellular. The alcohol (*S*)-**3c** was obtained with high conversions and selectivity from whole cells of fungi *P. raistrickii* CBMAI 931, *A. sydowii* CBMAI 935 and *P. oxalicum* CBMAI 1185 (82-94% *e.e.*), indicating that the enzymes were stereoselective reduction to chloroketone **2c** (Table 4). However, the reactions with enzyme broths provided only traces of chlorohydrin **3a**. These studies can be showed that the enzymes responsible for the bioreduction of chloroketones are intracellular, preferably.

The absolute *S*-configurations of the chlorohydrins **3a-c** were assigned via comparison with optical rotations values described in the literature (Table 5).

4. Conclusion

A simple protocol for asymmetric synthesis of chlorohydrins **3a-c** *in situ* was investigated. The reactions were conducted using α-acetophenones **2a-c** with NH_4Cl and

Oxone® followed by biocatalytic reduction from whole cells of marine-derived fungi under microwave irradiation and conventional heating.

In all cases, the conversions and enantiomeric excesses were dependent on the substrate structures, the type of marine fungus used, and experimental conditions (i.e., microwave irradiation or conventional heating). This reaction method involved chemical and biocatalytic methodologies and was efficient since it eliminated the purification process of chemical intermediates; the method improved overall yields and followed the principles of Green Chemistry.

Acknowledgements

The authors would like to acknowledge Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for their financial support to perform this research. The authors would also like to acknowledge Prof. R.G.S. Berlinck (Instituto de Química de São Carlos – USP) for providing the marine fungal strains.

References

Aguirre P.C., Bisogno, F.R., Orden, A.A., Kurina, S.M., 2015. Lyophilized *Rhodotorula* yeast as all-in-one redox biocatalyst: Access to enantiopure building blocks by simple chemoenzymatic one-pot procedures. J. Mol. Catal. B: Enzym. 114, 19-24.

Alvarenga, N., Birolli, W.G., Seleghim, M.H.R., Porto, A.L.M., 2014. Biodegradation of methyl parathion by whole cells of marine-derived fungi *Aspergillus sydowii* and *Penicillium decaturense*. Chemosphere. 117, 47-52.

Barbieri, C., Bossi L., D'Arrigo, P., Fantoni, G., Servi, S., 2001. Bioreduction of aromatic ketones: Preparation of chiral benzyl alcohols in both enantiomeric forms. J. Mol. Catal. B: Enzym. 11, 415-421.

Basavaiah, D., Das, U., Roy, S., 2009. Towards chiral diamines as chiral catalytic precursors for the borane-mediated enantioselective reduction of prochiral ketones. J. Chem. Sci. 121, 1003-1010.

Birolli, W.G., Ferreira, I.M., Alvarenga, N., Santos, D.A., de Matos, I.L., Comasseto, J.V., Porto, A.L.M., 2015. Biocatalysis and biotransformation in Brazil: An overview. Biotechnol. Adv. 33, 481-510.

Cahn, R.S. Ingold, C.K. Prelog, V., 1966. Specification of molecular chirality. Angew. Chem. Int. 5, 385-415.

Fardelone, L.C., Rodrigues, J.A.R., Moran, P.J.S., 2011. Chiral pharmaceutical intermediaries obtained by reduction of 2-halo-1-(4-substituted phenyl)-ethanones mediated by *Geotrichum candidum* CCT 1205 and *Rhodotorula glutinis CCT 2182.* Enzy. Res. 2011, 1-8 .

Ferreira, I.M., Rocha, L.C., Yoshioka, S.A., Nitschke, M., Jeller, A.H., Pizzuti, L., Seleghim, M.H.R., Porto, A.L.M., 2014. Chemoselective reduction of chalcones by whole hyphae of marine fungus *Penicillium citrinum* CBMAI 1186, free and immobilized on biopolymers. Biocatal. Agric. Biotechnol. 3, 358-364.

Ferreira, I.M., Meira, E.B., Rosset, I.G., Porto, A.L.M., 2015. Chemoselective biohydrogenation of α,β- and α,β,γ,δ-unsaturated ketones by the marine-derived fungus *Penicillium citrinum* CBMAI 1186 in a biphasic system. J. Mol. Catal. B: Enzym. 115, 59-65.

Ferreira, I.M., Nishimura, R.H.V., Souza, A.B., Clososki, G.C., Yoshioka, S. A., Porto A.L.M., 2014a. Highly enantioselective acylation of chlorohydrins using Amano AK lipase from *P. fluorescens* immobilized on silk fibroin–alginate spheres. Tetrahedron Lett. 55, 5062-5065.

Ferreira, I.M., Vasconcellos, S.P., Cruz, J.B., Comasseto, J.V., Porto, A.L.M., Rocha, L.C., 2015. Hydrogenation of bis-*α,β*-unsaturated enones mediated by filamentous fungi. Biocatal. Agric. Biotechnol. 4, 144-149.

Jimenez, D.E.Q., Ferreira, I.M., Birolli, W.G., Fonseca, L.P., Porto, A.L.M., 2016. Synthesis and biocatalytic ene-reduction of Knoevenagel condensation compounds by the marine-derived fungus *Penicillium citrinum* CBMAI 1186. Tetrahedron 72, 7317-7322.

Kour, D., Khajuria, R., Kapoor, K.K., 2016. Iodine-ammonium acetate promoted reaction between 2-aminopyridine and aryl methyl ketones: a novel approach towards the synthesis of 2-arylimidazo[1,2-*a*]pyridines. Tetrahedron Lett. 40, 4464-4467.

Kossuga, M.H. Stelamar, R., Xavier, C., Milanetto. M.C., Valle, M.Z., Pimenta, E.F., Morais, R.P., Carvalho, E., Mizuno. C. M., Coradello, L. F. C., Barroso, V.M., Vacondio, B., Javaroti, D.C.D., Seleghim, M.H.R., Cavalcanti, B.C., Pessoa, C., Moraes, M.O., Lima, B.A., Gonçalves, R., Bonugli-Santos, F.C., Sette, L.D., Berlinck, R.G.S., 2012. Evaluating methods for the isolation of marine-derived fungal strains and production of bioactive secondary metabolites. Rev. Bras. Farmacogn**.** 22, 257-267.

Lu, C., Luo, Z., Huang, L., Li, X., 2011. The Ru-catalyzed enantioselective preparation of chiral halohydrins and their application in the synthesis of (*R*)-clorprenaline and (*S*)-sotalol. Tetrahedron: Asymmetry 22, 722-727.

Martins, M.P., Ouazzani, J., Arcile, G., Jeller, A.H., Lima, J.P.F., Seleghim, M.H.R., Oliveira, A.L.L. Debonsi, H.M., Venâncio, T., Yokoya, N.S., Fujii, M.T., Porto, A.L.M., 2015. Biohydroxylation of (−)-Ambrox®, (−)-Sclareol, and (+)-Sclareolide by whole cells of Brazilian marine-derived fungi. Mar. Biotechnol. 17, 211-218.

Menezes, C.B.A. Bonugli-Santos, R. C., Miqueletto, P.B., Passarini, M.R., Silva, C.H., Justo, M.R., Leal, R.R., Fantinatti-Garboggini, F., Oliveira, V.M., Berlinck, R.G., Sette, L.D., 2009. Microbial diversity associated with algae, ascidians and sponges from the north coast of São Paulo state. Brazil. Microbiological Res. 165, 466-482.

Rocha, L.C., Ferreira, H.V., Pimenta, E.F., Berlinck, R.G.S., Selehim, M.H.R., Javaroti, D.C.D., Sette, L.D., Bonugli, R.C., Porto, A.L.M., 2009. Bioreduction of alpha-chloroacetophenone by whole cells of marine fungi. Biotechnol. Lett**.** 31, 1559-1563.

Rocha, L.C., Ferreira, H.V., Pimenta, E.F., Berlinck, R.G.S., Rezende, M.O.O., Landgraf, M.D., Seleghim, M.H.R., Sette, L.D., Porto, A.L.M., 2010. Biotransformation of alpha-bromoacetophenones by the marine fungus *Aspergillus sydowii*. Mar. Biotechnol. 12, 552-557.

Rocha, L.C., Souza, A.L., Rodrigues Filho, U.P., Campana Filho, S.P., Sette, L.D., Porto, A.L.M., 2012. Immobilization of marine fungi on silica gel, silica xerogel and chitosan for biocatalytic reduction of ketones. J. Mol. Catal. B: Enzym. 84, 160-165.

Rocha, L.C., Souza, Seleghim, M.H.R., Comasseto, J.V., Sette, L.D., Porto, A.L.M., 2015. Stereoselective bioreduction of α-azido ketones by whole cells of marine-derived fungi. Mar. Biotechnol. 1, 736-742.

Rodrigues, G.N., Alvarenga, N., Vancondio, B., De Vasconcellos, S.P., Passarini, M.R.Z., Seleghim, M.H.R. Porto, A.L.M., 2016. Biotransformation of methyl parathion by marine-derived fungi isolated from ascidian *Didemnum ligulum*. Biocatal. Agric. Biotechnol. 7, 24-30.

Soni, R. Hall, T.H. Morris, D.J. Clarkson, G.J. Owen, M.R. Wills, M., 2015. *N*-Functionalised TsDPEN catalysts for asymmetric transfer hydrogenation; synthesis and applications. Tetrahedron Lett. 56, 6397-6401.

Trincone A., 2011. Marine biocatalysts: enzymatic features and applications. *Mar. Drugs* 9, 478–499.

Vacondio, B., Birolli, W.G., Ferreira, I.M., Seleghim, M.H.R., Gonçalves, S., Vasconcellos, S.P., Porto, A.L.M., 2015. Biodegradation of pentachlorophenol by marine-derived fungus *Trichoderma harzianum* CBMAI 1677 isolated from ascidian *Didemnun ligulum*. Biocatal. Agric. Biotechnol. 4, 266-275.

Walton, A., Conerly, W., Pompeu, Y., Sullivan, B., Stewart, J., 2011. Biocatalytic reductions of Baylis-Hillman adducts. ACS Catal. 1, 989-993.

Wu, X-F., Min, C., Nyamzundui, E., Zhou, H-B., Dong, C., 2011. A novel C3-symmetric prolinol-squaramide catalyst for the asymmetric reduction of ketones by borane. Tetrahedron: Asymmetry 22, 1640-1643.

Yang, W., Xu, J., Xie, Y., Xu, Y., Zhao, G., Lin, G., 2006. Asymmetric reduction of ketones by employing *Rhodotorula* sp. AS2.2241 and synthesis of the beta-blocker (*R*)-nifenalol. Tetrahedron Asymmetry 17, 1769-1774.

Zampieri, D., de Paula. B., Zampieri, L., Vale, J., Rodrigues, J., Moran, P.J., 2013. Enhancements of enantio and diastereoselectivities in reduction of (*Z*)-3-halo-4-phenyl-3-buten-2-one mediated by microorganisms in ionic liquid/water biphasic system. J. Mol. Catal. B: Enzym. 85, 61-64.

Zhou, Z., Li, L., He, X., 2012. A simple and convenient method for direct alpha-chlorination of ketones with ammonium chloride and Oxone$^{(R)}$. Chin. Chem. Lett. 23, 1213-1216.

Highlight

- Synthesis of α-chloroketones with oxone® and NH_4Cl under microwave irradiation and conventional heating;
- Synthesis of α-chlorophenones *in situ* followed by biocatalytic reduction;
- Fungi marine used in the obtaining of chiral chlorohydrins.