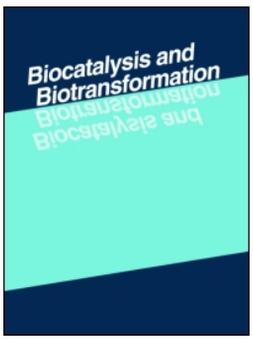


Biocatalysis and Biotransformation

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ibab20

Regioselective and chemoselective biotransformation of 2'-hydroxychalcone derivatives by marine-derived fungi

Iara Lisboa de Matos, Marcia Nitschke & André Luiz Meleiro Porto

To cite this article: Iara Lisboa de Matos, Marcia Nitschke & André Luiz Meleiro Porto (2021): Regioselective and chemoselective biotransformation of 2′-hydroxychalcone derivatives by marine-derived fungi, Biocatalysis and Biotransformation, DOI: 10.1080/10242422.2021.1956909

To link to this article: https://doi.org/10.1080/10242422.2021.1956909

BIOCATALYSIS AND BIOTRANSFORMATION
https://doi.org/10.1080/10242422.2021.1956909

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

Regioselective and chemoselective biotransformation of 2′-hydroxychalcone derivatives by marine-derived fungi

Iara Lisboa de Matos[a], Marcia Nitschke[b] and André Luiz Meleiro Porto[a]

[a]Laboratory of Biocatalysis and Organic Synthesis, São Carlos Institute of Chemistry, University of São Paulo, São Carlos, Brazil; [b]Laboratory of Microbial Biotechnology, São Carlos Institute of Chemistry, University of São Paulo, São Carlos, Brazil

ABSTRACT

Eight fungal strains (*Penicillium raistrickii* CBMAI 931, *Cladosporium* sp. CBMAI 1237, *Aspergillus sydowii* CBMAI 935, *Penicillium oxalicum* CBMAI 1996, *Penicillium citrinum* CBMAI 1186, *Mucor racemosus* CBMAI 847, *Westerdykella* sp. CBMAI 1679, and *Aspergillus sclerotiorum* CBMAI 849) mediated the biotransformation of the 2′-hydroxychalcone **1a**. The main products obtained were from hydrogenation, hydroxylation, and cyclization reactions. *Penicillium raistrickii* CBMAI 931 catalyzed the chemoselective reduction of **1a** to produce 2′-hydroxydihydrochalcone **2a** (72%) in 7 days of incubation in phosphate buffer (pH 7). *Aspergillus sydowii* CBMAI 935 promoted the hydroxylation of **1a** to yield 2′,4-dihydroxy-dihydrochalcone **5a** (c = 42%) in 7 days of incubation in phosphate buffer (pH 8). The reaction using *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 presented main cyclization products in phosphate buffer (pH 8), but the reactions with these fungi did not present enantioselectivity. Marine-derived fungi were effective and versatile biocatalysts for biotransformation of the 2′-hydroxychalcones yielding different products according to the conditions and microorganism used.

ARTICLE HISTORY
Received 18 February 2021
Revised 14 June 2021
Accepted 13 July 2021

KEYWORDS
Reduction reaction; marine fungi; biotransformation; 2′-hydroxychalcone; biocatalysis

Introduction

Chalcones are α,β-unsaturated compounds of natural occurrence that are easily found in fruits and vegetables (Zenger et al. 2015; Dan and Dai 2020). These compounds are biosynthetic precursors of many natural products belonging to the class of flavonoids, such as flavanones and flavanols (Zenger et al. 2015). The skeleton of chalcones (C6–C3–C6) consists of two aromatic rings connected by three α,β-unsaturated carbon atoms, allowing multiple substituents for them. In nature, these compounds are generally found with hydroxyl groups in their aromatic rings, but a variety of substituents may be synthetically incorporated into the backbone of chalcones.

The basic skeleton of chalcones has characteristics that attract the attention of both the chemical and biological point of view since the synthesis of compounds of this class is performed with simple procedures that allow obtaining multi-substituted compounds with various sites capable of (bio)transformation (Kozłowska et al. 2018; Li et al. 2018). These characteristics can assign a variety of biological activities, including antidiabetic, antihypertensive, antiretroviral, anti-inflammatory, antihistamine, antioxidant, antimalarial, and anticancer (Mahapatra and Bharti 2016; Kim et al. 2021).

Chalcone derivatives are a target for scientific studies in reason of the pharmacological properties that they have. In this perspective, the versatility of enzymes that catalyze many types of organic reactions has been used to obtain flavonoids and other chalcone derivatives. Chalcones may undergo biotransformation to yield a variety of compounds, among which include hydrogenation, hydroxylation, methylation, and cyclization reactions (Kozłowska et al. 2018). This diversity of products is a result of the various sites of transformation found in the structural framework of chalcones. For example, 2′-hydroxychalcone may undergo hydrogenation reactions of the double bond, a carbonyl reduction, cyclization for the formation of flavanones, the addition of substituents on the aromatic rings, and even the formation C–C bonds by Michael addition. Faced with so many transformation possibilities, 2′-hydroxychalcones are interesting

CONTACT André Luiz Meleiro Porto almporto@iqsc.usp.br Laboratory of Biocatalysis and Organic Synthesis, São Carlos Institute of Chemistry, University of São Paulo, Av. João Dagnone, 1100, Ed. Química Ambiental, J. Santa Angelina, São Carlos 13563-120, Brazil

Supplemental data for this article can be accessed here.

This article has been republished with minor changes. These changes do not impact the academic content of the article.

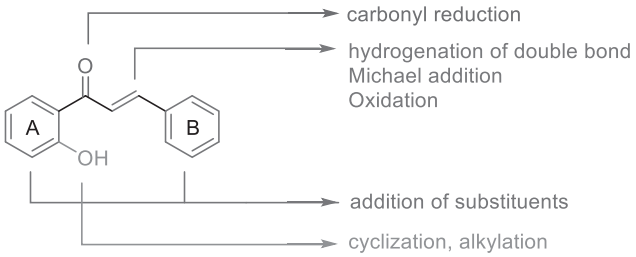


Figure 1. Possible reaction sites on the structural skeleton of 2′-hydroxychalcone.

substrates to study the biocatalytic potentialities using fungi isolated from the marine environment (Figure 1).

Enzymes may catalyze various reactions, including hydroxylation, glycosylation, hydrogenation, dehydrogenation, hydrolyses, *O*-methylation, *O*-acetylation, among others (Giri et al. 2001; Banerjee et al. 2012; Cao et al. 2015). In general, the reactions are performed using isolated enzymes, microorganisms (fungi, bacteria, and yeasts), and vegetable and animal cells. Biocatalytic reactions employing microorganisms show advantages, such as rapid microbial growth, large-scale enzyme production, being biodegradable, and the chemo-, regio-, and stereoselective control of biotransformation.

Works of literature have reported 2′-hydroxychalcones as starting material to produce flavanones. The conversion of 2′-hydroxychalcone to flavanone is performed using basic or acidic catalysts, amino acids, microwave radiation, and enzymes (Raghav et al. 2016). Fungi and bacteria were used for the biotransformation of 2′-hydroxychalcones to flavanones (Alarcón et al. 2013; Kostrzewa-Susłow et al. 2017). Cyclization products were also observed in the biotransformation of 3-(2′′,3′′-dimethoxyphenyl)-1-(2′-hydroxyphenyl)propenone(2′-hydroxy-2,3-dimethoxychalcone) by *Aspergillus alliaceus* UI 315 (Sanchez-Gonzalez and Rosazza 2004).

Marine-derived fungi have been studying targets by our research group for the biotransformation and biodegradation of various compounds. The fungus *Penicillium citrinum* CBMAI 1186 was used for the regioselective reduction of chalcones, enones, and Knoevenagel adducts (Ferreira et al. 2015; Jimenez et al. 2016). *Aspergillus sydowii* CBMAI 935, and *Penicillium decaturense* CBMAI 1234 promoted the biodegradation of methyl parathion (Alvarenga et al. 2014). The stereoselective bioreduction of α-azido ketones and acetophenone derivatives were also performed using marine-derived fungi (Ribeiro et al. 2015; Rocha et al. 2015; Mouad et al. 2016). Thus, these studies showed that marine organisms could be excellent sources of different enzymes capable of catalyzing reactions in organic molecules.

Strains of bacteria and fungi were used in biotransformation reactions of chalcones derivatives, but studies employing marine-derived fungi are scarce in the literature (Ferreira et al. 2014). In a recent study, we investigated the biotransformation of chalcones with fungi from the marine environment for the obtention of dihydrochalcones (de Matos et al. 2019). In the present paper, we report the biotransformation of 2′-hydroxychalcone by eight marine-derived fungi to produce flavonoid and 2′-hydroxychalcone derivatives.

Materials and methods

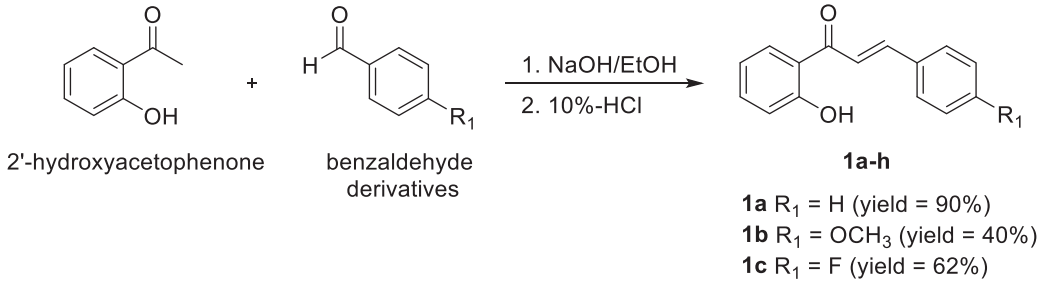
Chemical reagents and culture media

Benzaldehyde (99%), 2-Hydroxyacetofenone (99%), and 4-fluorobenzaldehyde (98%) were purchased from Sigma-Aldrich, and 4-methoxybenzaldehyde (98%) from Vetec. Sodium hydroxide (97%) and hydrochloric acid (37%) were obtained from Quemis (Brazil). The organic solvents, that is, ethylic alcohol, ethyl acetate, and dimethyl sulfoxide, were purchased from Synth. All reagents were used without further purification. The Malt Extract was obtained from Kasvi and Agar bacteriological from Himedia.

General methods

The manipulations involving the marine-derived fungi were performed in a laminar flow cabinet (Veco). All materials and culture media were previously sterilized in a vertical autoclave (Phoenix, Av-50) for 20 min at 121 °C. The artificial seawater was prepared according to: $CaCl_2{\cdot}2H_2O$ (1.36 g L^{-1}), $MgCl_2{\cdot}6H_2O$ (9.68 g L^{-1}), NaCl (30.00 g L^{-1}), Na_2HSO_4 (0.14 g L^{-1}), KCl (0.61 g L^{-1}), Na_2SO_4 (3.47 g L^{-1}), $NaHCO_3$ (0.17 g L^{-1}), KBr (0.10 g L^{-1}), $SrCl_2{\cdot}6H_2O$ (0.04 g L^{-1}), and H_3BO_3 (0.03 g L^{-1}).

The conversions of products were determined by Gas Chromatography/Mass Spectrometry (GC/MS). The analyses were performed on Shimadzu GC2010plus/MS2010plus in electron ionisation (EI, 70 eV) with a DB5 column (30 m × 0.25 mm × 0.25 one, J & W Scientific). The method conditions were as follows: initial oven temperature of 90 °C for 4 min, increased to 280 °C at 10 °C min^{-1}, and held for 5 min; finally, the temperature was increased to 300 °C at 10 °C min^{-1}, and held for 10 min. The total time of analysis was 40 min. The following conditions were used: injector temperature, 250 °C; interface temperature, 270 °C; injector split ratio of 1:20. Helium was used as carrier



Scheme 1. Synthesis of the 2′-hydroxychalcone derivatives by Claisen–Schimdt reaction.

gas with an initial flow of 0.75 mL min^{-1}, and the injection volume was 0.5 μL of solution with a concentration of 5.0 mg mL^{-1}. The fragment ions were detected in the range of 40–350 Da.

The chiral analyses were performed by high-performance liquid chromatography (HPLC-UV) in a Shimadzu2010 chromatograph: CBM-20A Controller LC-20AT pump, DGU-20A5 degasser, sampler SIL-20AHT, CTO-20A column oven, and SPD-M20A UV/VIS detector operating at 248 nm. The column used was a Phenomenex LUX® chiral column (0.46 cm × 25 cm; 5 μM) in isocratic mode with hexane and isopropanol (9.5:0.5) and flow rate of 0.5 mL min^{-1}. The total analysis time was 40 min.

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra of the purified compounds were recorded on Agilent Technologies 500/54 Premium Shielded (^{1}H NMR and ^{13}C at 500 and 125 MHz) spectrometer. The samples were solubilized in DMSO-d_6, and the chemical shifts were reported in ppm relative to an internal standard, tetramethylsilane (TMS). Coupling constants (*J*) were expressed in hertz (Hz).

Fourier transform infra-red (FTIR) spectra of the substrates and purified products were recorded on a Shimadzu IRAffinity 1 spectrometer model. Analyses were performed using KBr discs for solid samples. The spectra were recorded in transmittance, and the wavenumber of vibrational bands was performed at 4000–400 cm^{-1} (SM).

The optical rotation products were measured in $CHCl_3$ in a Jasco – Model P-2000 polarimeter equipped with a Na-lamp ($\lambda = 589$ nm), 1.0 cm cuvette, and 1.0 mL volume of solution at 23 °C. The concentration of compounds was reported in g/100 mL.

Synthesis of the chalcones

The 2-hydroxychalcone **1a** were synthesized, according to Ferreira et al. (2014). 2-hydroxyacetophenone (0.010 mol) and benzaldehyde (0.011 mol) were added in a flask containing ethanol (50 mL). After stirring on the magnetic plate (10 min) NaOH (5 mol L^{-1}, 5.0 mL) was added dropwise. The reaction mixture was stirred at room temperature for 12 h. Then, HCl (10%, 10 mL) was added to stop the reaction. The precipitate formed was filtered under vacuum and washed with cold water. The 2′-hydroxychalcone **1a** was obtained in good yield (90%) and characterized by NMR (^{1}H and ^{13}C), MS, and FT-IR. The identification was performed comparing spectroscopic data with data described in the literature (Supplementary material). The 4-methoxy-2′-hydroxychalcone **1b** and 4-fluoro-2′-hydroxychalcone **1c** were synthesized following the same experimental procedure (Scheme 1).

Culture of marine-derived fungi

Fungi (*Penicillium raistrickii* CBMAI 931, *Cladosporium* sp. CBMAI 1237, *Aspergillus sydowii* CBMAI 935, *Penicillium oxalicum* CBMAI 1996, *Penicillium citrinum* CBMAI 1186, *Mucor racemosus* CBMAI 847, *Westerdykella* sp. CBMAI 1679, and *Aspergillus sclerotiorum* CBMAI 849) were cultured according to the following procedure: in a 250 mL Erlenmeyer flask was added 10 small slices of solid stock culture (0.5 × 0.5 cm) bearing mycelia of fungi and 100 mL of liquid culture medium (Malt Extract in artificial seawater, 20 g L^{-1}). The mycelia of marine-derived fungi were maintained in an orbital shaker for seven days at 32 °C, shaking at 130 rpm.

Screening of marine-derived fungi for biotransformation reaction of the 2′-hydroxychalcone 1a

After 7 days of growth in the liquid medium, the mycelia were harvested by apparatus Buchner filtration, and wet mycelia were used in the biotransformation reactions. Screening for the biotransformation of 2′-hydroxychalcone **1a** was carried out in 50 mL Erlenmeyer flasks, each containing 20 mL of the medium consisting of Malt Extract (20 g L^{-1}) in

phosphate buffer (pH 7.0 and 8.0, Na_2HPO_4/KH_2PO_4, 0.1 mol L^{-1}), 2.5 g of the wet weight of mycelia of fungi, and 25 mg (0.11 mmol) of 2′-hydroxychalcone **1a** previously dissolved in 200 μL DMSO. The reactions were maintained for seven days in an orbital shaker at 32 °C and 130 rpm. After seven days of incubation, the mixture was removed, 20 mL of ethyl acetate was added, and the mixture was maintained for 30 min with magnetic stirring. Then, the mixture was centrifuged (15 min, 10,000 rpm), and the mycelia were separated by filtration. The liquid phase was extracted with ethyl acetate (3 × 10 mL). The organic phase was dried with anhydrous sodium sulphate, filtered, evaporated under reduced pressure, and analyzed by chromatographic methods (GC/MS). The reactions were performed in duplicate.

Isolation of the products of biotransformation from 2′-hydroxychalcone 1a by Aspergillus sydowii CBMAI 935

The extracts obtained from biotransformation reactions using *A. sydowii CBMAI 935* were purified using a chromatography column over silica gel flash using hexane and ethyl acetate (9.5:0.5 *v/v*). After purification, we obtained the products of 2′-hydroxydihydrochalcone **2a**, flavanone **3a**, and 2′,4-dihydroxydihydrochalcone **4a**, and determined their yields. The characterization of the products was performed using NMR (^1H and ^{13}C), IR, and GC/MS, and the data were confirmed with those reported in the literature (Supplementary material).

Results and discussion

Screening of marine-derived fungi for the biotransformation of 2′-hydroxychalcone 1a

Initially, 2′-hydroxychalcone **1a** was used for biotransformation reactions in the screening of eight strains of marine-derived fungi (*P. raistrickii* CBMAI 931, *Cladosporium* sp. CBMAI 1237, *A. sydowii* CBMAI 935, *P. oxalicum* CBMAI 1996, *P. citrinum* CBMAI 1186, *M. racemosus* CBMAI 847, *Westerdykella* sp. CBMAI 1679, and *A. sclerotiorum* CBMAI 849). The fungi were selected due to promising results obtained by our research group using these microorganisms in the reduction of chalcones and biotransformation reactions of pesticides (Alvarenga et al. 2014; Ferreira et al. 2014; de Matos et al. 2019).

The experiments were performed using fungal mycelia in phosphate buffer (Na_2HPO_4/KH_2PO_4 0.1 mol L^{-1}, pH 7 and 8) for 7 days. Phosphate buffer was used at two acidic values; pH 7 was selected because it is neutral and pH 8 because the seawater is slightly alkaline, with pH 7.4–8.5. GC-MS analyses showed the formation of three main products. 2′-Hydroxydihydrochalcone **2a** and flavanone **3a** were obtained in all experiments, and the 4,2′-dihydroxydihydrochalcone **4a** was produced only by *A. sydowii* CBMAI 935 (Table 1).

All of the marine-derived fungi used in the biotransformation of 2′-hydroxychalcone **1a** were able to promote the chemoselective hydrogenation of the C = C double bond of the α,β-unsaturated system of 2′-hydroxychalcone **1a**, with emphasis for fungi *P. raistrickii* CBMAI 931 (c = 72%, Entry 1, Table 1) and *A. sclerotiorum* CBMAI 849 (c = 81%, Entry 8, Table 1).

In general, the reaction condition employing phosphate buffer pH 7 was the one that most favoured the reduction of 2′-hydroxychalcone **1a**. At pH 7, the fungus *P. raistrickii* CBMAI 931 promoted 72% of conversion in the 2′-hydroxydihydrochalcone **2a** and 18% conversion in the flavanone **3a**. In phosphate buffer pH 8 the formation of compound **2a** (c = 46%) was somewhat lower. Meanwhile, there was an increase in the formation of flavanone **3a** with c = 46% (Entry 1, Table 1). The chemoselectivity of the reactions using the fungus *P. raistrickii* CBMAI 931 was studied by de Matos et al. (2019) from the biotransformation of halogenated 2′-hydroxychalcones.

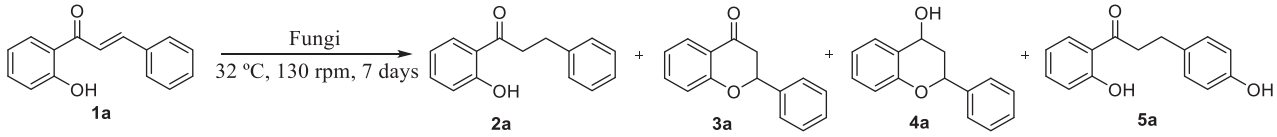
The reaction with *A. sclerotiorum* CBMAI 849 followed the same pattern of formation of the compounds. At pH 7, a higher form of 2′-hydroxydihydrochalcone **2a** (c = 81%) was obtained compared to flavanone **3a** (c = 7%). While at pH 8, there was an increase in the formation of flavanone **3a** (c = 23%) and a decrease in the formation of **2a** (c = 68%) (Entry 8, Table 1).

By HPLC analysis using a chiral column, it was observed that both *P. raistrickii* CBMAI 931 and *A. sclerotiorum* CBMAI 849 showed no selectivity for flavanone **3a**, presenting in the racemic form (Supplementary Material, SM).

The fungus *Westerdykella* sp. CBMAI 1679 also promoted the biotransformation of 2′-hydroxychalcone **1a** to 2′-hydroxydihydrochalcone **2a** with 67% and 48% conversions in phosphate buffer pH 7 and pH 8, respectively (Entry 5, Table 1).

Marine-derived fungi are sources of Ene reductases (ERs), which are the enzymes responsible for catalyzing (asymmetric) reductions of double bonds conjugated to electron-withdrawing groups. These enzymes are dependent on flavin mononucleotide (FMN) and are part of the family of old yellow

Table 1. Biotransformation of 2′-hydroxychalcone **1a** by marine-derived fungi in phosphate buffer (Na_2HPO_4/KH_2PO_4 0.1 mol L^{-1}, pH 7 and pH 8, 32 °C, 130 rpm, 7 days).



		Conversion (%)[a]							
		pH 7				pH 8			
Entry	Fungi	2[a]	3a	4a	5a	2a	3a	4a	5a
1	*P. raistrickii* CBMAI 931	72	18			46	46		
2	*Cladosporium* sp. CBMAI 1237	18	7			28	8	8 *trans* 5 *cis*	
3	*P. oxalicum* CBMAI 1996	49	9			17	45		
4	*A. sydowii* CBMAI 935	10	9		29	36	1		46 (26)[b]
5	*Westerdykella* sp. CBMAI 1679	67	6			48	8		
6	*P. citrinum* CBMAI 1186	13	22			8	51 (36)[b]		
7	*M. racemosus* CBMAI 847	8	20			8	47 (30)[b]		
8	*A. sclerotiorum* CBMAI 849	81	7			68	23		

[a]Determined by GC-MS (EI, 70 eV); [b]Isolated yield.

enzymes (OYEs) (Forejtníková et al. 2005; Skrobiszewski et al. 2013).

The fungus *Cladosporium* sp. CBMAI 1237 showed the best conversions in phosphate buffer pH 8, obtaining 28% of the **2a** product and 8% of flavanone **3a** (Entry 2, Table 1). *Cladosporium* sp. CBMAI 1237 also promoted the formation of two other compounds with low conversions, which were not isolated, but on low-resolution GC-MS analysis, it was suggested to treat the *cis* and *trans* isomers of flavan-4-ol **4a** (analysis confirmed by a synthetic standard). In the reactions of 2′-hydroxychalcone **1a** with *Westerdykella* sp. CBMAI 1679 and *Cladosporium* sp. CBMAI 1237, the flavanone **3a** was formed as a racemic mixture with 8% conversion (Entries 2 and 5, Table 1).

The fungus *A. sydowii* CBMAI 935 promoted the formation of three compounds, 2′-hydroxydihydrochalcone **2a** (c = 10%, pH 7), flavanone **3a** (c = 9%, pH 7), and a hydroxyl compound **5a** (c = 29%, pH 7) (Entry 4, Table 1). Reactions carried out at pH 8 showed the best conversions for 2′-hydroxydihydrochalcone **2a** (c = 36%) and hydroxylated product **5a** (c = 46%). The mass spectrum of hydroxylated compound **5a** showed the molecular ion $M^{+\bullet}$ 242 (34%), indicating the addition of a hydroxyl group to the dihydrogenated product **2a** ($M^{+\bullet}$ 226, 35%). The hydroxylated compound **5a** (isolated yield = 26%) was isolated by column chromatography, and its structure was confirmed by spectroscopic analysis. The compound was identified as 1-(2′-hydroxyphenyl)-3-(4-hydroxyphenyl)propan-1-one **5a**. Data on the characterization are presented in Supplementary Material. Alarcón et al. (2013) reported the formation of cyclization products (c = 27%) and hydroxylation (c = 22%) in the biotransformation reaction of 2′′,4′′-dimethoxy-2′-hydroxychalcone by *A. niger* fungus. The hydroxylation reactions of 2′-hydroxychalcone **1a** are being investigated in the laboratory.

Penicillium citrinum CBMAI 1186 and *M. racemosus* CBMAI 847 showed higher formations of the cyclized product **3a** when compared to the other fungi (Table 1). The cyclization of 2′-hydroxychalcone occurred due to the presence of the hydroxyl group at the 2′-position of the A-aromatic ring since the oxygen of the hydroxyl can bind to the Cβ of the double bond of the **1a** yielding the cyclized product **3a**. At pH 8, we obtained higher values of conversion for the formation of cyclized product **3a**, with 51% and 47% conversion for *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847, respectively (Entries 6–7, Table 1). The flavanone **3a** obtained in reactions from the biotransformation by *P. citrinum* CBMAI 1186 (isolated yield = 36%) and *M. racemosus* CBMAI 847 (isolated yield = 30%) were analyzed by HPLC-UV equipped with a chiral column, but we only observed a racemic mixture of enantiomers (Figure 2). Therefore, the flavanone cyclization reaction did not show enantioselectivity.

The presence of the hydroxyl group at the 2′-position in the 2′-hydroxychalcone **1a** ring A favours the

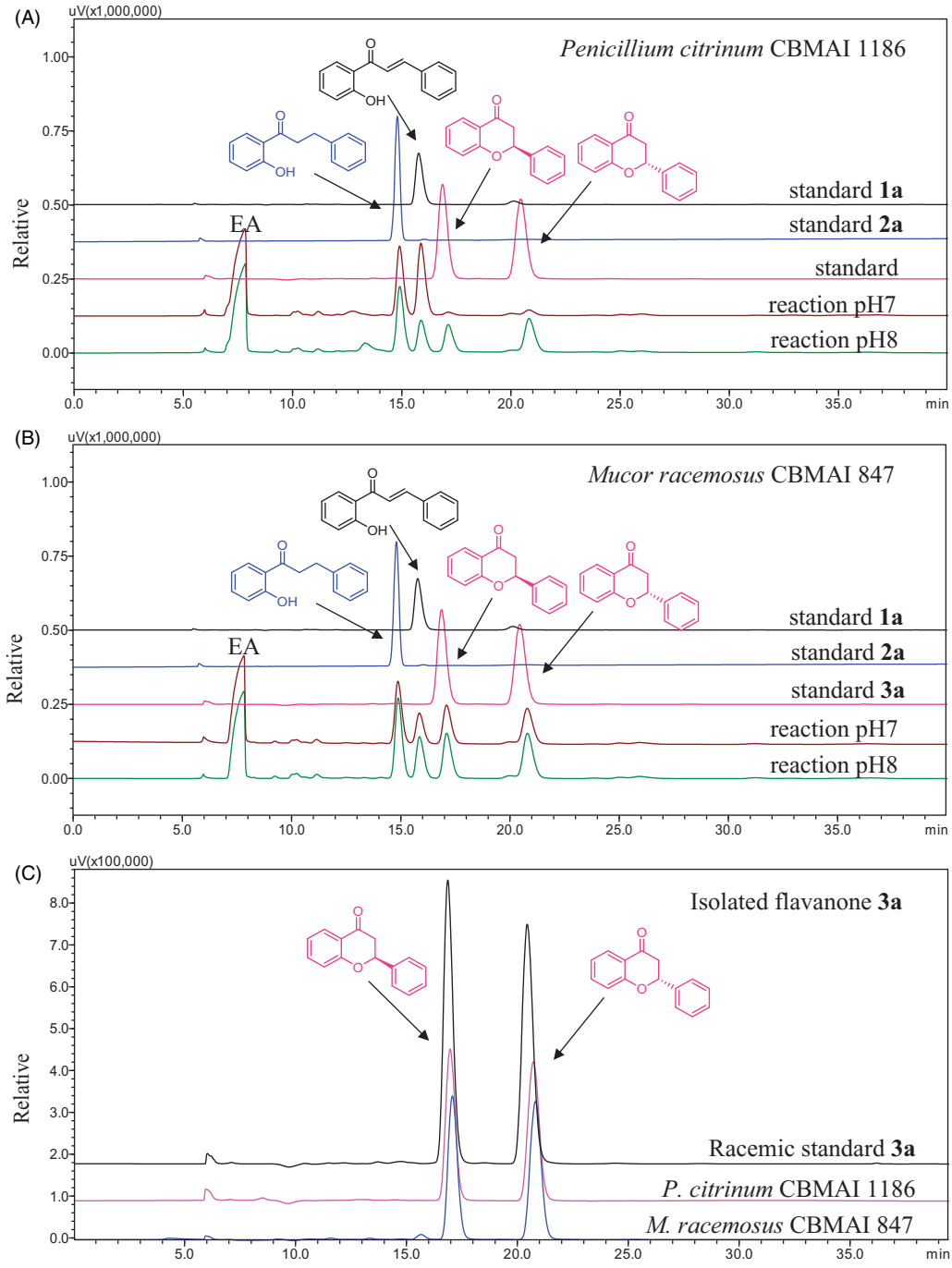


Figure 2. Chromatograms obtained by HPLC using chiral column: (A) reactions of **1a** by *P. citrinum* CBMAI 1186; (B) reactions of **1a** by *M. racemosus* CBMAI 847; (C) Flavanone **3a** isolated from the reactions of **1a** by *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 after 7 days of incubation. Analysis conditions: Chromatograms obtained by HPLC-UV (Lux® Phenomenex column, hexane/isopropanol 9.5: 0.5, 0.5 mL min^{-1}, 40 min, $\lambda = 246$ nm); **2a** (t_r = 14.8 min), **1a** (t_r = 15.9 min), (*S*)-**3a** (t_r = 17.2 min), (*R*)-**3a** (t_r = 20.9 min). *Note.* the peak at t_r = 7.5 min refers to the solvent residue ethyl acetate (EA).

chalcone cyclization for the formation of flavanone **3a**, since the oxygen of the hydroxyl can bind to the Cβ of the double bond. The fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 presented a higher form of the cyclized product **3a** when compared to the other fungi tested (Table 1). At pH 8, higher conversion values were obtained for the formation of cyclization product **3a**, with 51% and 47% for *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847, respectively.

The use of microorganisms containing specific or promiscuous enzymes may allow the formation of predominantly one enantiomer. Thus, abiotic controls and flavanone **3a** isolated in the biocatalytic reactions with

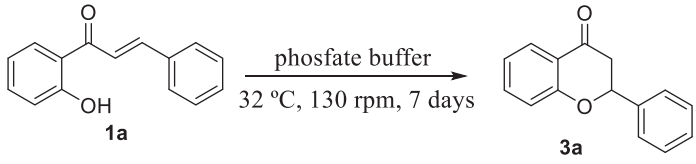
fungi *P. citrinum* CBMAI 1186 (yield = 36%) and *M. racemosus* CBMAI 847 (yield = 30%) were analyzed by HPLC using a chiral column, where it was possible to verify that the product **3a** was obtained in a racemic mixture.

However, it is known that 2′-hydroxychalcone **1a** can be converted to flavanone **3a** when in an aqueous solution (Jez and Noel 2002; Li et al. 2018). Thus, to evaluate the interference of the reaction medium, abiotic controls were performed for 2′-hydroxychalcone **1a**, where we observed spontaneous cyclization in phosphate buffer in all pH values tested (Table 2). Specifically, presenting significant cyclized in pH 7 (c = 24%) and pH 8 (c = 39%). Considering the proximity of the results obtained in the abiotic controls with those of the biotransformation reactions, we performed new experiments at pH values where the spontaneous cyclization could be minimized. Thus, abiotic controls were also carried out in phosphate buffer at pH 5 and 6.

From the abiotic controls it was observed that, with the increase in the pH of the medium (pH 7 to pH 8), there was also an increase in the cyclization of 2′-hydroxychalcone **1a** to flavanone **3a**. Given that the abiotic control in phosphate buffer (pH 6) showed the lower formation of flavanone **3a**, experiments were carried out with the fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 to evaluate the performance of these fungi enzymes under these conditions (Table 3).

In the reactions carried out in pH 6 phosphate buffer, low formations of **3a** were obtained for both *P. citrinum* CBMAI 1186 (c = 6%) and *M. racemosus* CBMAI 847 (c = 2%) (Table 3). These results indicated the strong influence of the reactional medium on the cyclization of compound **3a**. In contrast, the dihydrogenation product **2a** was favoured under these conditions and was obtained with 10% and 41% for *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847, respectively. As can be recognized, pH plays an important role in reactions involving enzymes, as they can affect the shape of an enzyme, as well as change the ionic state of the substrate and the enzymes involved in the reaction, resulting in a change in enzymatic activities and enantioselectivities (Krist et al. 1998; Lou et al. 2004).

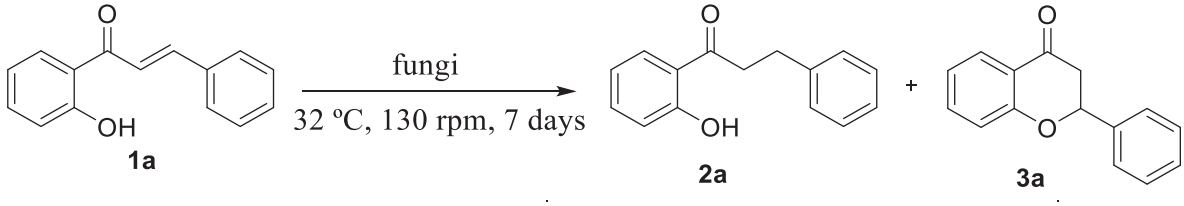
Table 2. Abiotic controls of the 2′-hydroxychalcone **1a** in phosphate buffer (Na_2HPO_4/KH_2PO_4 0.1 mol L^{-1}, 32°C, 130 rpm, 7 days).



pH	Conversion (%)[a] 3a	*ee*[b] (**3a**)
5.2	10	*rac*[c]
6.0	6	*rac*
7.0	24	*rac*
8.0	39	*rac*

[a]Determined by GC-MS; [b]*ee* = ennantiomeric excess obtained by HPLC chiral analysis; [c]*rac* = racemic product.

Table 3. Biotransformation of 2′-hydroxychalcone **1a** by marine-derived fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 (Na_2HPO_4/KH_2PO_4 0.1 mol L^{-1}, pH 6).



Marine-derived fungi	Conversions (%)[a] 2a	3a	*ee*[b] (**3a**)
P. citrinum CBMAI 1186	10	6	*rac*[c]
M. racemosus CBMAI 847	41	2	*rac*

[a]Determined by GC-MS (EI, 70 eV). [b]*ee* = ennantiomeric excess, obtained by HPLC chiral analysis; [c]*rac* = racemic product.

Studies in the literature showed that the fungus *Aspergillus niger* promoted the cyclization of a series of substituted 2′-hydroxychalcones, and yields ranged between 10% and 27%, and enantiomeric excesses were not observed (Alarcón et al. 2013). The fungus *Aspergillus alliaceus* UI 315 promoted the cyclization of 2′-hydroxy-2,3-methoxychalcone with low yields of 6–16% and without enantioselectivity (Sanchez-Gonzalez and Rosazza 2004). The cyclization of xanthohumol to the aurone and flavanone forms employing fungi has also been reported in the literature (Herath et al. 2003; Tronina et al. 2013, 2014)

The stereoselective cyclization of chalcones to obtain enantiomerically enriched flavanones is catalyzed by the enzyme chalcone isomerase (CHI). Cyclization studies of substituted 2′-hydroxychalcones using the CHI enzyme showed that deprotonation of the 2-hydroxyl group occurs easily and that the presence of electron-withdrawing groups in the B ring improves the reactivity of the monoanion (Jez and Noel 2002; Li et al. 2018).

Given the absence of enantioselectivity in the reactions using *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847, and the proximity of the conversion values obtained with the abiotic controls at pH 7 (c = 24%) and pH 8 (c = 39%), the cyclization of 2′-hydroxychalcone **1a** in the biotransformation reactions possibly occurred in the reaction medium or even with amino acids present in fungal cells/mycelia. Amino acids, such as L-proline, L-alanine, L-serine, and L-leucine, have been employed as additives in the intramolecular cyclization of 2′-hydroxychalcones in basic medium forming a racemic mixture of the corresponding flavanones (Tanaka and Sugino 2001; Jiang et al. 2011).

In conclusion, the biotransformation reactions with *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 were the most efficient in promoting the cyclization of substrate **1a** in the corresponding flavanone **3a** but did not present enantioselectivity. Moreover, the conversion values were close to those observed in the abiotic control. The condition that most favoured the reduction of the double bond occurred with the fungus *P. raistrickii* CBMAI 931 at pH 7.

Biotransformation reactions of para-substituted 2′-hydroxychalcones 1b–c by P. raistrickii CBMAI 931, P. citrinum CBMAI 1186, and M. racemosus CBMAI 847

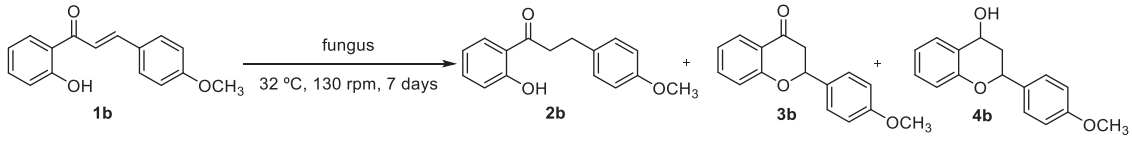
Based on the results obtained in the screening of marine-derived fungi, 3-methoxy-2′-hydroxychalcone **1b** and 4-fluoro-2′-hydroxychalcone **1c** were used in biotransformation reactions by fungi *P. raistrickii* CBMAI 931, *P. citrinum* CBMAI 1186, and *M. racemosus* CBMAI 847 in phosphate buffer (pH 7 and 8) for 7 days.

The biotransformation of compounds **1b** and **1c** mostly led to the formation of cyclization products and reduction of the C = C double bond. As expected, the presence of ERs was observed mainly in the biotransformation reactions using the fungus *P. raistrickii* CBMAI 931 by reducing the C = C double bond of synthetic 2′-hydroxychalcones **1b** and **1c**, with the formation of bioreduced products **2b** and **2c**. The reduction of the C = C double bond of **1b** was higher in the reaction performed at pH 7 with the formation of **2b** with 44% conversion (Entry 1, Table 4). The methoxy substituent is an electron-donor group, making the activated double bond have a more anionic character favouring the reduction of the C = C double bond and the formation of the 4-methoxy-2′-hydroxydihydrochalcone **2b**. At pH 8, we obtained 38% conversion of product **2b**. However, in the reaction performed with *P. raistrickii* CBMAI 931 at pH 8, compound **3b** was produced at 12% conversion.

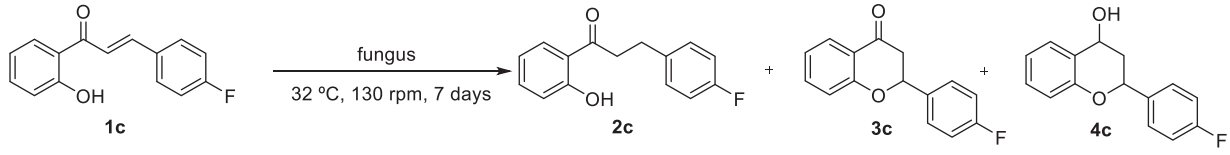
The biotransformation of 4-fluoro-2′-hydroxychalcone **1c** by fungus *P. raistrickii* CBMAI 931 led to the formation of 4-fluoro-2′-hydroxy-dihydrochalcone **2c** and flavanone **3c**. In pH 7phosphate buffer, we obtained compound **2c** at 29% conversion and compound **3c** at 18% conversion. Meanwhile, at pH 8, there was an increase in the formation of both products with the product **2c** at 30% conversion and **3c** at 23% conversion (Entry 5–6, Table 4). The flavanones **3b** and **3c** obtained in the biotransformation reactions of 2′-hydroxychalcones **1b** and **1c** were produced as a racemic mixture. The racemic mixture of flavanones **3b**–**c** was confirmed using a chiral column by HPLC analysis.

The biotransformation reactions of **1b** and **1c** using the fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 showed the low formation of 2′-hydroxy-dihydrochalcones **2b** (c = 0–2%) and **2c** (c = 2–4%) (Entries 2–3 and 6–7, Table 4). Cyclization of substrate **1b** in flavanone **3b** was favoured in the reactions carried out in pH 8 phosphate buffer. Flavanone **3b** was obtained with 19% conversion in the reaction using the fungus *P. citrinum* CBMAI 1186 and 23% conversion using the fungus *M. racemosus* CBMAI 847. Cyclization of **1c** to form flavanone **3c** was also favoured in the basic medium. However, higher conversions with 26% and 30% formation were observed using the fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847, respectively. The presence of the

Table 4. Biotransformation of 2′-hydroxychalcones **1b** to **1c** by marine-derived fungi in phosphate buffer (Na_2HPO_4/KH_2PO_4 0.1 mol L^{-1}, pH 7, pH 8, 32 °C, 130 rpm, 7 days).



		Conversion (%)[a]						
		pH 7				pH 8		
Entry	Fungi	2b	3b	4b	NI	2b	3b	4b
0	Abiotic control of **1b**		9				18	
1	*P. raistrickii* CBMAI 931	44	9			38	12	
2	*P. citrinum* CBMAI 1186	2	11			1	19	2 *cis*
3	*M. racemosus* CBMAI 847	2	11		8		23	1 *cis*



		2c	3c	4c	2c	3c	4c
4	Abiotic control of **1c**		11			27	
5	*P. raistrickii* CBMAI 931	29	18	2 *cis*	30	23	
6	*P. citrinum* CBMAI 1186	3	19	3 *cis*	2	26	2 *cis*
7	*M. racemosus* CBMAI 847	4	7		4	30	1 *cis*

[a]Determined by GC-MS (EI, 70 eV).

fluorine atom in the *para* position of ring B increases the electron deficiency in the α,β-unsaturated system, favouring the nucleophilic attack of the oxygen and consequently allowing the formation of the cyclized product. At pH 8, there was a low conversion in the compounds **4b** and **4c** that may result from the hydroxylation of flavanones **3b** and **3c**.

In contrast, the abiotic controls of the reactions also formed flavanones **3b** and **3c** with significant conversion values (c = 9–27%) that were very close to those obtained in the biotransformation reactions (c = 9–30%). In both cases, flavanones **3b** and **3c** were obtained as racemic mixtures, making it difficult to understand the mechanisms by which the reaction occurs, whether enzymatic or not. It is important to note that despite the spontaneous cyclization of the 2′-hydroxychalcones **1b** and **1c**, the ERs present in the fungus *P. raistrickii* were efficient in favour of the hydrogenation of the C = C double bond for the formation of the 2′-hydroxydihydrochalcones **2b** and **2c**.

Conclusion

The screening of marine-derived fungi presented promising results regarding the biotransformation of 2′-hydroxychalcone **1a**. The main products obtained were the reduction of the C = C double bond and hydroxylation of the B-ring of 2′-hydroxychalcones **1a–c**. The cyclization products obtained in the reactions with the fungi *P. citrinum* CBMAI 1186 and *M. racemosus* CBMAI 847 did not show enantioselectivity. *Penicillium raistrickii* CBMAI 931 efficiently mediated the biotransformation of 2′-hydroxychalcone **1a** as a source of enoate reductase enzymes by reducing the C = C double bond with chemo- and regioselective controls. The fungus *A. sydowii* CBMAI 935 was found to be a source of oxidoreductases by promoting the hydroxylation of 2′-hydroxychalcone **1a** to 1-(2′-hydroxyphenyl)-3-(4-hydroxyphenyl)propan-1-one **5a** at a yield of 26%.

This paper showed that the fungi used were able to perform biotransformation reactions and the results

evidenced the importance of conducting studies involving the biotransformation of 2′-hydroxychalcone derivatives.

Acknowledgements

Iara Lisboa de Matos thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the provision of a scholarship (CAPES Process no. 1313652). 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.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The present work was financially supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP/Proc. 2013/21642-0, 2016/20155-7 and 2014/18257-0) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001.

ORCID

Iara Lisboa de Matos http://orcid.org/0000-0001-5459-5829
Marcia Nitschke http://orcid.org/0000-0001-6107-4183
André Luiz Meleiro Porto http://orcid.org/0000-0002-0857-2340

References

Alarcón J, Alderete J, Escobar C, Araya R, Cespedes CL. 2013. *Aspergillus niger* catalyzes the synthesis of flavonoids from chalcones. Biocatal Biotransform. 31(4):160–167.

Alvarenga N, Birolli WG, Seleghim MHR, Porto ALM. 2014. Biodegradation of methyl parathion by whole cells of marine-derived fungi *Aspergillus sydowii* and *Penicillium decaturense*. Chemosphere. 117(1):47–52.

Banerjee S, Singh S, Rahman LU. 2012. Biotransformation studies using hairy root cultures - a review. Biotechnol Adv. 30(3):461–468.

Cao H, Chen X, Jassbi AR, Xiao J. 2015. Microbial biotransformation of bioactive flavonoids. Biotechnol Adv. 33(1): 214–223.

Dan W, Dai J. 2020. Recent developments of chalcones as potential antibacterial agents in medicinal chemistry. Eur J Med Chem. 187:111980.

de Matos IL, Nitschke M, Porto ALM. 2019. Hydrogenation of halogenated 2′-hydroxychalcones by mycelia of marine-derived fungus *Penicillium raistrickii*. Mar Biotechnol. 21(3): 430–439.

Ferreira IM, Meira EB, Rosset IG, Porto ALM. 2015. Ketones by the marine-derived fungus *Penicillium citrinum* CBMAI 1186 in a biphasic system. J Mol Catal B: Enzym. 115: 59–65.

Ferreira IM, Rocha LC, Yoshioka SA, Nitschke M, Jeller AH, Pizzuti L, Seleghim MHR, Porto ALM. 2014. Chemoselective reduction of chalcones by whole hyphae of marine fungus *Penicillium citrinum* CBMAI 1186, free and immobilized on biopolymers. Biocatal Agric Biotechnol. 3(4):358–364.

Forejtníková H, Lunerová K, Kubínová R, Jankovská D, Marek R, Kareš R, Suchý V, Vondráček J, Machala M. 2005. Chemoprotective and toxic potentials of synthetic and natural chalcones and dihydrochalcones in vitro. Toxicology. 208(1):81–93.

Giri A, Dhingra V, Giri C, Singh A, Ward OP, Narasu ML. 2001. Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects. Biotechnol Adv. 19(3):175–199.

Herath WHMW, Ferreira D, Khan IA. 2003. Microbial transformation of xanthohumol. Phytochemistry. 62(5):673–677.

Jez JM, Noel JP. 2002. Reaction mechanism of chalcone isomerase. pH dependence, diffusion control, and product binding differences. J Biol Chem. 277(2):1361–1369.

Jiang H, Zheng X, Yin Z, Xie J. 2011. An efficient catalytic synthesis of flavanones under green conditions. J Chem Res. 35(4):220–221.

Jimenez DEQ, Ferreira IM, Birolli WG, Fonseca LP, Porto ALM. 2016. Synthesis and biocatalytic ene-reduction of Knoevenagel condensation compounds by the marine-derived fungus *Penicillium citrinum* CBMAI 1186. Tetrahedron. 72(46):7317–7322.

Kim H-K, Lee H-Y, Riaz TA, Bhattarai KR, Chaudhary M, Ahn JH, Jeong J, Kim H-R, Chae H-J. 2021. Chalcone suppresses tumor growth through NOX4-IRE1α sulfonation-RIDD-miR-23b axis. Redox Biol. 40:101853.

Kostrzewa-Susłow E, Dymarska M, Guzik U, Wojcieszyńska D, Janeczko T. 2017. *Stenotrophomonas maltophilia*: a gram-negative bacterium useful for transformations of flavanone and chalcone. Molecules. 22(11):1830.

Kozłowska J, Potaniec B, Żarowska B, Anioł M. 2018. Microbial transformations of 40-methylchalcones as an efficient method of obtaining novel alcohol and dihydrochalcone derivatives with antimicrobial activity. RSC Adv. 8(53):30379–30386.

Krist KA, Ross T, Mcmeekin TA. 1998. Final optical density and growth rate; effects of temperature and NaCl differ from acidity. Int J Food Microbiol. 43(3):195–203.

Li J, Chen R, Wang R, Liu X, Xie K, Chen D, Dai J. 2018. Biocatalytic access to diverse prenylflavonoids bycombining a regiospecific *C*-prenyltransferase anda stereospecific chalcone isomerase. Acta Pharmaceutica Sinica B. 8(4): 678–686.

Lou WY, Zong MH, Zhang YY, Wu H. 2004. Efficient synthesis of optically active organosilyl alcohol via asymmetric reduction of acyl silane with immobilized yeast. Enzyme Microb Technol. 35(2–3):190–196.

Mahapatra DK, Bharti SK. 2016. Therapeutic potential of chalcones as cardiovascular agents. Life Sci. 148:154–172.

Mouad AM, De Oliveira ALL, Debonsi HM, Porto ALM. 2016. Bioreduction of fluoroacetophenone derivatives by endophytic fungi isolated from the marine red alga *Bostrychia radicans*. Biocatalysis. 1(1):141–147.

Raghav N, Garg S, Ravish I. 2016. Conversion of 2′-substituted chalcones in the presence of BSA as evidenced by ^{1}H NMR studies. Int J Biol Macromol. 85:23–28.

Ribeiro SS, Ferreira IM, Lima JPF, de Sousa BA, Carmona RC, Dos Santos AA, Porto ALM. 2015. Fast microwave-assisted resolution of (±)-cyanohydrins promoted by lipase from *Candida antarctica*. J Brazil Chem Soc. 26(7):1344–1350.

Rocha LC, Seleghim MHR, Comasseto JV, Sette LD, Porto ALM. 2015. Stereoselective bioreduction of α-azido ketones by whole cells of marine-derived fungi. Mar Biotechnol. 17(6):736–742.

Sanchez-Gonzalez M, Rosazza JPN. 2004. Microbial transformations of chalcones: hydroxylation, *O*-demethylation, and cyclization to flavanones. J Nat Prod. 67(4): 553–558.

Skrobiszewski A, Ogórek R, Pląskowska E, Gładkowski W. 2013. *Pleurotus ostreatus* as a source of enoate reductase. Biocatal Agric Biotechnol. 2(1):26–31.

Tanaka K, Sugino T. 2001. Efficient conversion of 2′-hydroxychalcones into flavanones and flavanols in a water suspension medium. Green Chem. 3(3):133–134.

Tronina T, Bartmańska A, Milczarek M, Wietrzyk J, Popłoński J, Rój E, Huszcza E. 2013. Antioxidant and antiproliferative activity of glycosides obtained by biotransformation of xanthohumol. Bioorg Med Chem Lett. 23(7):1957–1960.

Tronina T, Bartmańska A, Popłoński J, Huszcza E. 2014. Transformation of xanthohumol by *Aspergillus ochraceus*. J Basic Microbiol. 54(1):66–71.

Zenger K, Dutta S, Wolff H, Genton MG, Kraus B. 2015. In vitro structure-toxicity relationship of chalcones in human hepatic stellate cells. Toxicology. 336:26–33.