*J. Microbiol. Biotechnol.* (2012), **22**(6), 832–837
http://dx.doi.org/10.4014/jmb.1112.12014
First published online March 21, 2012
pISSN 1017-7825 eISSN 1738-8872

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

© The Korean Society for Microbiology and Biotechnology

# Oxidative Potential of Some Endophytic Fungi Using 1-Indanone as Substrate

**Fill, Taicia Pacheco, José Vinicius da Silva, Kleber Thiago de Oliveira, Bianca Ferreira da Silva, and Edson Rodrigues-Fo***

*Departament of Chemistry, Federal University of São Carlos, CP 676, 13.565-905, São Carlos, SP, Brazil*

Received: December 7, 2011 / Revised: January 20, 2012 / Accepted: January 21, 2012

**The oxidative potential of the fungus *Penicillium brasilianum*, a strain isolated as endophytic from a Meliaceae plant (*Melia azedarach*), was investigated using 1-indanone as substrate to track the production of monooxygenases. The fungus produced the dihydrocoumarin from 1-indanone with the classical Baeyer–Villiger reaction regiochemistry, and (-)-(*R*)-3-hydroxy-1-indanone with 78% ee. Minor compounds that had resulted from lipase and SAM activities were also detected. The biotransformation procedures were also applied using a collection of *Penicillium* and *Aspergillus* fungi obtained from *M. azedarach* and *Murraya paniculata*. The results showed that Baeyer–Villiger were mostly active in fungi isolated from *M. azedarach*. Almost all fungi tested produced 3-hydroxy-1-indanone.**

**Keywords:** Biotransformation, BV oxidation, 1-indanone, endophytic, *Penicillium brasilianum*, *Aspergillus*

The Baeyer–Villiger (BV) oxidation of cyclic or acyclic ketones, converting them to the corresponding lactone and esters, is one of the major reactions of organic chemistry. Typical oxidants for these reactions are *m*-chloroperbenzoic acid (*m*CPBA), trifluoroperacetic acid, peroxybenzoic acid, and hydrogen peroxide [4]. However, the instability, toxicity, and lack of enantioselectivity concerning the oxidants have inspired researchers to explore the enzymatic BV oxidation that has been shown to be an efficient strategy for more than 10 years [8, 9]. These enzymes, now termed "Baeyer–Villiger monooxygenases" (BVMOs), are flavin-containing enzymes that use NAD(P)H and molecular oxygen in order to catalyze the nucleophilic oxygenation of ketones, as well as the electrophilic oxygenation of various heteroatoms such as sulfur, selenium, nitrogen, and phosphorous [11]. BVMOs produced by different microbial sources are described to convert substrates significantly different from their natural ones [10]. The conversions usually occur with high enantio- and/or regioselectivity, while using environmentally friendly conditions [8–10], and can be scaled to hundred liters fermentation [1, 2].

BVMOs are produced by numerous bacteria, such as those belonging to genera *Acinetobacter*, *Arthrobacter*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Streptomyces*, and *Xanthobacter*, and by fungi, such as *Curvularia, Aspergillus, Cunninghamella, Cylindrocarpon,* and *Dreschlera* genera [5, 11]. The Baeyer–Villiger reaction can be performed using isolated enzymes or intact cells of living microorganisms. Both approaches (pure enzyme or whole cells) have advantages and disadvantages. Although the use of intact cells is cheaper, the producing microorganism may be pathogenic. Its large-scale cultivation may be a dangerous process; it will depend on special laboratory equipment and requires very well-trained microbiologists. Therefore, efforts should be done in order to find new safety sources of enzymes.

Our research group has been involved with biochemical studies of symbiotic microorganisms such as those living in association with health plants. One of the plants chosen for these studies, *Melia azedarach* (Meliaceae), is a good producer of degraded triterpenoids known as limonoids [19]. The triterpene degradation in this plant appears to occur in a series of steps, including Baeyer–Villiger-like oxidation and structural rearrangements, resulting in limonoid molecules [20]. From health tissues of this Meliaceae, we isolated a collection of endophytic fungi [17]. One of these, identified as *Penicillium brasilianum*, surprisingly produced a series of compounds of the meroterpene class, whose molecular structures closely resemble limonoid molecules, at least in the putative enzymes used for their

**Corresponding author*
Phone: +55-16-3351-8053; Fax: +55-16-3351-8350;
E-mail: edinho@pq.cnpq.br

# Supplementary data for this paper are available on-line only at http://jmb.or.kr.

biosynthesis [3, 16]. In fact, studies on the biosynthesis of meroterpenes produced by *Aspergillus* and *Penicillium* species suggested that α-ketol rearrangements and Baeyer–Villiger oxidations are the main enzymatic reactions in these biosynthetic processes [16]. This finding may constitute further endorsement of the ability of endophytic microorganisms to exchange genetic information during their association with the host plants, resulting in the codification of specific enzymes that may be involved in the biosynthesis of some secondary metabolites similar or identical to those produced by the isolated organisms [7, 18].

The above briefly described biochemical plant–fungus interactions represent our approaches to discovering interesting enzymes. Therefore, the aim of the present work was to investigate the activity of BVMOs produced by the fungus *P. brasilianum*, using an exogenous substance, 1-indanone, as a probe molecule, which is an adequate substrate for lactonization and its products are easily detected by many analytical techniques. Additionally, different microorganisms isolated as endophytes in our group were evaluated to check the presence of BVMOs.

## Materials and Methods

### General Experimental Procedures

Gas chromatography mass spectrometry analyses were performed on a QP2010 Shimadzu system equipped with a J&W Scientific DB-1 capillary column (30 m × 0.25 mm) with helium as the carrier gas. Mass spectra were acquired at 70 eV. $^1$H NMR spectra were recorded on BRUKER DRX-200 and 400 spectrometers, using deuteron chloroform ($CDCl_3$) as the solvent and TMS as the internal standard. Thin-layer chromatography (TLC) was performed using precoated plates (aluminum foil, silica gel 60 $F_{254}$ Merck, 0.25 mm) and visualized by UV at 254 nm. Merck 60 silica gel (100–200 mesh) was used for flash chromatography. Substrate 1-indanone, sodium bicarbonate, and potassium iodide were purchased from Aldrich, and *m*-chloroperbenzoic acid from Fluka; and fungi growth media were purchased from commercial suppliers and used as provided. All solvents were distilled before use.

### Production of Lactone Dihydrocoumarin (2) for Analysis Correlation by NMR and Gas Chromatography

Sodium bicarbonate (3.83 g) was added slowly together with *m*-chloroperbenzoic acid (4.37 g) to a stirred solution of 1-indanone (1.0 g, 7.57 mmol) dissolved in 60 ml of dichloromethane. The reaction was completed in 24 h as monitored using TLC [silica gel, ethyl acetate/hexane, 25:75 (v:v)]. The reaction mixture was stopped with 20 ml of potassium iodide (10% solution) and 20 ml of $NaHSO_3$ solution. The mixture was extracted twice with ethyl acetate. The combined organic layers were washed with water and dried over sodium sulfate. After filtering, the solvent was evaporated. The product was purified by flash chromatography [silica gel, ethyl acetate/hexane 10:50 (v:v)]. The yield was 95%. GC-MS (EI): 132 ($M^{+\cdot}$); $^1$H NMR (in $CDCl_3$): δ 7.38 (d, $J = 8$ Hz, 1H), δ 7.29 (d, $J = 8$ Hz, 1H), δ 7.25 (d, $J = 8$ Hz, 1H ), δ 7.00 (d, $J = 8$ Hz, 1H), δ 3.10 (t, 2H), δ 2.70 (t, 2H).

### Synthesis of 3-Hydroxy-1-Indanone (3) for Enantiomeric Separation and Analysis of Enantiomeric Excess in Bioproducts

1-Indanone (1.0 g, 7.57 mmol) was added to a recipient containing 50 ml of distilled water. To this suspension was added 1.33 g (7.57 mmol) of *N*-bromosuccinimide. The reaction mixture was stirred at room temperature under irradiation from a 40 W incandescent light bulb. After 3 h, the mixture was extracted twice with 50 ml of ethyl ether, and then the solvent was evaporated. The product was purified by flash chromatography [silica flash, ethyl ether/hexane, 30:70 (v:v)], and identified by GC-MS and NMR spectroscopy. GC-MS (EI): 132 ($M^{+\cdot}$); $^1$H NMR (in $CDCl_3$): δ 7.76 (m, H-7), δ 7.72 (m, H-5), δ 7.70 (m, H-6), δ 7.50 (t, $J = 8$ Hz, H-4), δ 5.45 (dd, $J =$ 2.9 and 6.8, H-3), δ 3.10 (dd, $J = 6.8$ and 19.0, H-2a), and δ 2.60 (dd, $J = 2.9$ and 19.0, H-2b).

Enantiomeric separation was performed by HPLC, using a Shimadzu chromatographic system equipped with diode array detector (DAD). A Chiralcel OD-H HPLC column (250 × 4.60 mm, 5 μm particle size) was used for separation. The HPLC mobile phase flow rate was set at 0.8 ml/min with an isocratic elution (hexane:isopropyl alcohol, 80:20).

### Biotransformation of 1-Indanone Using Growing Cells

The *Penicillium* used in the present work is deposited (LaBioMi024) at the Laboratório de Bioquímica Micromolecular de Microorganismos (LaBioMMi) of the Departamento de Química at Universidade Federal de São Carlos, São Carlos, Brazil. The strain was maintained on potato-dextrose agar (PDA) slants. The inoculum was prepared by suspending spores from 7-day-old cultures in water.

Erlenmeyer flasks (250 ml) containing 100 ml of Czapek medium, consisted of glucose (30.0 g), $NaNO_3$ (3.0 g), $K_2HPO_4$ (1.0 g), $MgSO_4$ (0.5 g), KCl (0.5 g), $FeSO_4$. $7H_2O$ (0.01 g), and yeast extract (20.0 g) in one liter of distilled water, and 20 mg of 1-indanone dissolved in 1 ml of acetone, were inoculated with spores or a disk (5 mm) from a culture plate of the microorganism. Five flasks with medium but without 1-indanone were kept as controls. Growth was carried out in static conditions during a certain period of time. The cultures were filtered and then extracted three times with ethyl acetate. The organic layer was dried with anhydrous $Na_2SO_4$ and evaporated. The crude extracts were separated and purified by chromatography techniques using silica gel. An aliquot of each extract was analyzed by GC-MS.

A slightly modified procedure was conducted in order to evaluate the influence of the substrate on the microorganism growth after 3 days of incubation. The microorganism was cultivated under stirring in an orbital shaker (120 rpm) for 3 days. Then, a solution of 1-indanone at 20.0 mg in 1 ml of acetone was added to the biocatalytic system and maintained under stirring for 10 days. The reaction was worked up as described above.

### Biotransformation of 1-Indanone Using Resting Cells

The fungus was inoculated to the same medium described above, but with no 1-indanone added. After 3 days of incubation in an orbital shaker (120 rpm), the mycelium was separated from the liquid medium by filtration. Then 100.0 ml of phosphate buffer (pH 7.0) was added to the mycelium and a solution of 20 mg 1-indanone in 1.0 ml of acetone was added to the resting cells and maintained

under stirring for 10 days. The extraction work-up and analyses were as mentioned above.

**Screening of Different Endophytic Microorganisms for BV Activity**
Different species of *Penicillium* and *Aspergillus* were screened to verify BV activity. All the fungi tested are deposited at the Laboratório de Bioquímica Micromolecular de Microorganismos (LaBioMMi) of the Departamento de Química at Universidade Federal de São Carlos, São Carlos, Brazil.

The tested strains were maintained on PDA slants. After 7 days of growing, Erlenmeyer flasks (125 ml) containing 30 ml of the previously described medium and 10 mg of 1-indanone dissolved in 1.0 ml of acetone were inoculated with spores of the microorganism. Flasks with medium and without 1-indanone were kept as controls. Growth was carried out in static conditions during 15 days and the extraction followed the above described procedures. The production of dihydrocoumarin and hydroxyindanone was evaluated using HPLC/MS/MS, performed in a WATERS HPLC (Alliance 2695) connected to a DAD (Alliance 2996) coupled to a MICROMASS QuattroLC triplequadrupole tandem mass spectrometer equipped with Z-ESI ion source. Single reaction monitoring (SRM) experiments (149 > 103 for hydroxyindanone; 147 > 107 for dihydrocoumarin) were performed in order to selectively detect both oxidation products. A phenyl-hexyl (Phenomenex) HPLC column (250 × 4.60 mm, 5 µm particle size) was used for separation. The HPLC mobile phase flow rate was set at 1.0 ml/min. The elution was performed with methanol (0.1% TFA) and water (0.1% TFA) for 25 min with gradient elution as follows: 0−10 min 10−30% B, 10−20 min 30−50% B, 20−25 min 50−100% B elution. The ESI capillary voltage was set at 3.80 kV and cone voltage at 25 V; the ion-source temperature was held at 100°C. For acquisitions of product ion spectra, argon was used in the collision cell ($1.3 \times 10^{-3}$ mbar) and the precursor ions were accelerated at 10 eV.

## RESULTS AND DISCUSSION

### Compounds Identification

The BVMO enzyme activity was firstly investigated using *Penicillium brasilianum* owing to previous chemical works that showed that this fungus produced a series of meroterpenes-containing lactone groups. To investigate if the fungus could express this enzymatic activity in an exogenous ketone, after 3 days of microorganism growth, an acetone solution of 1-indanone (20 mg/ml) was added (triplicate). After further 3 or 10 days of growth, the bioreaction was extracted and small aliquots of the products were analyzed by TLC (Fig. S1) and GC-MS. It was observed that in 3 days, 1-indanone was only partially consumed and only one product was formed. In 10 days, only trace amount of indanone was observed, and additional products were detected.

The replicates were combined, and then the product that appeared during the first 3 days of experiment (compound **2**), and also the major compound detected after 10 days (compound **3**), were purified by preparative TLC. Compound **2** showed the same $^1$H NMR spectrum (Fig. S2) profile observed for 1-indanone, with signals for four aromatic hydrogens and two methylene groups, indicating that it is a biotransformation product. The two methylene groups were detected at almost the same position (multiplets at δ 2.70 and 3.10) as for indanone, but the four aromatic hydrogens were shielded by ca. 0.5 ppm (δ 7.38, 7.29, 7.25, and 7.00), probably due to the loss of the anisotropic carbonyl effect. These data, along with the MS spectrum, which showed the addition of one extra oxygen atom in the indanone molecule ($M^{+\cdot}$ at *m/z* 132 for indanone and 148 for compound **2**), are indicative that the fungus performed a Baeyer−Villiger reaction in **1**, with the conventional regiochemistry. Moreover, the dihydrocoumarin was synthesized from 1-indanone using *m*CPBA in dichloromethane, and the purified product showed exactly the same spectral data as compound **2**.

The second product, obtained as a major compound after 10 days of fermentation, was an isomer of **2** ($M^{+\cdot}$ at *m/z* 148, suggested molecular formula $C_9H_8O_2$), but showed to possess a little higher polarity compared with lactone **2**. The $^1$H NMR acquired for **3** (Fig. S3) showed the four aromatic hydrogens at almost the same position observed for 1-indanone (**1**) (δ 7.76, 7.72, 7.70, and 7.50), which indicates that the alkylphenone moiety was kept in this molecule. On the other hand, the signals corresponding to the two methylene groups were substituted by a clear AMX 3-spins system at δ 5.46 (dd, $J = 2.9$ and 6.8 Hz, H-3), 3.10 (dd, $J = 6.8$ and 19 Hz, H-2a), and 2.62 ($J = 2.9$ and 19 Hz, H-2b). These data are in agreement with those found for 3-hydroxyindanone [6] and differs from those of 2-hydroxyindanone [12].

In order to evaluate whether the hydroxylation of 1-indanone by *P. brasilianum* occurred as a simple racemic benzylic oxidation or by an enzymatic process with enantiomeric distinction, we studied the resolution of the stereoisomers of 3-hydroxyindanone by liquid chromatography using a chiral phase column. A racemic mixture was synthesized from 1-indanone and *N*-bromosuccinamide (NBS) in water, using an adaptation of the method proposed by Podgoršeka *et al.* [14]. The 3-hydroxyindanone, which gave exactly the same NMR and MS spectroscopic data as the one produced by the fungus, was obtained in a "one-pot" reaction, and probably came from the nucleophilic substitution by water in the very reactive brominated position. A polysaccharide-based chiral stationary phase column was able to resolve the two 3-hydroxyindanone enantiomers (alpha value = 1.16; Res. 2.12) (Fig. S4). The sample isolated from the biotransformation experiment was analyzed in the same conditions and gave two chromatographic peaks whose areas are 89:11 related (78% ee). The measured optical rotation of the mixture ($[\alpha]_D$) gave -14.7°. Therefore, the fungus produced the levorotatory as the major isomer. Resnick *et al.* [15] described the biotransformation of 1-indanone and 2-indanone to hydroxyindanones with

bacterial strains expressing naphthalene dioxygenase (NDO) and toluene dioxygenase. A *Pseudomonas* sp. strain, expressing NDO was able to oxidize 1-indanone to a mixture of 3-hydroxy-1-indanone (91%) and 2-hydroxy-1-indanone (9%). The (*R*)-3-hydroxy-1-indanone was formed in 62% ee (*R*:*S*, 81:19), whereas the 2-hydroxy-1-indanone was racemic. The purified NDO enzyme also oxidized 1-indanone and 2-indanone to the same products [15].

The GC-MS analysis of the extracts from the 10 days fermentation experiment showed the presence of minor compounds **4** and **5**, which were identified by interpretation of their EIMS spectra and comparison with the NIST library. Compound **4** probably arose from enzymatic transesterification by a lipase, and **5** from an alkylation by *S*-adenosyl methionine (SAM). Thus, this finding suggests that 1-indanone serves as a good probe molecule to track multiple enzymatic processes during fermentations.

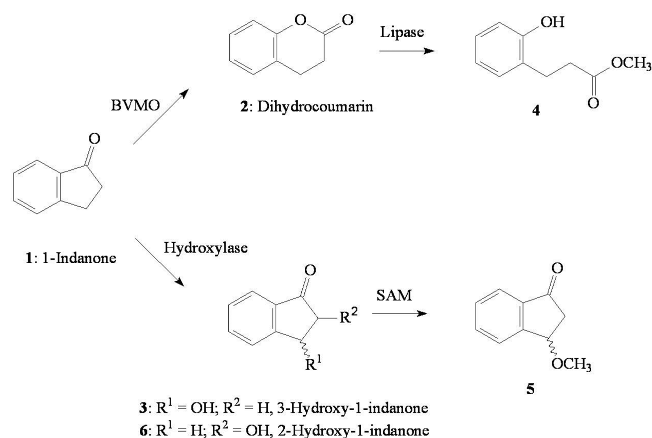


**Fig. 1.** Biotransformation scheme of 1-indanone followed by *P. brasilianum* and other endophytic fungi isolated from *M. azedarach* and *M. paniculata*.

**Different Biotransformation Processes Using *P. brasilianum***

The experiments described above were performed using *P. brasilianum* growing cells, with the substrate added in the medium prior to fungus inoculation. We also tested the highest substrate concentration that could be added without fungus growth inhibition. Thus, 1-indanone was added at 20, 50, and 100 mg/ml. The growth was evaluated after 10 days by visual inspection of the mycelial mass compared with controls (no 1-indanone adding). The fungus grew like the control in the presence of 20 mg/ml 1-indanone, at ca. 70% of control with 50 mg/ml, and was almost 100% inhibited with 100 mg/ml. When 1-indanone was added at 20 mg/ml after the fungus was grown for 3 days and then for 10 further days of fermentation, it was 100% consumed and the same products **2–5** were obtained, with a little excess of **3**, and **4**, and **5** as minor products.

Fermentation with fungus cell suspension was also tested for 1-indanone biotransformation. Thus, 3-day mature cells were produced using the same medium of the experiments described above, filtered, and resuspended in phosphate buffer at pH 7.0. After 10 days of incubation, the experiment was extracted and analyzed by HPLC-MS/MS. Since all the conditions were kept almost the same as for other experiments, the suspended cells also produced the dihydrocoumarin **2** and 3-hydroxyindanone (**3**). Only a small amount of the precursor compound (**1**) rested in the medium after 10 days. This result represents an interesting guide for BVMO extraction and studies, since a clean reaction was achieved with, penicillic acid being the only parallel metabolite detected in this experiment.

According to Mihovilovic *et al.* [9], monooxygenases are dependent on cofactors, which complicate their utilization in organic chemistry as pure enzymes. Whole-cell fermentations offer a different approach to overcoming the cofactor obstacle, since living organisms provide natural recycling systems for all factors required. In addition, the difficult process of protein purification could be avoided, and applications would not be limited by possible enzyme instability. Microorganisms are, in many cases, easily cultivated and stored [8, 9]. This implies that there will always be needs for efforts to find new MO producers. In this sense, *P. brasilianum* was found to be a promising source for these enzymes.

**Screening of Different Endophytic Microorganisms for MO Activity**

The structural similarities of meroterpenes produced by *P. brasilianum* and the limonoids found in its host plant suggest a close biochemical intimacy between the two associated organisms [3, 16, 20]. There are many reports in the literature showing the production of secondary metabolites by endophytic microorganisms with structures exactly the same or similar to those found in their host plants [7, 18]. This inspired us to check whether BV oxidations are particular to *P. brasilianum* or a common biochemical process for other endophytic fungi. The screening was performed with fungi especially from genera *Penicillium* and *Aspergillus*, isolated in our laboratory from the *Melia azedarach* (Meliaceae) [17] and *Murraya paniculata* (Rutaceae) [13] plant families. 1-Indanone was used as the substrate in growing cells fermentation, and the formation of the two oxidized products **2** and **3** were monitored by HPLC-ESI-MS/MS (Fig. S2 and S3).

Although compounds **2** and **3** are constitutional isomers, their product ion spectra, using the $[M+H]^+$ as precursor ion (*m/z* 149) (Fig. 5SA and 5SB), contained ions at *m/z* 107 and 103 as base peaks for **2** and **3**, respectively (Fig. S6). Thus, it was possible to detect both compounds using the SRM transitions 149 > 107 for **2** and 149 > 103 for **3**. Using these transitions and the described chromatographic conditions, the 3-hydroxyindanone (**2**) was detected at r.t.

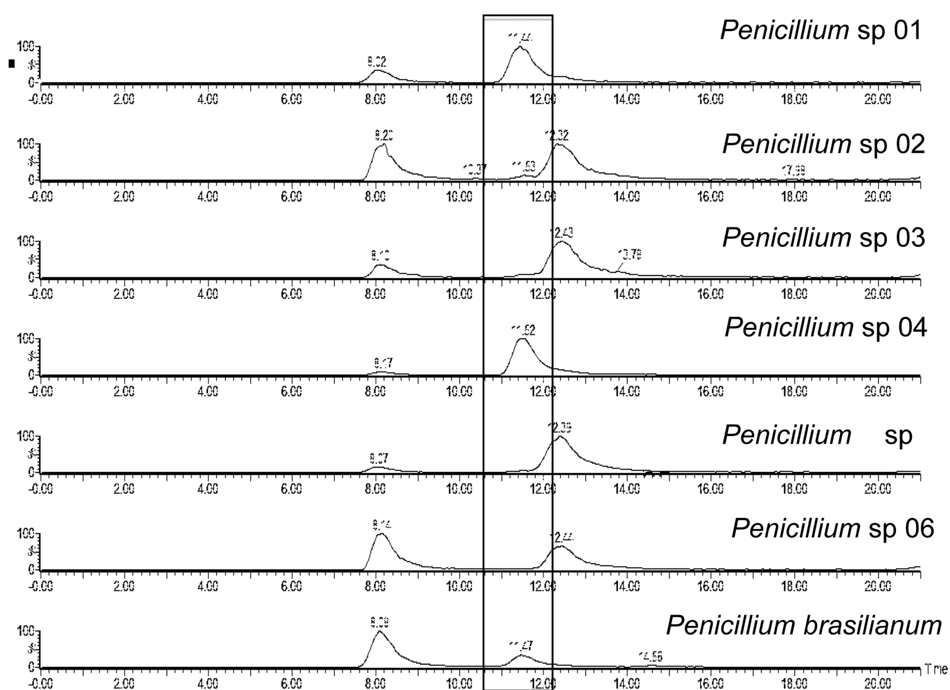


**Fig. 2.** Detection of the dihydrocoumarin **2** (ca. 11.50 min) in biotransformation experiments using endophytic *Penicillium* species.

8.04 min, and the dihydrocoumarin **2** at 11.50 min for *P. brasilianum* extracts (Fig. 2). Besides *P. brasilianum*, two other non-identified *Penicillium* and one *Aspergillus* (*A. aculeatus*) species, both collected in *M. azedarach*, also produced the BV product **2**; all of the *Penicillium* and *Aspergillus* species tested produced the 3-hydroxy-1-indanone (**3**) from 1-indanone; *A. flavus* produced exclusively the 3-hydroxy-1-indanone. Four *Penicillium* (*P. brasilianum* not

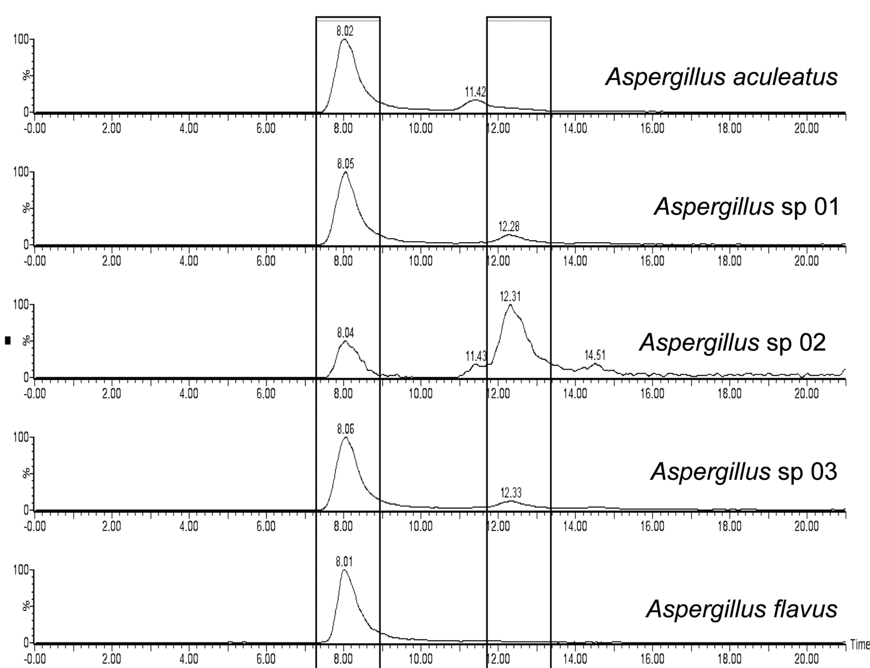


**Fig. 3.** Detection of 3-hydroxy-1-indanone (**3**) (ca. 8.04 min) and 2-hydroxy-1-indanone (**6**) (r.t. ca. 12.30 min) in biotransformation experiments using endophytic *Aspergillus* species.

included) and one *Aspergillus* species also produced a compound with retention time nearby **2** (ca. 12.30 min), whose MS/MS spectrum is very similar to the one obtained for **3** (Fig. S5C), but with an ion at *m/z* 131 ($[M+H-H_2O]^+$) almost as intense as the base peak at *m/z* 103. Therefore, this compound was suggested to be the 2-hydroxy-1-indanone (**6**).

In summary, experimental conditions using whole cells to evaluate the formation of MO oxidation products from 1-indanone were studied. *P. brasilianum* was able to produce the dihydrocoumarin, probably by using BVMO, and (-)-(*R*)-3-hydroxy-1-indanone in 78% ee. Minor products that resulted from other enzymes activities were detected, indicating that 1-indanone is a good probe molecule to track different enzymes in fungi.

Although not exclusively, the production of the BV product appeared to be more frequent in the tested fungi isolated from *M. azedarach*. This plant species is a good producer of terpenoids containing esters and lactone groups. Our findings described here may indicate that exploitation of associations between plant and microorganisms is a good tool when one is looking for specific enzymes. In this case, the fungus *P. brasilianum* and the plant *M. azedarach* developed common abilities to convert ketones in lactones groups, and this conversion can be reproduced *in vitro*. The structure of plant secondary metabolites may reveal the enzymatic ability of the microorganisms associated with their producers.

## Acknowledgments

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for financial support and research fellowships.

## References

1. Alphand, V., G. Carrea, R. Wohlgemuth, R. Furstoss, and J. M. Woodley. 2003. Towards large-scale synthetic applications of Baeyer-Villiger monooxygenases. *Trends Biotechnol.* **21:** 318–323.
2. Baldwin, C. V. F. 2008. The first 200-L scale asymmetric baeyer-villiger oxidation using a whole-cell biocatalyst. *Org. Proc. Res. Develop.* **12:** 660–665.
3. Fill, T. P. and E. Rodrigues-Fo. 2007. Four additional meroterpenes produced by penicillium sp found in association with melia azedarach. possible biosynthetic intermediates to Austin. *Z. Naturforsch.* **62b:** 1035–1044.
4. Hassall, C. H. 1957. *The Baeyer-Villiger Oxidation of Aldehydes and Ketones,* Organic Reactions Vol. 9, John Wiley & Sons, Inc., New York.
5. Jiyeoun, P., D. Kim, S. Kim, J. Kim, K. Bae, and C. Lee. 2007. The analysis and application of a recombinant monooxygenase library as a biocatalyst for the baeyer-villiger reaction. *J. Microbiol. Biotechnol.* **17:** 1083–1089.
6. Joly, S. and M. S. Nair. 2001. Efficient enzymatic kinetic resolution of 4-hydroxytetralone and 3-hydroxyindanone. *Tetrahedron Asymmetry* **12:** 2283–2287.
7. Kusari, S., M. Lamshöft, S. Zühlke, and M. Spiteller. 2008. An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J. Nat. Prod.* **71:** 159–162.
8. Leisch, H., K. Morley, and P. C. K. Lau. 2011. Baeyer-villiger monooxygenases: More than just green chemistry. *Chem. Rev.* **111:** 4165–4222.
9. Mihovilovic, M. D., B. Müller, and P. Stanetty. 2002. Monooxygenase-mediated baeyer-villiger oxidations. *Eur. J. Org. Chem.* 3711–3730.
10. Mihovilovic, M. D. 2006. Enzyme Mediated Baeyer-Villiger Oxidations. *Curr. Org. Chem.* **10:** 1265–1287.
11. Mlochowski, J., W. P. Czoch, M. P. Ottlik, and H. W. M. Lochowska. 2011. Non-metal and enzymatic catalysts for hydroperoxide oxidation of organic compounds. *Open Catal. J.* **4:** 54–82.
12. Murahashi, S. I., S. Noji, T. Hirabayashia, and N. Komiya. 2005. Manganese-catalyzed enantioselective oxidation of C-H bonds of alkanes and silyl ethers to optically active ketones. *Tetrahedron Asymmetry* **16:** 3527–3535.
13. Pastre, R., A. M. R. Marinho, E. Rodrigues-Fo, A. Q. L. Souza, and J. O. Pereira. 2007. Diversidade de policetídeos produzidos por espécies de *Penicillium* isoladas de *Melia azedarach* e *Murraya paniculata*. *Quim. Nova* **30:** 1867–1871.
14. Podgoršeka, A., S. Stavbera, M. Zupanb, and J. Iskra. 2006. Visible light induced 'on water' benzylic bromination with *N*-bromosuccinimide. *Tetrahedron Lett.* **47:** 1097–1099.
15. Resnick, S. M., D. S. Torok, K. Lee, J. M. Brand, and G. T. Gibson. 1994. Regiospecific and stereoselective hydroxylation of 1-indanone and 2-indanone by naphthalene dioxygenase and toluene dioxygenas. *Appl. Environ. Microbiol.* **60:** 3323–3328.
16. Santos, R. M. G. and E. Rodrigues-Fo. 2002. Meroterpenes from penicillium sp found in association with *Melia azedarach*. *Phytochemistry* **61:** 907–912.
17. Santos, R. M. G., E. Rodrigues-Fo, W. C. Rocha, and M. F. S. Teixeira. 2003. Endophytic fungi from *Melia azedarach*. *World J. Microbiol. Biotechnol.* **19:** 767–770.
18. Stierle, A. and G. A. Strobel. 1995. The search for a taxol-producing microorganisms among endophytic fungi of the pacific Yew, *Taxus brevifolia*. *J. Nat. Prod*. **58:** 1315–1324.
19. Tan, Q. G. and X. D. Luo. 2011. Meliaceous limonoids: Chemistry and biological activities. *Chem. Rev.* **111:** 7437–7522.
20. Waterman, P. G. and M. F. Grundon. 1983. *Chemistry and Chemical Taxonomy of the Rutales*, 1st Ed. Academic Press, London.