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## Structural modification of *trans*-cinnamic acid using *Colletotrichum acutatum*

### Modificación estructural de ácido *trans*-cinámico empleando *Colletotrichum acutatum*

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#### Abstract

The biotransformation of *trans*-cinnamic acid by whole cells of the Colombian native phytopathogenic fungus *Colletotrichum acutatum* was studied. Initially, fungitoxicity of this compound against *C. acutatum* was evaluated; *trans*-cinnamic acid exhibited a moderate to weak toxicity against the microorganism and apparently a detoxification mechanism was present. Then, in order to study such mechanism and explore the capacity of this fungus to biotransform *trans*-cinnamic acid into value-added products, the microorganism was incubated with the substrate using three different culture media (Czapeck-Dox, Sabouraud and PDB) at room conditions. Using Czapeck-Dox medium, whole cultures of *C. acutatum* reduced *trans*-cinnamic acid, first to aldehydes (*trans*-cinnamaldehyde and 3-phenylpropanal), then to alcohols (cinnamyl alcohol and 3-phenyl-1-propanol). Subsequently, these alcohols were transformed to the corresponding acetyl esters. Nevertheless, some of these products were absent or present at different concentration when culture medium was changed. The results suggest a mechanism of detoxification in which the  $\alpha,\beta$ -unsaturated carbonyl system is affected. Besides, the formed metabolic products are useful compounds used as fragrances and flavors. Therefore, metabolism of *trans*-cinnamic acid using *C. acutatum* can provide new potential metabolic targets to control *C. acutatum* as well as a simple and efficient way to obtain flavor compounds

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and perfumes, such as cinnamyl alcohol and 3-phenyl-1-propanol, and their acetyl esters.

----- **Keywords:** Biocatalyst, phytopathogenic fungus, metabolic pathway, culture media

## Resumen

Se estudió la biotransformación de ácido *trans*-cinámico mediante células completas del hongo fitopatógeno nativo colombiano *Colletotrichum acutatum*. Inicialmente, se evaluó la fungitoxicidad de este compuesto contra *C. acutatum*; el ácido *trans*-cinámico exhibió una toxicidad moderada a débil contra el microorganismo y aparentemente se presentó un mecanismo de detoxificación. Luego, para estudiar tal mecanismo y explorar la capacidad de este hongo para biotransformar el ácido *trans*-cinámico en productos con valor agregado, el microorganismo se incubó con el sustrato usando tres medios de cultivo diferentes (Czapeck-Dox, Sabouraud y PDB) a condiciones ambientales. Usando el medio Czapeck-Dox, los cultivos completos de *C. acutatum* redujeron el ácido *trans*-cinámico, primero a aldehídos (*trans*-cinamaldehído y 3-fenilpropanal), luego a alcoholes (alcohol cinámico y 3-fenil-1-propanol). Posteriormente, estos alcoholes fueron transformados a los correspondientes ésteres de acetilo. Sin embargo, algunos de estos productos estuvieron ausentes o presentes a una concentración diferente cuando se cambió el medio de cultivo. Los resultados sugieren un mecanismo de detoxificación en el cual el sistema carbonílico  $\alpha,\beta$ -insaturado es afectado. Por otra parte, los productos metabólicos formados son compuestos útiles usados como fragancias y sabores. Por consiguiente, el metabolismo del ácido *trans*-cinámico usando *C. acutatum* puede proporcionar nuevos blancos metabólicos para controlar *C. acutatum* así como también una forma simple y eficiente para obtener sabores y perfumes, tales como el alcohol cinámico y el 3-fenil-1-propanol, y sus ésteres de acetilo.

----- **Palabras clave:** Biocatalizador, hongo fitopatógeno, ruta metabólica, medios de cultivo

## Introduction

Biocatalysis or biotransformation encompasses the use of biological systems to catalyze the conversion of one compound to another. The catalyst part can thereby consist of whole cells, cellular extracts, or isolated enzyme(s). If the conversion is developed by a free and/or immobilized enzyme, it means biocatalysts, but if these transformations take place by the whole cell (with the correct enzyme) we talk about biotransformation [1]. Although the current

interest in applying biotransformations in organic synthesis is mainly related to the preparation of enantiopure compounds, these can also be used to perform transformations of achiral functional groups. The reason is that biotransformations are carried out usually at room temperature and atmospheric pressure, avoiding the use of extreme reaction conditions, and minimizing problems of isomerization, racemization, epimerization or transposition [2]. Therefore, biotransformations attract considerable attention due to its simple, cheap and benign methodologies that combines

green chemistry with high efficiency [3]. Besides, biotransformation experiments using phytopathogenic fungi provide information on the detoxification mechanism used by these microorganisms and give an indication of the structural modifications that may be necessary if substrates of this type are to be further developed as selective fungal control agents [4].

On the other hand, phenylpropenoides and cinnamates can potentially serve as a good source of starting material for the production of value-added compounds. Several studies have demonstrated that valuable aroma and flavoring compounds, and pharmaceutical intermediates, are produced as intermediates in the degradation pathways of such phenylpropenoides and cinnamates [5,6]. Thus, biotransformation of these compounds seems to be a reasonable alternative to produce raw materials for different industries. Also, products of such bioconversions are considered natural [7], which gives them better perspectives of use than synthetic counterparts.

In addition, several phenylpropenoides and cinnamates have been reported possessing antifungal activity [8,9] and have been suggested to be effective to control postharvest pathogens [9]. Nevertheless, knowledge about microbial metabolism of these compounds by phytopathogenic fungi is still limited. Understanding potential biofungicide metabolism in microorganisms is necessary for fungicide development as well as for safe and efficient use.

In this sense, filamentous phytopathogenic fungi have high potential for the biotransformation of compounds with aromatic structure; however, cellular pathways and metabolic processes involved must be known better. This paper reports for the first time the capability of the fungus *C. acutatum*, a cosmopolitan filamentous phytopathogenic fungus to biotransform *trans*-cinnamic acid into value-added products. A possible metabolic pathway of the biotransformation and culture medium effect is also discussed.

## Experimental

### Analytical methods

Thin layer chromatography (TLC) was made on precoated plates (Si 60 F<sub>254</sub>, 0.25 mm, Merk). Mixtures of *n*-hexane:EtOAc were used as mobile phase. Column chromatography (CC) employed silice gel 60 (Merck) and Sephadex LH-20. Gas chromatography (GC) was performed on a Hewlett-Packard 6890 (Agilent Technologies) gas chromatograph coupled with a HP 5973 MSD with a HP-5 column (30 m x 0.25 mm i.d.; coating thickness 0.25 µm). Chromatographic conditions were: column temperature, 50-250°C at 10°C/min and keep it five minutes; injector temperature, 150°C; detector temperature, 280°C; carrier gas, N<sub>2</sub> at 1 mL/min. Relative composition of the individual constituent was determined from the peaks average area. EI-MS measurements were obtained using gas chromatography-mass spectrometry (GC-MS). Substances were identified by comparison of their spectroscopic properties with those of reference substances and by comparison with the NIST 2002 Mass Spectral Library.

### Biological and chemical materials

*C. acutatum* strain was provided by the Laboratory of Phytopathology (Universidad Nacional de Colombia-Medellín). The fungus was isolated from diseased *Solanum betaceum* cav. Sendt (tamarillo) fruits, and characterized through morphological and molecular data by Dr. Afanador-Kafuri. The fungus was maintained in a Potato Dextrose Agar (PDA) medium at 24±2°C, and monthly subcultured in Petri dishes. To evaluate the antifungal activity, previously sterile Petri dishes measuring 15 cm in diameter were inoculated with 1 mL of a spore suspension of the fungus. The suspension was uniformly spread over the medium using a bent glass rod. After that, the inoculated medium was incubated at 25°C for 48 h. A mycelial disc of 5 mm of diameter was used for antifungal test. The substrate for biotransformation, *trans*-cinnamic acid (A),

was purchased from Alfa Aesar, and compounds cinnamyl alcohol (B) and 3-phenyl-1-propanol (C) were obtained from Sigma-Aldrich Co. Bacto Agar was obtained from Becton, Dickinson and Co. Yeast extract was from Oxoid Ltd. Peptone from casein (pancreatically digested) was acquired from Merck KGaA.

### **Antifungal bioassay**

In order to investigate the toxicity of (A) against *C. acutatum*, the poisoned food technique described by Velasco et al. [6] was used. Different concentrations (50-700 µg/mL) of (A) dissolved in acetone (2 µL/mL) were diluted in Petri dishes with PDA. All concentrations were tested in triplicate, and the results are shown as mean values of colony diameters [ $\pm$  standard deviation (SD)]. Petri dishes with acetone were used as the control. The Petri dishes were incubated at room temperature and the diameter of the mycelial growth was measured each 24 hours. The incubation was stopped when the mycelial mass of control Petri dishes had almost filled it (ca. 288 h). The relative growth inhibition of the treatment compared to the control was calculated as percentage, using the formula: Inhibition (%) =  $\{1 - \text{radial growth of treatment (mm)} / \text{radial growth of control (mm)}\} \times 100$  (1)

### **Preculture of *C. acutatum***

The microorganism was inoculated into 1.0 L Erlenmeyer flasks, containing 500 mL of Czapeck-Dox liquid medium. Erlenmeyer flasks were shaken (reciprocating shaker, 120 rpm) at room temperature for 168 h. Mycelia were recovered by filtration, and washed with H<sub>2</sub>O to inoculate in a new culture medium with the substrate for the biotransformation and time-course experiments.

### **Preparative biotransformation**

Mycelium of *C. acutatum* was transplanted into four 1.0 L Erlenmeyer flask containing 500 mL of sterilized Czapeck-Dox culture medium

and the substrate (at 400 µg/mL). Cultivation and biotransformation was carried out at room temperature and stirring (120 rpm) for 336 h. After the incubation period, culture medium and mycelia were separated by filtration. Mycelia were discarded and culture medium was used to isolate the metabolic products. Control was carried out in order to verify the presence of similar compounds on the fungus culture (without substrate).

### **Isolation and identification of metabolic products**

The culture medium was saturated with NaCl, refrigerated, filtered and extracted with CH<sub>2</sub>Cl<sub>2</sub> (3x2.0 L). Afterward, the medium was acidified to pH 2 with 1.0 M HCl, and extracted again with CH<sub>2</sub>Cl<sub>2</sub> (2x2.0 L). Both organic extracts were mixed, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated in vacuum, and the crude extract was chromatographed on a silice gel column. Elution was performed with an *n*-hexane-EtOAc gradient system. Several fractions were collected and separated into 4 groups (I-IV) according to TLC profiles. Fractions II and III were fractionated by size-exclusion column chromatography over Sephadex LH-20 (100x2 cm) using *n*-hexane-CH<sub>2</sub>Cl<sub>2</sub>-MeOH (50:25:25, v/v) as eluent to yield metabolic compounds (B) and (C); these compounds were isolated and identified by spectroscopic analysis, and comparison with authentic samples. Spectral data and retention times of (B), (C), (D), and (E) are in good agreement with those observed in the literature [5, 6], corresponding to cinnamyl alcohol, 3-phenyl-1-propanol, cinnamyl acetate, and 3-phenyl propyl acetate, respectively. In addition, many minor metabolites were detected.

### **Time-course experiments and effect of culture medium**

Portions of 1 mL of the mycelia were transferred to inoculate seven 500 mL flasks, each containing 125 mL of Czapeck-Dox medium and the substrate (A). Cultivation was carried out at room

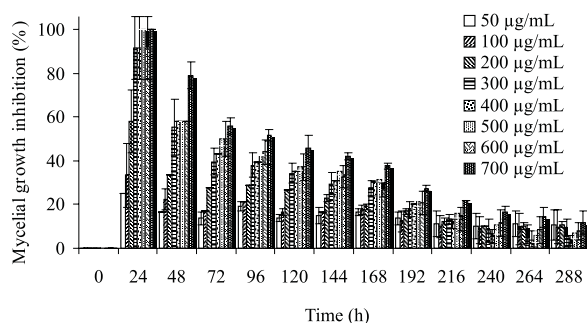


temperature and stirring (reciprocating shaker, 120 rpm) for 336 h. The culture medium from each flask was removed every 48 hours and then, it was saturated with NaCl, refrigerated, filtered and extracted with  $\text{CH}_2\text{Cl}_2$ , and the solvent was subsequently evaporated. These extracts were analyzed by TLC and GC-MS. The ratios between the substrate and metabolic products were determined on the basis of GC peak areas. Control cultivation with no substrate was also performed. Further, time-course experiments using Sabouraud and PDB liquid media were carried out. Cultivation and analyses were performed under the same conditions described for the Czapeck-Dox medium.

## Results and discussion

### Fungitoxicity bioassay

In order to determine the concentration to use in the biotransformation process, the toxicity of (A) against *C. acutatum* was examined. Overall, compound (A) displayed a moderate to weak activity against the fungus, as can be seen in figure 1. The inhibition of *C. acutatum* growth was depending of concentration. Complete inhibition activity of *C. acutatum* on exposure to (A) for 24 h was observed from 300 to 700  $\mu\text{g/mL}$ . However after that period, the inhibitory effect was strongly decreased at all the concentrations evaluated.



**Figure 1** Antifungal activity of (A) on in vitro grown *C. acutatum*

Cheng et al. [10] have reported that (A) shows a remarkable antifungal activity against *L.*

*sulphureus* and *L. betulina*, with  $\text{IC}_{50}$  values of 87.4 and 55.8  $\mu\text{g/mL}$ , respectively. Authors suggested that the acid group and the conjugated double bond are important features to exhibit the strong antifungal action. A similar observation was also noted by different authors in previous studies [11,12].

As shown in figure 1, the inhibitory effect of (A) diminished with time, a fact that suggests that the fungus has a detoxification mechanism. In order to study this mechanism and explore the biotechnological potential of *C. acutatum* to biotransform (A) into value-added products, the microorganism was incubated with the substrate at 400  $\mu\text{g/mL}$  during 336 h. Such concentration was able to inhibit nearly 90% of fungal growth for 24 h, retaining an inhibition percentage of about 40% after 120 h.

### Isolation and identification of metabolic products

To isolate the main metabolic products, a preparative incubation of (A) in Czapeck-Dox liquid medium using *C. acutatum* was performed. During biotransformation, a pleasant, sweet and floral odor was perceived, indicating the presence of aroma compounds. A comparison through TLC and GC among the extract obtained from the biotransformation and the control, showed that *C. acutatum* transformed (A) into various metabolites. Two metabolic products (B) and (C) were isolated, and their structures were elucidated on the basis of spectral data, corresponding to cinnamyl alcohol, and 3-phenyl-1-propanol, respectively. Lower amounts of (D) and (E) were observed; these compounds were detected by means of TLC and GC analysis, and their mass spectral and retention times were consistent with those reported to cinnamyl acetate and 3-phenyl propyl acetate, respectively. Additionally, other minor metabolites were detected.

It is noteworthy that *C. acutatum* was able to reduce the carboxylic group without affect the double bond to give the major product, cinnamyl alcohol (B). This compound is valuable in

perfumery for its odor and fixative properties. It is a component of some flower compositions (lilac, and hyacinth); in aromas is used for cinnamon notes and for rounding of fruit aromas [13]. Otherwise, reduction of carboxylic acid to aldehydes and subsequently alcohols is, biologically, a difficult process due to the very low redox potential required for the reaction (-600 mV) [14]. For this reason, the reduction of non-activated carboxylic acids has been described only for a limited number of mesophilic microorganisms. *Nocardia* [15], *Clostridium formicoaceticum* [16], and some fungi [17] reduce aromatic carboxylic acids to alcohols. *C. formicoaceticum* [16] also reduce aliphatic carboxylic acids. Therefore, biocatalytic reductions of carboxylic acids are attractive and constitute a good alternative to chemical methods. In general, chemical methods for carboxylic acid reductions are limited, and they usually require prior derivation and product deblocking with reactants containing functional groups [18].

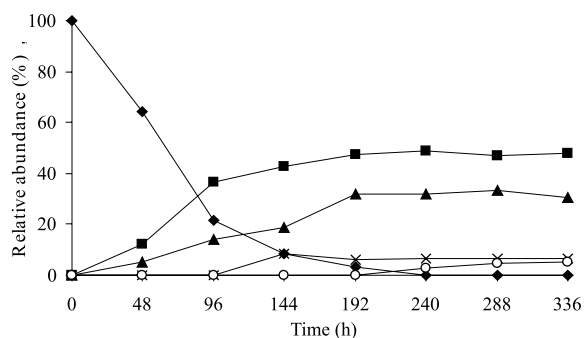
The biotransformation of (A) by *C. acutatum* also allowed the isolation of 3-phenyl-1-propanol (C), resulting from the reduction of the double bond C-C and the carbonyl group. Metabolite (C) was the second major metabolite through the bioprocess. It has a sweet, balsamic and floral odor and is also used as a cosmetic and perfume ingredient [13]. Simultaneously, alcohols (B) and (C) were esterified by the microorganism to generate the corresponding acetates (D) and (E). These esters have been employed as block of construction of flavors and are widely used in the production of perfumes [13]. Compound (D) occurs in cassia oil and is a colorless liquid with a sweet-flowery-fruity, slightly balsamic odor.

In addition, recent studies about the structure-antifungal activity relationship of cinnamaldehyde congeners have shown that compounds having an aldehyde group or an acid group, and a conjugated double bond, possesses much stronger antifungal activity [10]. The authors reported that (B) and (D) were less inhibitory to fungal growth than

(A) against *L. betulina* and *L. sulphureus* at the concentration of 100 µg/mL. Thus, the formation of (B), (C), (D) and (E) from (A) by *C. acutatum* suggests a mechanism of detoxification in which the  $\alpha,\beta$ -unsaturated carbonyl system is affected. Indeed, the MIC value obtained at 24 h of incubation (defined as the lowest compound concentration exhibiting approximately 50% reduction of growth compared with the control) for (A), gave the lowest value followed by (B) and (C), respectively (*Data not shown*). Therefore, (A) was the most active against *C. acutatum*, followed by (B) and (C). Based on these findings, it is possible to postulate that one of the possible modes of action of (A) may be due to its role as Michael-type acceptor for biological nucleophiles. In contrast, compounds lacking the  $\alpha,\beta$ -unsaturated carbonyl system, such as (B), (C), were found to be less active.

#### **Time-course experiments and influence of the culture medium**

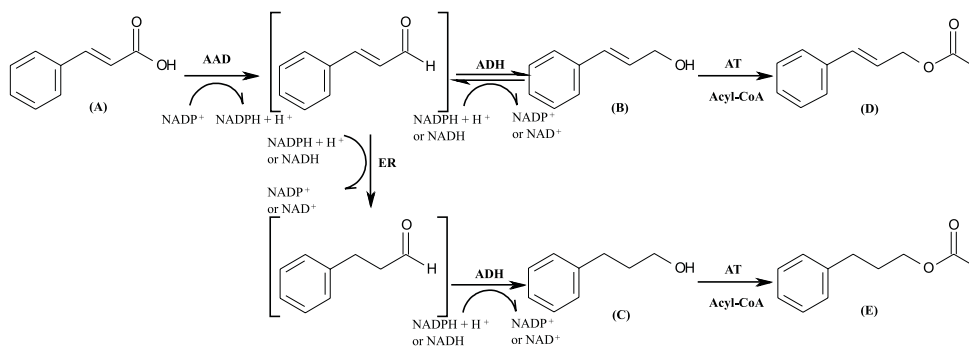
In this test, (A) was incubated with the microorganism during 336 h. Every 48 h, the medium from one flask was removed and extracted, and then analyzed through TLC and GC. All the metabolic products and the substrate were quantitatively measured through GC. As it is shown in figure 2, (A) was mainly transformed into (B). After 144 h about 90% of (A) was modified. Under the conditions used, the alcohol (B) reached about 47% of the products in 192 h and continued stable until the end of process. In the same way, the alcohol (C) reached about 31% at 192 h, and then its concentration remained almost unchanged. The increase in relative abundance of (B) and (C) coincided with the decline of (A). Additionally, the metabolic compounds (D) and (E) increased slowly after 96 and 192 h, respectively, but no one of these metabolites obtained a considerable concentration at the end of the evaluation (only 7 and 6% in the order given).



**Figure 2** Time course for the biotransformation of (A) by *C. acutatum* in liquid medium culture Czapeck-Dox: (♦) *trans*-cinnamic acid (A); (■) cinnamyl alcohol (B); (▲) 3-phenyl-1-propanol (C); (x) cinnamyl acetate (D); (○) 3-phenyl propyl acetate (E)

The metabolic pathways for the biotransformation of (A) by *C. acutatum* were proposed according to the time course experiment and the structures of the metabolites identified. Whole cultures of *C. acutatum* reduce the carboxylic acid (A) to *trans*-cinnamaldehyde, then to alcohols [(B) and (C)] and subsequently to the corresponding

acetyl esters [(D) and (E)]. The reduction reaction has been proposed to occur sequentially, and it involves at least three separate enzymes [19]. These enzymes are an aryl-aldehyde oxidoreductase carboxylic acid reductase, AAD that converts (A) into cinnamaldehyde, an alcohol dehydrogenase aldehyde reductase ADH that converts cinnamaldehyde into the alcohol (B), and an acyl transferase (AT) that catalyzes the formation of acetyl ester (D) from the alcohol product [15]. Furthermore, the reduction of the double bond from *trans*-cinnamaldehyde, via 3-phenylpropanal (formed by the action of the enoate reductase, ER), and further over-reduction of the saturated aldehyde leads finally to the formation of the saturated alcohol (C) [20], as described in figure 3. Although the formation of (C) from (B) by *C. acutatum* has been recently reported, such process results of the reversibility of the interconversion *trans*-cinnamaldehyde-cinnamyl alcohol [21], through the sequence [(B)-(trans-cinnamaldehyde)-(3-phenylpropanal)-(C)]. Finally, alcohols (B) and (C) were acetylated through esterification by acyl transferases (AT) to produce (D) and (E).



**Figure 3** Possible metabolic pathways of *trans*-cinnamic acid (A) by *C. acutatum*

It is noteworthy that during the conversion of (A) by *C. acutatum* no aldehydes were detected. This phenomenon was also observed for the reduction of acids by, e.g. *C. thermoaceticum* [22], *N. asteroides* JCM 3016 [23], and several fungi [17]. However, Correa et al. [5] recently reported that *trans*-cinnamaldehyde, the intermediate of the reduction of (A), was quickly

transformed to (B) and (C) by *C. acutatum*. This indicates that the fungus was able to reduce the aldehyde to the alcohol, and saturate the double bond. In this sense, the lack of aldehydes during biotransformation of (A) by *C. acutatum* indicated that the second reaction, the reduction of *trans*-cinnamaldehyde, was faster than the reduction of (A). Therefore, it is very likely that for (A),



the reduction to aldehyde is the rate-limiting step of the conversion of acids to alcohols. Moreover, the low accumulation of aldehydes in the culture medium is probably due to its higher cell toxicity [24]. In fact, cinnamaldehyde has been recognized by displaying antibacterial and antifungal properties [25], including against *C. acutatum* [5].

As can be seen from figure 3, the intermediate *trans*-cinnamaldehyde is reduced through two pathways: (1) reduction of the aldehyde to the allylic alcohol (which is not a substrate for enoate reductases) by the action of an alcohol dehydrogenase (ADH) and the coenzymes NADH and NADPH [26], and (2) saturation of the C=C bond by an ER to furnish the saturated aldehyde. Overall, the chemoselectivity in the bioreductions of the bonds C=C versus C=O by whole cells of *C. acutatum* is poor, which is due to the presence of competing enzymes [6]. Because enoate reductases and alcohol dehydrogenases depend on the same nicotinamide cofactor, redox-decoupling of both enzyme activities is hardly possible [20, 26]. However, the formation of (B) as the major metabolite throughout the process suggests that the conversion [(*trans*-cinnamaldehyde) to (B)] is slightly faster than [(*trans*-cinnamaldehyde) to (C)].

The ability to *C. acutatum* to reduce (A) is an interesting aspect, due to that microbiological reduction of carboxylic acids is an unusual and potentially useful biocatalytic reaction, which has not yet been widely examined and exploited [27]. This article is the first report on the capacity of *C. acutatum* to reduce a carboxylic acid to value-added products. Nevertheless, knowledge about the properties of each enzyme involved in serial reactions is essential to establishing useful whole-cell biocatalytic processes.

Due that the efficient conversion of carboxylic acids to alcohols or their corresponding acetyl esters is an attractive whole-cell reaction sequence for the biocatalytic synthesis of fragrances and flavors, a complementary study of the culture medium effect on biotransformation of (A) was

carried out. Therefore, (A) was incubated with the microorganism using PDB and Sabouraud media. Biotransformation on PDB showed to be slower than on Czapeck-Dox medium; substrate (A) was gradually converted (about 70% after 240 h), mainly to metabolites (B) and (C). During the first 192 h, traces of these alcohols were detected. Then, relative abundances of (B) and (C) increasing to about 25% at 288 h and remained constant until 336 h. It seems remarkable that in PDB medium, compounds (D) and (E) were not detected. Instead, other metabolites (e.g. phenylacetic acid and benzaldehyde) were found at very low relative abundances (<5%). Additionally, the biotransformation using Sabouraud demonstrated to be faster than on Czapeck-Dox medium. Substrate (A) was rapidly converted by *C. acutatum* (>95% after 96 h). Metabolite (C) was the main metabolic product throughout the process; it presented a relative abundance of 50% at 144 h. However, at 240 h and after, (C) was not detected. Under the conditions used, the alcohol (B) was only found at 216 h, reaching a relative abundance of approximately 5%. Similar to biotransformation of (A) by *C. acutatum* on PDB, the compounds (D) and (E) were absent on Sabouraud. Instead, three compounds with a molecular ion of 154 amu, corresponding to hydroxylated derivatives of (C), were found. It seems remarkable that in Sabouraud medium, the conversion of (A) by the fungus was more selective and slower toward the formation of (C) than in Czapeck-Dox. Also, it seems noteworthy that some minor metabolites detected on Sabouraud and PDB were not detected on Czapeck-Dox.

Such specificity of the medium for transformation has also been previously reported [28, 6]. Authors suggest that the difference in the compounds production in each medium means that the enzymes presents in the microorganism are induced in different way, due to the suitability of each medium for the production of some specific metabolites. Thus, the Czapeck-Dox medium, rich in minerals, could be providing metal ions needed to some catalytic processes (such as

cofactors or Lewis acids), favoring certain stages involving the ADH and Acyl-transferases. In addition, the culture medium possibly influences the physiological status of the fungus, which in turn, could induce differences in efficiency towards the formation of some products. However, further investigations are needed to determine how the composition of culture medium affects the enzymatic behavior.

## Conclusions

In conclusion, according to the results described herein, the *trans*-cinnamic acid presents a moderate to weak antifungal activity against *C. acutatum*. Also, a detoxification mechanism was established. Results obtained from biotransformation experiments demonstrate the ability of the phytopathogenic fungus to transform *trans*-cinnamic acid. Thus, using Czapeck-Dox medium, two compounds were isolated and identified: cinnamyl alcohol, and 3-phenyl-1-propanol, and two products were also detected by GC: cinnamyl acetate, and 3-phenyl propyl acetate. Therefore, *C. acutatum* was able to reduce *trans*-cinnamic acid, first to aldehydes, and then to alcohols. Further, these alcohols were transformed to the corresponding acetyl esters. It suggests a mechanism of detoxification in which the  $\alpha,\beta$ -unsaturated carbonyl system is modified. Interestingly, the products formed are valuable aroma and flavoring compounds, which opens good prospects for production of these through biotechnological processes using *C. acutatum*. Unfortunately, using PDB and Sabouraud media, some of these products were absent or present at a lower concentration. Besides, others metabolites were detected. Further investigations are needed to produce these metabolites in large quantities by improving the conditions of the biotransformation.

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## References

1. J. Leresche, H. Meyer. "Chemocatalysis and biocatalyst (biotransformation): some thoughts of a chemist and of a biotechnologist". *Org. Process. Res. Dev.* Vol. 10. 2006. pp. 572-580.
2. H. Luna. "Aplicación de la biocatálisis a la preparación de intermediarios para la síntesis de fármacos". *J. Mex. Chem. Soc.* Vol. 48. 2004. pp. 211-219.
3. K. Faber, R. Patel. "Chemical biotechnology: A happy marriage between chemistry and biotechnology: asymmetric synthesis via green chemistry". *Curr. Opin. Biotechnol.* Vol. 11. 2000. pp. 517-519.
4. M. Daoubi, R. Galán, A. Benharref, I. Collado. "Screening study of lead compounds for natural product-based fungicides: antifungal activity and biotransformation of 6a, 7a-Dihydroxy- $\beta$ -himachalene by *Botrytis cinerea*". *J. Agric. Food Chem.* Vol. 53. 2005. pp. 6673-6677.
5. Y. Correa, D. Durango, C. García. "Transformación microbiana del arilpropanoide cinamaldehído con el hongo fitopatógeno *Colletotrichum acutatum*". *Vitae.* Vol. 16. 2009. pp. 83-91.
6. R. Velasco, J. Gil, C. García, D. Durango. "Production of 2-phenylethanol in the biotransformation of cinnamyl alcohol by the plant pathogenic fungus *Colletotrichum acutatum*". *Vitae.* Vol. 17. 2010. pp. 272-280.
7. E. Shimoni, U. Ravid, Y. Shoham. "Isolation of a *Bacillus* sp. capable of transforming isoeugenol to vanillin". *J. Biotechnol.* Vol. 78. 2000. pp. 1-9.
8. S. Zacchino, S. López, G. Pezzenati, R. Furlán, C. Santecchia, L. Muñoz, F. Giannini, A. Rodríguez, R. Enriz. "In vitro evaluation of antifungal properties of phenylpropanoids and related compounds acting against dermatophytes". *J. Nat. Prod.* Vol. 62. 1999. pp. 1353-1357.
9. D. Sivakumar, R. Wijeratnam, R. Wijesundera, M. Abeysekere. "Control of postharvest diseases of rambutan using cinnamaldehyde". *Crop Prot.* Vol. 21. 2002. pp. 847-852.
10. S. Cheng, J. Liu, E. Chang, S. Chang. "Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi". *Bioresource Technol.* Vol. 99. 2008. pp. 5145-5149.
11. S. Chang, P. Chen, S. Chang. "Antibacterial activity of leaf essential oils and components from *Cinnamomum osmophloeum*". *J. Ethnopharmacol.* Vol. 77. 2001. pp. 123-127.

12. H. Lee, S. Cheng, S. Chang. "Antifungal property of the essential oils and their constituents from *Cinnamomum osmophloeum* leaf against tree pathogenic fungi". *J. Sci. Food Agric.* Vol. 85. 2005. pp. 2047-2053.
13. H. Surburg, J. Panten. *Common fragrance and flavor materials: Preparation, Properties and Uses*. 5<sup>th</sup> ed. Ed. Wiley-VCH Verlag GmbH. Weinheim (Germany). 2006. pp. 7-175.
14. R. Thauer, K. Jungermann, K. Decker. "Energy conservation in chemotropic anaerobic bacteria". *Bacteriol. Rev.* Vol. 41. 1977. pp. 100-180.
15. Y. Chen, J. Rosazza. "Microbial transformation of ibuprofen by a *Nocardia* species". *Appl. Environ. Microbiol.* Vol. 60. 1994. pp. 1292-1296.
16. L. Fraisse, H. Simon. "Observations on the reduction of non-activated carboxylates by *Clostridium formicoaceticum* with carbon monoxide or formate and the influence of various viologens". *Arch. Microbiol.* Vol. 150. 1988. pp. 381-386.
17. H. Arfmann, W. Abraham. "Microbial reduction of aromatic carboxylic acids". *Z. Naturforsch.* Vol. 48c. 1993. pp. 52-57.
18. A. He, T. Li, L. Daniels, I. Fotheringham, J. Rosazza. "*Nocardia* sp. carboxylic acid reductase: cloning, expression, and characterization of a new aldehyde oxidoreductase family". *Appl. Environ. Microbiol.* Vol. 70. 2004. pp. 1874-1881.
19. T. Li, J. Rosazza. "The carboxylic acid reduction pathway in *Nocardia*. Purification and characterization of the aldehyde reductase". *J. Industrial Microbiol. Biotechnol.* Vol. 25. 2000. pp. 328-332.
20. M. Hall, B. Hauer, R. Stuermer, W. Kroutil, K. Faber. "Asymmetric whole-cell bioreduction of an  $\alpha,\beta$ -unsaturated aldehyde (cital): competing prim-alcohol dehydrogenase and C-C lyase activities". *Tetrahedron: Asymmetr.* Vol. 17. 2006. pp. 3058-3062.
21. R. Stuermer, B. Hauer, M. Hall, K. Faber. "Asymmetric bioreduction of activated C=C bonds using enoate reductases from the old yellow enzyme family". *Curr. Opin. Chem. Biol.* Vol. 11. 2007. pp. 203-213.
22. H. Simon, H. White, H. Lebertz, J. Thanos. "Reduktion von 2-enoaten und alkanooaten mit kohlenmonoxid oder formiat, viologen und *Clostridium thermoaceticum* zu gesättigten säuren und ungesättigten bzw. gesättigten alkoholen". *Angew. Chem.* Vol. 99. 1987. pp. 785-787.
23. N. Kato, H. Konishi, K. Uda, M. Shimao, C. Sakazawa. "Microbial reduction of benzoate to benzyl alcohol". *Agric. Biol. Chem.* Vol. 52. 1988. pp. 1885-1886.
24. R. Villa, F. Molinari. "Reduction of carbonylic and carboxylic groups by plant cell cultures". *J. Nat. Prod.* Vol. 71. 2008. pp. 693-696.
25. D. Sivakumar, R. Wijeratnam, R. Wijesundera, M. Abeysekere. "Control of postharvest diseases of rambutan using cinnamaldehyde". *Crop Prot.* Vol. 21. 2002. pp. 847-852.
26. J. Carballeira, M. Quezada, P. Hoyos, Y. Simeó, M. Hernaiz, A. Alcántara, J. Sinisterra. "Microbial cells as catalysts for stereoselective red-ox reactions". *Biotechnol. Adv.* Vol. 29. 2009. pp. 686-714.
27. J. Rosazza, T. Li. *Carboxylic acid reductase and methods for use of the same*. U.S. patent 5795759. August 18. 1998.
28. S. Gurram, N. Kollu, G. Sivadevuni, M. Solipuram. "Biotransformation of albendazole by *Cunninghamella blakesleeana*: influence of incubation time, media, vitamins and solvents". *Iran. J. Biotechnol.* Vol. 7. 2009. pp. 205-215.