Beauveria spp: evaluation of different strains by biotransformation of quercetin and rutin for biotechnological purpose

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1. Introduction

Beauveria spp belongs to moniliaceae family, Deuteromycotina subdivision, Hyphomycetes class. Scientific investigations has been indicated their potential use as biological control agents for several insect pests, as an alternative to harmful chemical insecticides (Athayde et al., 2001). *Beauveria bassiana* is the most common species of *Beauveria*. It is an entomophatogenic fungi that perform many different complex conversion (Van den Brink et al. 1998). In order, Grogan & Holland (2000), Haufe et al. (2002), Olivo et al. (2003) and others reported that *B. bassiana* ATCC 7159 has been used on more than 300 different substrates biotransformation. Biochemical evidences indicates that these reactions are often mediated by cytochrome P450 and enzymes of phase II metabolism respectively.

The process of biotransformation using the whole cell has attracted more attention for the production of pharmaceutical derivatives, mostly result from the costs of the latter enzyme isolation, purification and stabilization (Mulinacci et al. 2005) and for allow the preparation of derivatives with high stereo and regio-selectivity under environmental friendly conditions constituting an alternative to chemical reagents (Keppler et al. 2005). Chemical means involves complicated protection-deprotection procedures and it is rather difficult to obtain good yields from this procedure. Further more, the use of biotransformation to mimic mammalian metabolism is well known (Ma et al. 2006) and we can observe oxidation, reduction (similar to hepatic phase I in animal metabolism) or conjugation (phase II reactions) by enzymatic system of the microorganisms (Azerad, 1999).

Flavonoids are polyphenolic metabolites in plants and have been proposed to exert beneficial effects in a multitude of disease states (Havsteen, 2002). Accumulating evidence suggests that flavonoids exert cellular effects, may be mediated by their interactions with specific proteins central to intracellular signaling pathways (Williams et al. 2004, Walle 2004). Quercetin, a flavonoid compound, inhibits protein kinases, DNA topoisomerase, regulate gene expression and has been shown that is important in the transcriptional activation of cytochrome P450 enzymes, it is possible that cells exposed to quercetin have higher levels of phase I detoxification enzymes (Havsteen, 2002). It has been proposed that low concentrations of quercetin (nanomolar to low micromolar) could lead to expression of survival genes and defensive genes, including that of phase II detoxifying enzymes, such as UDP-glucuronosyltransferase (Williams et al. 2004).

In this work, 10 strains of filamentous fungi from Brazilian biomes named IP3a, IP6, IP8, IP11, IP94, IP98, IP129, IP132, IP147, IP153 and *Beauveria bassiana* ATCC 7159 were studied. The aim was to explore the biotransformation potential of the fungi *Beuaveria* spp collected in different environment, specially, to identify microorganisms isolates for obtain similar metabolites with that observed in mammalian metabolism or new compounds with

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interesting biological activities. For this purpose we applied quercetin and rutin, quercetin plant derivative (rhamnoglycoside of quercetin) as standard substrate.

2. Experimental

2.1 Chemicals

Quercetin and rutin were purchased from Sigma Chemical Company, St. Louis, MO (USA). Others analytical reagents grade were applicable.

2.2 Microorganisms

A total of ten isolates of *Beauveria* spp named: IP3a, IP6, IP8, IP11, IP94, IP98, IP129, IP132, IP147, IP153 and *Beauveria bassiana* ATCC 7159 were used in the present study. *Beauveria* spp isolates were collected from the soil of Brazilian biome in the Midwest region. All isolates were maintained in potato agar and lyophilized after morphological evaluation.

2.2.1 Morphological evaluation of Beauveria spp

All isolates of *Beauveria* spp and *Beauveria bassiana* ATCC 7157 were cultured on potato agar. The macroscopic characteristics were described through the observation of anverse and reverse masses aspect after seven days culture. Microscopic characteristics (microculture technique) were available after five days under proper conditions and growth rate and pigment color production in liquid medium culture also were observed (Hegedus & Khachatourians, 1995).

2.3 Culture and biotransformation process

Inoculated 0.5 mL of each *Beauveria* spp and *Beauveria bassiana* ATCC 7159 conidia suspension, from seven days culture, in a 100 mL of liquid medium peptone dextrose soybean meal - PDSM (peptone 5 g, dextrose 20 g, soybean meal 5 g, dihydrogen potassium phosphate 5 g, sodium chloride 5 g, yeast extract 3 g in 1L of distilled water) in Erlenmeyer flasks. Experiments were maintained in shaker under 200 rpm at 29 ± 2 °C for 72 hours. A solution of quercetin was added to the cultures to a final concentration of 0.5 g/liter. After substrate administration the same conditions were maintained. Samples of the supernatant were taken in the range 24 - 96 h, every 24 h. The samples were saturated with sodium chloride, extracted with ethyl acetate, followed by centrifugation. The organic extracts were dried on $MgSO_4$, evaporated and then directly analyzed by electrospray ionization (ESI) mass spectrometer (MS). The same procedures were made in parallel for the rutin substrate.

2.4 ESI-MS analysis

The organic phase of each strain biotransformation process was used without no additional purification or preparation steps. They were resuspended in 500 µL of HPLC-grade methanol before injection into ESI/MS. A API 1200L quadrupole MS/MS instrument (VARIAN, inc.) equipped with an electron ion source was used for analysis of masses of the breakdown products, at the following operating conditions: the mobile phase was methanol, drying gas temperature 400 ºC, flow rate 0.5 mL/min, nebulizer pressure 23-24 psi, capillary voltage 40V, needle 5000V, shield 600V. The flow rate of the electrospray solutions to the ion source was 5 µL/min, nitrogen was used as collision gas. All mass spectrometer was carried out in the positive ionization mode and were recorded in scan mode in the mass range from 250 to 1000 Th. A VARIAN Workstation system software was used for data acquisition and processing.

3. Results and discussion

Beauveria colonies morphology were evaluated and at the beginning was observed a white color changing to yellow or pink. It grows as a filamentous fungi form and produced conidia globose or ovoid in shape.

Biotransformation were carried out in a 250 mL flask containing 100 mL of PDSM, after 3 days the supernatant of culture broth of isolates: IP6, IP132, IP147 and IP153 turned pink. To this and another's cultures were added each substrate. The ability to produce pink, yellow and green pigments has been used as a taxonomic feature in classification of *Beauveria*

isolates. These pigments should be associated with fungal antimicrobial activity (Hegedus & Khachatourians, 1995).

3.1 Quercetin biotransformation process

The data presented in Table 1 shows the molecular ion of relevant quercetin metabolites, obtained by the biotransformation process.

Table 1- Data of quercetin derivatives formed in supernatant of incubation by *Beauveria* spp strains and *Beauveria bassiana* ATCC 7159, range 24 – 96 hour.

Quercetin derivatives ESI-MS	**Strains**										
	IP 3a	IP 6	IP 8	IP 11	IP 94	IP 98	IP 129	IP 132	IP 147	IP 153	*Beauveria bassiana* ATCC 7159
Quercetin *m/z* (301-303)	+	+	+	+	+	+	+	+	+	+	+
Methylation *m/z* (315-316)	+	+	+	+	+	+	+	+	+	+	+
Sulphation *m/z* (380-383)	+	+	+	+	+	+	+	+	+	+	+
Monoglucuronidation *m/z* (477-479)	+	+	+	+	+	+	+	+	+	+	+
Methylation and Monoglucuronidation *m/z* (491-492)	+	+	+	+	+	+	+	+	+	+	+
Rutin *m/z* (606-608)	+	+	+	+	+	+	+	+	+	+	+

+ = quercetin derivatives produced by strains.

ESI-MS spectrum revealed that, quercetin was extensively metabolized by fungal enzymes and irrelevant differences were observed in the distribution of the quercetin derivatives at the range 24 and 96 h after substrate contact in all strains analyzed. The time courses of metabolite accumulation were similar. In general, the conjugates in 24 h, produced the molecular ion (*m/z* 383) suggesting quercetin sulphation (Fig 4), *m/z* (479) monoglucuronidation and *m/z* (492) monoglucuronidation and sulphation. The molecular ion (*m/z* 607) consisted of rutin (quercetin glycosides) suggesting that a glucose and rhamnose molecule were introduced to quercetin. The molecular ion (*m/z* 315) and (*m/z* 303 $[M^{+}1]^{-}$) corresponding a methylation and quercetin aglycone respectively. Adduct ions resulting from methanol addition was also observed (329 amu). The analyses in 48, 72 and 96 hours were similar for all strains.

3.2 *Rutin biotransformation process*

The data obtained by quercetin and rutin biotransformation process were the same. In mammalian, cleavage of flavonoids has been shown to be brought by the colonic microorganism, Herath et al. (2006). The present research was performed on a rutin with only 5% of quercetin in their composition specification. Comparing the results is clear that the reactions processed in both biotransformation were similar. In this way the obtain of the similar mass spectra pattern for rutin and quercetin we can speculate that, rutin was firstly cleavage by fungi enzymes resulting in quercetin aglycon, that performed the same biotransformation process.

By analysis of the ESI-MS profiles of the samples culture broth and comparing it with to previous study on the enzymatic derivatization of flavonoids was possible to confirm that reactions occurred on the aglycon structure. The hydroxyl groups of quercetin were targets for glucosidation, glucuronidation, sulphation and methylation in the synthesis process of metabolites. The molecular ions observed in ESI-MS results, are in consonance with LC-MS

analysis and H NMR data reported by Oliveira & Watson (2000), O'Leary et al. (2003), by Woude and coworkers (2004) who determinate, in unequivocal way, 14 different phase II mono and mixed conjugates of quercetin in various biological systems. The study presented by Woude et al. (2004) allows concluded that, the plasma phase II metabolic pattern in mammalian, result of interplay of different organs with metabolizing capacity specially the liver and the small intestine (Table 2). The same pattern just was observed in previous experiments with most microorganisms, including *Beauveria bassiana* ATCC 7159, by Herath et al. (2006) when tested 3- and 7-hydroxiflavones biotransformation.

Table 2- Data of mass spectrum fragment ions resulting from studies of quercetin metabolism in various biological systems by different authors.

ES mass Spectrum	Quercetin Metabolite	References
301-303	Quercetin	
315-317	3'-*O*-methylquercetin	Woude et al. (2004)
	4'-*O*-methylquercetin	O'Leary et al (2003)
381-383	Quercetin-7-*O*-sulphate	Woude et al. (2004)
	Quercetin-3'-*O*-sulphate	O'Leary et al (2003)
395	3'-*O*-methylquercetin-7-*O*-sulphate	Woude et al. (2004)
	4'-*O*-methylquercetin-3-*O*-sulphate	O'Leary et al (2003)
477-479	Quercetin-7-*O*-glucuronide	Woude et al. (2004)
	Quercetin-3-*O*-glucuronide	O'Leary et al (2003)
491-493	3'-*O*-methylquercetin-7-glucuronide	Woude et al. (2004)
	4'-*O*-methylquercetin-7-glucuronide	O'Leary et al (2003)
		Oliveira & Watson (2000)

On the ongoing work glucuronidation, sulphation and methylation was indeed the metabolic pathway in quercetin and rutin biotransformation by *Beauveria* spp isolates and *Beauveria bassiana* ATCC 7159, indicating that the fungi screened express phase II metabolic enzymes, it could be established inducible enzymes process.

4. Conclusion

The present investigation leads to the conclusion that the filamentous fungi collected in Brazilian biome, employed in these experiment, are capable to present a wide range of phase II biotransformation patterns. They offer significant potential for use in microbial models to mimic mammalian metabolism and represent a powerful biotechnological research tools. All fungi isolates carried out similar pattern of microbial transformation, concerning an indirect evidence of the same enzyme system perform.

Work is in progress to collect more information about *Beauveria* spp strains by molecular characterization.

5. References

1-Athayde ACR, Ferreira UL, Lima EALA 2001. Fungos Entomopatogênicos: uma alternativa para o controle do carrapato bovino – *Boophilus microplus*. Biotecnologia Ciência & Desenvolvimento 21:12-15.

2-Azerad R 1999. Microbial Models for Drug Metabolism. *Advances in Biochemical Engineering/Biotechnology* 63: 163 – 218.

3-Grogan, G.J., Holland, H.L., 2000. The biocatalytic reactions of *Beauveria* spp*. Journal of Molecular Catalysis B: Enzimatic* 9:1-32.

4-Haufe, G., Wölker D., Fröhlich R. 2002. Selectivity of biohydroxylation with *Beauveria bassiana* of trans-2-fluorocycloalkyl N-phenylcarbamates. *Journal of Organic Chemistry* 67(9): 3022-3028.
5-Havsteen, B.H., 2002. The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics* 96:67-202.
6-Hegedus DD, Khachatourians GG 1995. The impact of biotechnology on hiphomycetous fungal insect biocontrol agents. *Biotechnology Advances* 13: 455-490.
7-Herath W., Mikell J.R., Hale A.L., Ferreira D., Khan I.A. 2006. Microbial Metabolism. Matabolites of 3- and 7-Hydroxyflavones. *Chem. Pharm. Bull* 54(3): 320-324.
8-Keppler Artur F, Porto André L M, Schoenlein-Crusius Iracema H, Comasseto João V, Andrade Leandro H 2005. Enzymatic evaluation of different *Aspergillus* strains by biotransformation of cyclic ketones. *Enzyme and Microbial Technology*, 36: 967-975.
9-Ma Xiao-chi, Ye Min, Wu Li-jun, Guo De-an 2006. Microbial transformation of curdione by *Mucor spinosus*. *Enzyme and Microbial Technology*, 38: 367-371.
10-Mulinacci Nadia, la Marca Giancarlo, Innocenti Marzia, Vincieri Franco F, Crespi-Perellino Nicoletta, Minghetti Anacleto 2005. Cell cultures of *Ajuga reptans* L. to bioconvert emodin and aloe-emodin: an HPLC/ESI/MS investigation. *Enzyme and Microbial Technology*, 36: 399-408.
11-O'Leary, K.A., Day, A.J., Needs, P.W., Mellon, F.A., O'Brien, N.M., Williamson, G., 2003. Metabolism of quercetin-7- and quercetin-3-glicuronides by an *in vitro* hepatic model: the role of human β-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. *Biochemical Pharmacology* 65:479-491.
12-Oliveira E. J., Watson D. G. 2000. In vitro glucuronidation of kaempferol and quercetin by human UGT-1A9 microsomes. *FEBS Letters*, 471: 1-6.
13-Olivo H. F., Peeples T. L., Rios M. Y., Velazquez F., Kim, J. W., Narang S 2003. Microbial C-hydroxilation and β-4-*O*-methylglucosidation of methylbenzamide 7-azanorbomane ethers with *Beauveria bassiana*. *Journal of Molecular Catalysis B: Enzimatic*, 21: 97-105.
14-Van den Brink HJM, van Gorcom RFM, van den Hondel CAMJJ, Punt PJ 1998. Cytochrome P450 Enzyme Systems in Fungi. *Fungal Genetics and Biology,* 23: 1-17.
15-Walle T 2004. Absorption and metabolism of flavonoid. *Free Radical Biology & Medicine* 36:829-837.
16-Williams RJ, Spencer JPE, Rice-Evans C 2004. Flavonoids antioxidants or signaling molecules? *Free Radical Biology & Medicine* 36:838-849.
17-Woude Hester van der, Boersma Marelle G, Vervoot Jacques, Rietjens Ivonne M C M 2004. Identification of 14 Quercetin Phase II Mono and Mixed Conjugates and Their Formation by Rat and Human Phase II in Vitro Model Systems. *Chem. Res. Toxicol*. 17: 1520-1530.