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Catechin Degradation by Several Fungal Strains Isolated from Mexican Desert

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Abstract Eleven fungal strains isolated previously from the Mexican desert were evaluated for their capacity to use catechin as carbon source in submerged cultures. At 2 g/L of catechin, all strains grew better than the control strain, *Aspergillus niger* Aa-20. *Aspergillus niger* PSH and *Penicillium commune* EH2 degraded 79.33% and 76.35% with degradation rates of 0.0065 and 0.0074 g/L.h, respectively, when an initial catechin concentration of 3 g/L was used. Obtained results demonstrated the potential biotechnological capacity of these fungal strains to use condensed tannins as carbon source.

Key Words: catechin; degradation; fungal strains; submerged culture.

Condensed and hydrolysable tannins are water-soluble polyphenols recalcitrant to biodegradation [1]. They are present in plants playing important roles as resistant agents to microbial decomposition, mainly due to the ability of these molecules to inhibit microbial growth by binding strongly to proteins, and polysaccharides like cellulose and pectin [2-5]. Condensed tannins are more resistant to microbial decomposition, while hydrolysable tannins are more easily degraded by some microorganisms [6-10]. Condensed tannins are polymers of catechin or similar flavans connected by carbon linkages and only a very limited number of microorganisms have been reported to degrade them, mainly bacteria [11, 12]. Information about fungal catechin degradation is scarce [1, 13]. For this reason, the mechanism of condensed tannin degradation is not clear, especially by fungi [1]. The present study was undertaken to evaluate the potential of eleven fungal strains isolated from the Mexican desert [14] to degrade catechin. Previously, fungal strains were studied on a physiological and molecular level. Molecular characterization included the amplification of deoxyribonucleic acid by polymerase chain reaction and use of IGS (inter-genetic sequences) and RAPD's (random amplified polymorphic DNA) markers [15] and physiological study was conducted to know the growth rates on several supports or media and polysacharidase production profiles (including inulinases, rhamnogalacturonases, pectinases, amylases, celulases and tannases among others) [16].

All strains were isolated from the Mexican desert and characterized for their capacity to produce tannase [17]. Spores (stored at –20 °C in cryoprotect blocks) of the eleven fungal strains (Table 1) were tested in a first selective step, using as criterion the maximum catechin degradation value. In a second step, two selected strains were tested under higher catechin concentration conditions. *Aspergillus niger* Aa-20 was used as control strain [9,18]. Culture medium composition was similar to that reported by Antier et al., [19] using a mixture of glucose (Sigma) and catechin (Sigma-Aldrich) as carbon source at 2 g/L (each one) in the first experimental step, and 1 and 3 g/L respectively, in the second experimental step, only with the selected strains. A carbon/nitrogen ratio of 9.7 was used in all experiments, due mainly to carbon source effect on tannase production [9]. All experiments were carried out in Erlenmeyer flasks (250 mL) with 50 mL of culture media. Culture conditions were: inoculation level, 5 x10^6 spores per flask; incubation temperature, 30 °C; agitation rate, 200 rpm; initial pH 5.5 and a culture time of 95 h. All experiments were conducted in triplicate.

Catechin content was evaluated by the reverse phase HPLC method developed by Ramirez-Coronel and Augur

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Table 1. Fungal strains and their growth forms on medium with catechin at 2 g/L.

Identification Number	Name of strains	Growth form at the end of the culture	Aspect of the broth at the end of the culture
1	*Penicillium commune* GH2	Mycelial	Brown
2	*Penicillium commune* EH2	Mycelial	Turbidity (yellowish)
3	*Penicillium commune* EH3	Mycelial	Turbidity (yellowish)
4	*Penicillium commune* ESS	Mycelial	Turbidity (yellowish)
5	*Aspergillus terrícola* PSS	Pellets	Very clear
6	*Aspergillus ornatus* ESH	Pellets	Very clear
7	*Aspergillus rugulosa* NS4	Disrupted pellets	Turbidity (yellowish)
8	*Aspergillus fumigatus* GS	Big pellets	Clear
9	*Aspergillus rugulosa* NH4	Big pellets	Clear
10	*Aspergillus niger* PSH	Mycelial	Brown
11	*Aspergillus niger* GH1	Pellets	Clear
Control	*Aspergillus niger* Aa-20	Small pellets	Clear

[20]. Fungal biomass was determined gravimetrically by dry weight. Biomass was evaluated in the second step, only with the selected strains. Kinetic changes of pH were evaluated potentiometrically. All results were statistically analyzed by mean value comparison using the Tukey's test.

All tested fungal strains, showed a capacity to grow on a medium with catechin and glucose at 2 g/L each one, exhibiting several growth forms (Table 1). Fungal strains exhibiting mycelial biomass formation were associated with higher catechin degradation as compared to those producing pellets (Tables 1 and 2). Kinetics of catechin degradation by the eleven fungal strains is shown in Fig. 1. It is important to note that only one strain (*Penicillium commune* EH2) grows slow producing low levels of biomass under these conditions; however, it degraded a higher catechin concentration in relation to control strain, *A. niger* Aa-20. *A. niger* PSH was the best degrader in initial stages of culture, between 20 and 30 hours of culture (Fig. 1).

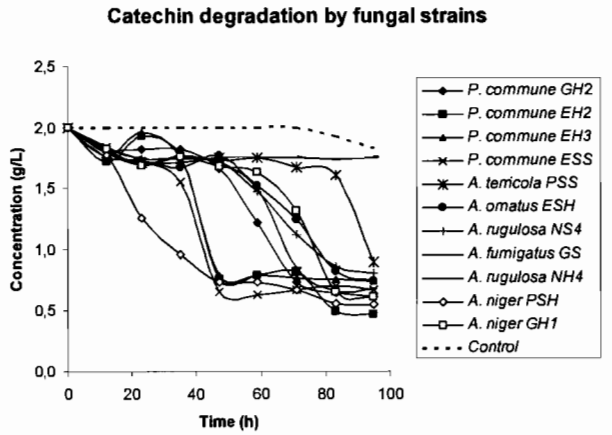

Fig. 1. Catechin degradation by several fungal strains using an initial catechin concentration of 2 g/L.

Two strains of *Penicillium commune* (EH3 and ESS) showed a particular degradation pattern, because catechin degradation was faster than other strains, over the first 35–47 h. Table 2 shows the catechin degradation rate and the percent of catechin degradation. It is clear that all strains have a higher capacity to degrade catechin in comparison with the control strain, *A. niger* Aa-20, which has been characterized as a good tannin-degrading fungus [8,9]. It is important to consider that, while hydrolysable tannins can be utilized by several microorganisms, only very few members of the genus *Aspergillus* and *Penicillium* have been reported to grow on condensed tannins derived from catechin [3, 21, 23]. In addition, *Psalliata campestris* was found to oxidize catechin [22] and *Calvatia gigantea* was reported with a better capacity to degrade catechin [13]. However, most of the fungal strains evaluated in the present study, shown from 2 up to 32 times higher catechin degradation rates that *C. gigantea* [13] and *Aspergillus fumigatus* [3]. This can be attributed to special tannase activities presents in the wild strains isolated from the Mexican desert.

Table 2. Catechin degradation rate and percent of degradation by Fungal strains

Identification number of Strains	Catechin degradation rate (g/L.h)	Percent of egradation
1	0.0041	69.1
2	0.0264	76.4
3	0.0271	62.7
4	0.0319	66.5
5	0.0011	55.2
6	0.0011	62.8
7	0.0027	59.5
8	0.0019	12.7
9	0.0018	65.9
10	0.029	72.3
11	0.003	69.4
Control	0.0004	8.4

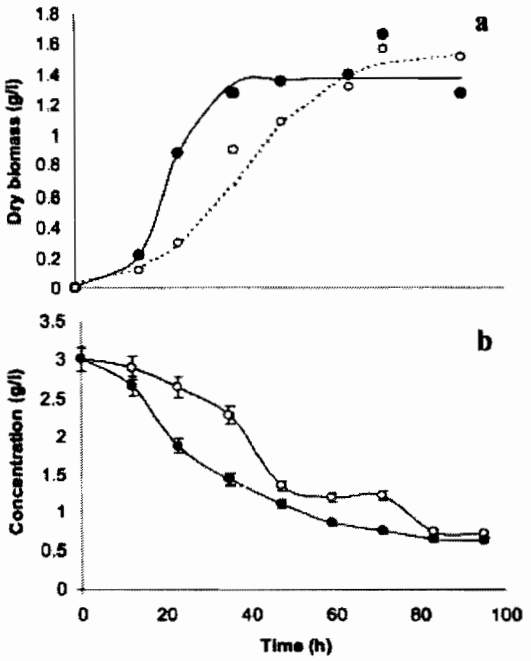

Fig. 2. Growth of *Aspergillus niger* PSH (●) and *Penicillium commune* EH2 (○) on catechin at 3 g/L (a). Solid and dotted lines represent the modeled growth of *Aspergillus* and *Penicillium*, respectively, using the logistic equation.

Further studies were carried out with two of the best catechin degraders, namely Aspergillus PSH and Penicillium EH2. Strains were grown using a culture medium with 3g/L of catechin and glucose at 1 g/L. Fig. 2a shows biomass production by each fungal strain, over time. The *Aspergillus* strain grew in pellets, oxidizing the culture broth, probably due to an efficient phenoloxidase system (laccase, peroxidase, tyrosinase). The Penicillium strain grew as diffuse mycelium without oxidizing the culture broth (Table 1).

Kinetic parameters associated with fungal growth were estimated using the logistic equation as reported previously [9]. Maximum growth value (X_{max}) for *Penicillium* was slightly higher than those obtained for *Aspergillus* and, μ (specific growth rate in h^{-1}) value was higher in the latter, its growth being significantly faster than that of *Penicillium* (Fig. 2a). Growth results reveal the ability of these fungal strains to utilize specific condensed tannins at high concentrations.

Fig. 2b shows the degradation of catechin over time by both strains. Catechin degradation rate and percent of degradation were calculated at 95h of culture. *Aspergillus* strains degraded a higher amount of catechin (79.33%) than *Penicillium* (76.35%) with degradation rates of 0.0065 and 0.0074 g/L.h, respectively. Both strains show interesting potential regarding catechin degradation. The present study is the first work that reports on fungal degradation of catechin using a high initial concentration (3 g/L) and points to the possibility of using these fungal strains for the fermentation of plant extracts and hydrolysates containing such phenolic compounds,(i.e, coffee pulp and residues of *Larrea tridentata Cov*, etc.).

In conclusion, Aspergillus niger PSH and Penicillium commune EH2 are promising strains for future biotechnological applications. Both strains can utilize specific condensed tannins as carbon source and could be used to degrade tannins present in coffee pulp. Transformation of such waste products could help solve the growing problem of agro industrial waste accumulation in coffee-growing countries such as Mexico, Columbia or Brazil. Use of higher initial catechin concentrations and related enzymatic activities are under investigation.

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REFERENCES

1. Bhat, T.K., B. Singh and P.O. Sharma. 1998. Microbial degradation of tannins-a current perspective. *Biodegradation.* 9: 343-357.
2. Benoit, R.E., R.L. Starkey and J. Basaraba. 1968. Effect of purified plant tannin on decomposition of some organic compounds and plant materials. *Soil Sci.* 105: 153-158.
3. Lewis, J.A. and R.L. Starkey. 1969. Decomposition of plant tannins by some soil microorganisms. *Soil Sci.* 107: 235-241.
4. Mueller-Harvey, I. and A.B. McAllan. 1992. Tannins: their biochemistry and nutritional properties. *Ad. Plant Cell Biochem. Biotechnol.* 1: 151-217.
5. Mueller-Harvey, I. 2001. Analysis of hydrolysable tannins. *Anim. Feed Sci. Technol.* 91: 3-20.
6. Lewis, J.A. and R.L. Starkey. 1968. Vegetable tannins, their decomposition and effects on decomposition of some organic compounds. *Soil Sci.* 106, 241-247.
7. Lekha, P.K. and B.K. Lonsane. 1997. Production and applications of tannin acyl hydrolase: state of the art. *Adv. Appl. Microbiol.* 44: 215-260.
8. Aguilar, C.N., C. Augur, G. Viniegra-González and E. Favela-Torres. 2000. *Influence of carbon source on tannase production by Aspergillus niger* Aa-20 in solid state culture. In: *Coffee Biotechnology and Quality*; Sera, T., Soccol, C.R., Pandey, A. and Roussos, S. (eds). Kluwer Academic Publishers. pp: 461-469.
9. Aguilar, C.N., C. Augur, E. Favela-Torres and G. Viniegra-González. 2001. Production of tannase by Aspergillus niger

Aa-20 in submerged and solid state fermentations: influence of glucose and tannic acid. *J. Ind. Microbiol. Biotechnol.* 26: 296-302.

10. Aguilar, C. N. and G. Gutiérrez-Sánchez. 2001. Sources, properties, and potential uses of tannin acyl hydrolase (3.1.1.20). *Food Sci. Technol. Int.* 7: 373-382.
11. Field, J.A. and G. Lettinga. 1992. Biodegradation of tannins. In: *Metal ions in biological systems.* Sigel, H. (ed). Volume 28. Degradation of environmental pollutants by microorganisms and their metalloenzymes. Marcel Dekker Inc. New York. pp: 61-97.
12. Deschamps, A.M. 1989. Microbial degradation of tannins and related compounds. In: *Plant cell wall polymers biogenesis and biodegradation.* Lewis, N.G. and Paice, M.G. (eds). American Chemical Society, Washington, D.C. pp: 559-566.
13. Galiotou-Panayotou, M. and B.J. Macris. 1986. Degradation of condensed tannins by *Calvatia gigantea. Appl. Microbiol. Technol.* 23: 502-506.
14. Cruz-Hernández, M., R. Rodríguez-Herrera, C.N. Aguilar, J.C. Contreras-Esquivel and F. Lara. 2001. Isolation and morphological characterization of fungal tannin-degrading strains. XXII Annual Meeting. Mexican Academy of research and teaching on Chemical Engineering, Mazatlán, Sin., Mexico,
15. Padilla, V. 2003. Molecular characterization of tannin-degrading fungal strains isolated from the semiarid region of Coahuila. Thesis. Universidad Autónoma de Coahuila, Saltillo, México. (In spanish).
16. Lafuente Castañeda, C.B. 2004. Biochemical characterization and evaluation of industrial potential of fungal strains. Thesis. Universidad Autónoma de Coahuila, Saltillo, México. (In spanish).
17. Cruz-Hernández, M. 2002. Isolation and characterization of fungal strains from Mexican desert. M.Sc. Thesis. School of Chemistry. Universidad Autónoma de Coahuila. Unidad Saltillo, México.
18. Aguilar, C.N., C. Augur, E. Favela-Torres and G. Viniegra-González. 2001. Induction and repression patterns of fungal tannase in solid state and submerged cultures. *Proc. Biochem.* 36: 571-578.
19. Antier P., A. Minjares, S. Roussos, M. Raimbault and G. Viniegra-González. 1993. Pectinase-hyperproducing mutants of *Aspergillus niger* C28B35 for solid-state fermentation of coffee pulp. *Enzyme Microb. Technol.* 15: 254-260.
20. Ramirez-Coronel, A. and C. Augur. 2003. Degradation of catechin, a major constituent of coffee pulp by filamentous fungi. *Journées du réseau de mycologie*. 2, 98-100. Nancy, France.
21. Grand, W.D. 1976. Microbial degradation of condensed tannins. *Science.* 193: 1137-1139.
22. Mahadevan, A. and S.N. Sivaswamy. 1985. Tannins and microorganisms. In: *Frontiers in applied microbiology.* Mukerji, K.G. Pathak, N.C. and Singh, V.P. (eds). Print House, Lucknow. pp: 327-347.
23. Ramirez-Coronel, A. 2002. Microbial degradation of catechin, a tannin present in coffee. Xe colloques de l'ecole doctorale des sciences de la vie et de la santé. Les Universités d'Aix-Marseille, I, II et III. Fance, 28-33.

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