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Catechin Degradation by Several Fungal Strains Isolated from Mexican Desert

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Abstract Eleven fungal strains previously isolated 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 strains, *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 where they play 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 hydrolyzable 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, mainly bacteria, have been reported to degrade them [11, 12]. Information about fungal catechin degradation is scarce [1, 13]. For this reason, the mechanism of condensed tannin degradation is not clear, especially in fungus [1]. The present study was undertaken to evaluate the potential of eleven fungal strains isolated from the Mexican desert [14] in the degradation of catechin. Previously, the fungal strains were molecularly and physiologically studied. Molecular characterization included the amplification of DNA by PCR (polymerase chain reaction) and use of IGS (inter-genetic sequences) and RAPD (random amplified polymorphic DNA) markers [15]. Physiological study was conducted to determine the growth rates on several supports or media as well as the production profile of polysaccharides (including inulinases, rhamnogalacturonases, pectinases, amylases, celulases, and tannases among others) [16].

All strains were isolated from the Mexican desert and characterized by their capacity to produce tannase [17]. Spores (stored at -20°C in cryoprotect blocks) of the eleven fungal strains tested (Table 1) were used in the first selective step, using as criterion the maximum catechin degradation value. In the second step, two selected strains were tested under higher catechin concentration conditions. *Aspergillus niger* Aa-20 was used as the 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, 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 as follows: inoculation level, 5×10^6 spores per flask; incubation temperature, 30°C; agitation rate, 200 rpm; initial pH, 5.5; and culture time 95 h. All experiments were conducted in triplicates.

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]. Catechin was determined in both experimental steps. Fungal biomass was determined gravimetrically as 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 for comparison using the Tukey's test.

All tested fungal strains showed a capacity to grow on medium with catechin and glucose at 2 g/l each, exhibiting several growth forms (Table 1). Those fungal strains with mycelial biomass formation were associated with higher catechin degradation than those grown in 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) grew in slowly, 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 h of culture (Fig. 1). Two strains of *Penicillium commune* (EH3 and ESS) showed a particular catechin degradation pattern, because their degradations were faster than any other strains during 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 only very few members of the genus *Aspergillus* and *Penicillium* have been reported to grow on condensed tannins derived from catechin, while hydrolysable tannins can be utilized by several microorganisms [3, 21, 23]. In addition, *Psalliata campestris* was found to oxidize catechin [22] and *Calvatia gigantea* was reported to have better capacity to degrade catechin [13]. However, most of the fungal strains were evaluated in the present study, shown from 2- to 32-fold higher catechin degradation rates than *C. gigantea* [13] and *Aspergillus fumigatus* [3]. This might be attributed to special tannase activities in the

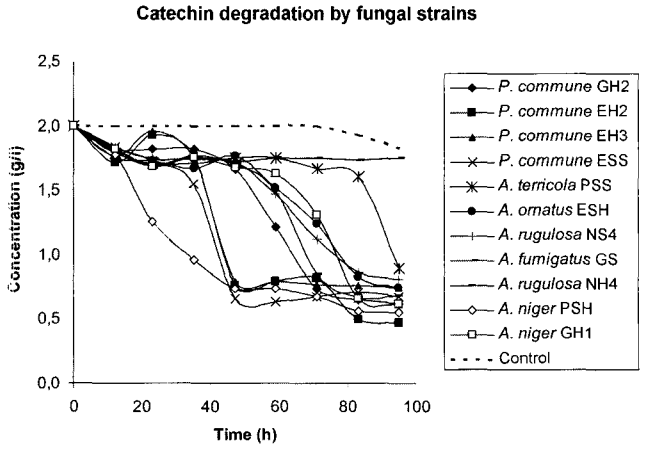

Fig. 1. Catechin degradation by several fungal strains using an initial catechin concentration of 2 g/l.

Table 2. Catechin degradation rate and percent of degradation by the tested fungal strains.

Identification number of strains	Catechin degradation rate (g/l/h)	Percent of degradation
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

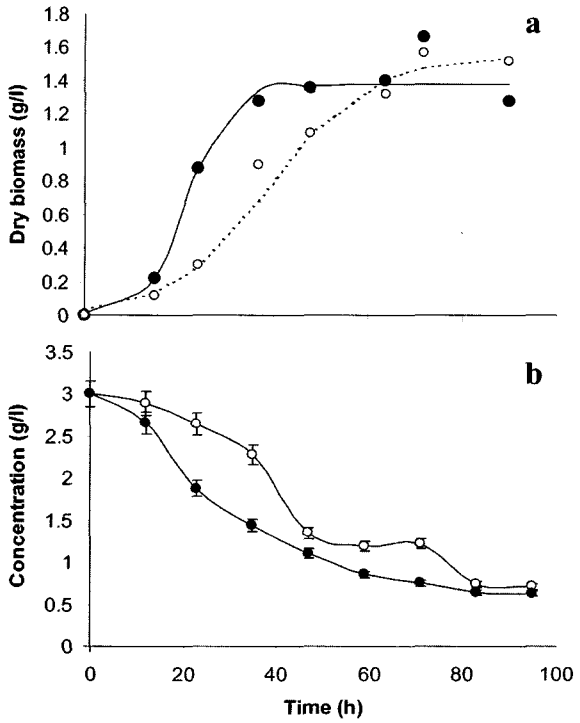

Fig. 2. Growth of *Aspergillus niger* PSH (°‹) and *Penicillium commune* EH2 (°¤) on catechin at 3 g/l (a). Solid and dotted lines represent the modeled growth of *Aspergillus* and *Penicillium*, respectively, using the logistic equation. Catechin degradation by *Aspergillus niger* PSH and *Penicillium commune* EH2 on a medium with catechin at 3 g/l (b).

presence of the wild strains isolated from the mexican desert.

Using a culture medium with 3 g/l of catechin and 1 g/l of glucose, two strains were selected from the better strains. One strain represented the genus *Aspergillus* (PSH) whereas the other one represented the genus *Penicillium* (EH2). Figure 2a shows the results of the fungal growth, where the *Aspergillus* strain was grown in pellets oxidizing the culture broth probably due to the fungus using its phenoloxidase system (laccase, peroxidase and tyrosinase), while *Penicillium* was grown as a diffuse mycelium without oxidizing the culture broth.

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, the μ (specific growth rate in h^{-1}) value was higher because its growth was significantly faster than *Penicillium* (Fig. 2a). The growth results revealed the ability of this fungal strains to utilize condensed tannins at high concentrations.

The catechin degradation rate and percent of degradation were calculated for 95 h of culture. Figure 2b shows that under these conditions, *Aspergillus* strains degraded higher amount of catechin (79.33%) than *Penicillium* (76.35%) with degradation rates of 0.0065 and 0.0074 g/l/h, respectively. This experimental section demonstrated the potential capacity of both fungal strains for catechin degradation. It is the first work that reports the fungal degradation of catechin using high initial concentration (3 g/l) and infers the possibility of using this fungal strains for the fermentation of plant tissue extracts and hydrolysates containing these high antinutritional phenolic compounds (i.e, coffee pulp and residues of *Larrea tridentata Cov*, etc.).

In conclusion, *Aspergillus niger* PSH and *Penicillium commune* EH2 exhibited their potential for biotechnological interest because they can utilize condensed tannins as carbon source and they could be used to degrade tannins directly in residues of coffee pulp, thus providing a good alternative to solve the big problem that represents the accumulations of this agroindustrial byproduct in some countries like Mexico, Colombia or Brazil. Use of higher initial catechin concentrations and the related enzymatic activities are under investigation.

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