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BRIEF REPORT

Hydrolytic enzyme activities in shiitake mushroom (*Lentinula edodes*) strains cultivated on coffee pulp

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Abstract Hydrolytic enzyme production (cellulases, laminarinases and xylanases) was studied in cultures of *Lentinula edodes* on sterilized coffee pulp. Samples of substrate colonized by mycelia were taken after 7, 14, 21, 28 and 35 days of incubation at 25 °C (W1 to W5) and during the fruiting period at different stages: formation of primordia (PF), first harvest (H) and one week after the first harvest (PH). The enzymatic activity was lower during the early mycelial growth and showed higher levels during the formation and development of fruiting bodies. During the reproductive stage of the fungus, the samples were subjected to a soaking treatment; however, it was not possible to relate this soaking treatment to the increase in enzyme production. The levels of enzymatic activity suggest that secretion of the studied enzymes does not influence the adaptability of the strains to the substrate.

PALABRAS CLAVE
Hongos comestibles;
Residuos de café;
Producción de enzimas;
Celulasas;
Laminarinasas;
Xilanasas

Actividad de las enzimas hidrolíticas en cepas del hongo shiitake (*Lentinula edodes*) cultivadas en pulpa de café

Resumen Se estudió la producción de enzimas hidrolíticas (celulasas, laminarinasas y xilanasas) en cultivos de *Lentinula edodes* en pulpa de café estéril. Se tomaron muestras de sustrato colonizado por el micelio después de 7, 14, 21, 28 y 35 días de incubación a 25 °C (W1 a W5) y durante el período de fructificación en diferentes etapas: formación de primordios (PF), primera cosecha (H) y una semana después de la primera cosecha (PH). La actividad enzimática fue menor al inicio del crecimiento micelial y mostró mayores niveles en la formación y el desarrollo de basidiomas. Durante la etapa reproductiva del hongo, las muestras se sometieron a un tratamiento de remojo. Sin embargo, no fue posible relacionar este tratamiento con el

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aumento de la producción de enzimas. Los niveles de actividad enzimática sugieren que la secreción de las enzimas estudiadas no influye en la capacidad de adaptación de las cepas al sustrato.

The mushroom commonly known as shiitake (*Lentinula edodes* [Berk.] Pegler) is widely appreciated for both its delicate flavor and medicinal properties. Its cultivation began in China around the year 1100 A.D., currently being the second most commercially produced species, exceeded only by the white button mushroom (*Agaricus bisporus* [Lange] Imbach)[1]. The improvement in cultivation techniques has enabled the production of shiitake in substrates that are very different from those in its natural habitat. The modern cultivation of this species is mainly based on the use of different substrates that are rich in lignin and cellulose, which are subjected to different thermal disinfection treatments such as autoclave sterilization and steam pasteurization[1,7].

Coffee pulp is an agroindustrial byproduct available in considerable quantities in tropical and subtropical regions[5]. Despite the fact that various studies have been conducted on coffee pulp for the cultivation of edible species[10,15], this substrate is still not used intensively. The organic nature and composition of this material makes coffee pulp an ideal substrate for processing with microorganisms and for the generation of products of high aggregate value. The use of coffee pulp has been proposed in the generation of silage, biogas, vermicompost, fodder and ethanol, among other products[5]. Coffee pulp has been used in the experimental cultivation of shiitake; however, as with other species, there are no data regarding commercial cultivation on this agroindustrial byproduct.

Due to differences in the chemical composition of substrates utilized in the cultivation of shiitake, it is necessary to select genotypes that present suitable characteristics for growth and production of fruiting bodies in a given substrate. The capacity of a species to grow in a particular lignocellulosic substrate depends on its ability to utilize the majority of the components of the substrate as nutritive elements[2]. This is determined by the capacity of the fungus to synthesize the necessary hydrolytic and oxidative enzymes.

The enzymes produced for the degradation of lignin and cellulose, and for the transformation of these materials into low molecular weight compounds that are easily assimilated by the mushroom, are induced by the presence of different components in the substrates[13]. Enzyme production has been widely studied in shiitake, especially when cultivated in substrates based on, or derived from, wood[3,14]. In addition, the spectrum of enzymes obtained during the complete cycle of cultivation of shiitake on wheat straw has been studied[4]. However, there is still a lack of information regarding enzyme production during cultivation on coffee pulp. Therefore, the objective of this study was to quantify hydrolytic enzyme production during cultivation on this substrate and to determine its possible role in the degradation of the substrate and sporome production, with the aim of optimizing the use of this residue for shiitake cultivation.

The following commercial strains of *L. edodes* were studied: IE-40, IE-105, IE-124, IE-171, IE-242 (cross of IE-244 × IE-245), IE-243, IE-244 IE-245 and IE-246. All these strains are deposited in the strain collection of the Instituto de Ecología (Xalapa, Mexico) and, for the purposes of this study, were sown in a potato dextrose agar medium (PDA, Bioxon, Becton Dickinson and Company, Queretaro, Mexico) and incubated at 25 °C in darkness.

The strain inoculum was produced with hydrated sorghum seeds (≈55% moisture). The seeds were placed in polypropylene bags for autoclave sterilization (121 °C, 1 h) (Aesa, Model 300, Mexico). Sterile seeds were inoculated with the mycelium of each of the cultures previously sown in PDA. The inoculated samples were incubated at 25 °C in darkness.

The coffee pulp was collected from a local coffee bean processing plant, sundried (to ≈20% moisture) and stored at ambient temperature until use. For sowing the fungus, the substrate was rehydrated in water for 12 h, and the excess water was drained until the substrate reached a moisture content of 65%. Substrate samples of 500 g (dry weight) were prepared, placed in polypropylene bags and sterilized at 121 °C for 1 h. Twenty-five grams of inoculum (5% of the inoculation percentage) were added to each sample and distributed homogenously throughout the sample. Eight replicates were prepared per strain and were all incubated at 25 ± 2 °C in darkness.

In order to evaluate the enzymatic activity of the fungi during the reproductive stage, some strains were randomly selected. Following incubation, the plastic bags were removed and the substrate blocks containing mycelium were subjected to two watering treatments: (1) rewetting (R), which consisted in submerging the complete block in water at 10 °C for 12 h, and (2) no rewetting (NR). The blocks were then kept under suitable environmental conditions for fruiting body development: alternate 12 h periods of light/darkness, temperature of 23 ± 3 °C and relative humidity of 85 ± 5%. The number of days required for primordia formation and production of the first harvest as well as the fresh weight of the basidiomes collected were recorded. These data were used to determine the biological efficiency of the different strains.

Samples were taken after 7, 14, 21, 28 and 35 days of incubation (samples W1 toW5). During the fruiting period, samples were taken at the following stages: primordia formation (PF), first harvest (H) and one week after the first harvest (PH). The enzymatic extracts were prepared in 50 ml flasks by placing 1 g of substrate with mycelium in 10 ml of