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ORIGINAL ARTICLE

Response of ligninolytic macrofungi to the herbicide atrazine: dose-response bioassays

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Abstract
The effect of atrazine concentrations on mycelial growth and ligninolytic enzyme activities of eight native ligninolytic macrofungi isolated in Veracruz, México, were evaluated in a semi-solid culture medium. Inhibition of mycelial growth and growth rates were significantly affected (p = 0.05) by atrazine concentrations (468, 937, 1875, and 3750 mg/l). In accordance with the median effective concentration (EC_{50}), *Pleurotus* sp. strain 1 proved to be the most tolerant isolate to atrazine (EC_{50} = 2281.0 mg/l), although its enzyme activity was not the highest. *Pycnoporus sanguineus* strain 2, *Daedalea elegans* and *Trametes maxima* showed high laccase activity (62.7, 31.9, 29.3 U mg/protein, respectively) without atrazine (control); however, this activity significantly increased (p < 0.05) (to 191.1, 83.5 and 120.6 U mg/protein, respectively) owing to the effect of atrazine (937 mg/l) in the culture medium. *Pleurotus* sp. strain 2 and *Cymatoderma elegans* significantly increased (p < 0.05) their manganese peroxidase (MnP) activities under atrazine stress at 468 mg/l. The isolates with high EC_{50} (*Pleurotus* sp. strain 1) and high enzymatic activity (*P. sanguineus* strain 2 and *T. maxima*) could be considered for future studies on atrazine mycodegradation. Furthermore, this study confirms that atrazine can increase laccase and MnP activities in ligninolytic macrofungi.

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PALABRAS CLAVE
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Lacasa;
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Estrés oxidativo;
Hongos de la pudrición blanca

Respuesta de macrohongos ligninolíticos al herbicida atrazina: bioensayos dosis-respuesta

Resumen
Se evaluó el efecto de diferentes concentraciones de atrazina sobre el crecimiento micelial y la actividad enzimática de ocho macrohongos ligninolíticos aislados en Veracruz, México. La inhibición del crecimiento micelial y la tasa de crecimiento diaria fueron significativamente ($p < 0,05$) afectadas por todas las dosis de atrazina (468, 937, 1875 y 3750 mg/l) adicionadas al medio de cultivo. De acuerdo con la concentración efectiva media (CE_{50}), *Pleurotus* sp. cepa 1 fue el aislamiento más tolerante a la atrazina (CE_{50} = 2281 mg/l), aunque sus actividades enzimáticas no fueron altas. *Pycnoporus sanguineus* cepa 2, *Daedalea elegans* y *Trametes maxima* mostraron actividades altas de lacasa (62,7, 31,9 y 29,3 U mg/proteína, respectivamente) en ausencia de atrazina (control); estas actividades se incrementaron ($p < 0,05$) significativamente (191,1, 83,5 y 120,6 U mg/proteína, respectivamente) en presencia de atrazina (937 mg/l) en el medio de cultivo. *Pleurotus* sp. cepa 2 y *Cymatoderma elegans* incrementaron significativamente ($p < 0,05$) sus actividades de manganeso peroxidasa (MnP) bajo la concentración de 468 mg/l de atrazina. Los aislamientos con alta CE_{50} (*Pleurotus* sp. cepa 1) y alta actividad enzimática (*P. sanguineus* cepa 2 y *T. maxima*) podrían ser considerados para futuros estudios en la micodegradación de atrazina. Además, el presente estudio confirma que la atrazina puede incrementar las actividades lacasa y MnP en macrohongos ligninolíticos.

Introduction

During the last century, farming and agricultural activities released many persistent and toxic chemical pesticides into the environment including insecticides, fungicides, nematicides, rodenticides and herbicides[21]. They comprise a variety of molecules possessing different properties that confer some degree of environmental persistence and mobility, as well as different toxic, carcinogenic, mutagenic and teratogenic potentials[10,17,21]. Such substances may also affect the endocrine systems of non-targeted organisms, including humans[38].

The use of herbicides in Mexico increased strongly in the last decade, where according to recent studies more than 45% of pesticides marketed were herbicides, trazine being the most frequently used[7]. The use of atrazine is not regulated and is widely used in Mexican agriculture, where the application rates range from 0.1 to 4 kg/ha/year. At these application rates, the annual consumption of atrazine in Mexico has been estimated to be around 1078 tons/year with an annual consumption increase of 10%[18,19].

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a selective herbicide that belongs to the s-triazine family, which contains an aromatic hexameric and symmetrical ring, constituted by three carbon and three nitrogen atoms in alternate positions[18]. Atrazine is used to control broadleaf and grass weeds in corn, sorghum, sugar cane, coffee crops and conifer reforestation. Atrazine kills susceptible plants by binding to the quinone protein in photosystem II and inhibits photosynthetic electron transport[36].

Despite its apparent biodegradability, atrazine has led to the contamination of terrestrial ecosystems and can be found and measured in ground and surface waters in many countries[7,30]. The removal of atrazine from the environment is an ecological responsibility, and finding a safe and economical method is both a major concern for land management agencies and a challenge to science[13]. Bioremediation with microbial organisms is one approach. In the last two decades, the use of ligninolytic macrofungi and their enzymes in the mycoremediation of environmental contaminants has become a promising solution[40]. This fungal ability to bioremediate is generally attributed to the production of extra-cellular ligninolytic enzymes such as laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP), which are substrate non-specific enzymes and are able to degrade a wide range of recalcitrant compounds including several herbicides that are structurally related to lignin[3-5,9,39].

The first step to establish future studies of atrazine degradation through mycoremediation in contaminated sites is finding tolerant strains to atrazine[28]. The selection through dose-response bioassay is considered a way to identify these strains, which could be used in subsequent studies on soil mycoremediation[8,29]. With this in mind, we proposed the hypothesis that tolerant strains will not show mycelial growth inhibition and reduction in their enzyme activities upon exposure to the herbicide, therefore they will obtain a high mean effective concentration (EC_{50}). To corroborate this hypothesis, the goals of this work were to assess mycelial growth rates and ligninolytic enzyme activities under atrazine stress in eight native ligninolytic macrofungi isolated from Veracruz, Mexico.