Rev Argent Microbiol. 2016;xxx(xx):xxx–xxx

asociación argentina de microbiología

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

BRIEF REPORT

Detection of manganese peroxidase and other exoenzymes in four isolates of *Geastrum* (Geastrales) in pure culture

Francisco Kuhar[a,*], **Valeria C. Castiglia**[b], **Juan C. Zamora**[c]

[a] *Centro de Investigación y Extensión Forestal Andino Patagónico – CONICET, Argentina*
[b] *Laboratorio de Micología Experimental, Departamento de Biodiversidad y Biología Experimental, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina*
[c] *Departamento de Micología, Real Jardín Botánico, CSIC, Madrid, Spain*

Received 17 May 2016; accepted 7 September 2016

KEYWORDS
Cellulase;
Xylanase;
Laccase;
Lipase;
Litter degradation

Abstract Knowledge regarding the enzymatic machinery of fungi is decisive to understand their ecological role. The species of the genus *Geastrum* are known to grow extremely slowly in pure culture, which makes it difficult to evaluate physiological parameters such as enzyme activity. Qualitative assays were performed on isolates of four species of this genus, showing evidence of laccase, cellulase, pectinase, amylase and lipase activity and suggesting that a wide range of carbon sources can be exploited by these species. For the first time in this genus, quantitative assays verified manganese peroxidase activity (up to 0.6 mU/g) in 30-day old cultures, as well as laccase, β-glycosidase and β-xylosidase activities.

PALABRAS CLAVE
Celulasa;
Xilanasa;
Lacasas;
Lipasa;
Degradación de hojarasca

Detección de manganeso peroxidasa y otras exoenzimas en 4 aislamientos de *Geastrum* (Geastrales) en cultivo puro

Resumen El conocimiento de la maquinaria enzimática de un hongo es decisivo para entender su rol ecológico. Las especies del género *Geastrum* son conocidas por su crecimiento extremadamente lento en cultivos puros, lo que hace difícil la evaluación de parámetros fisiológicos como las actividades enzimáticas. Se realizaron ensayos cualitativos sobre aislamientos de 4 especies de este género, mostrando evidencias de actividades lacasa, celulasa, pectinasa, amilasa y

* Corresponding author.
E-mail address: fkuhar@gmail.com (F. Kuhar).

http://dx.doi.org/10.1016/j.ram.2016.09.002

Please cite this article in press as: Kuhar F, et al. Detection of manganese peroxidase and other exoenzymes in four isolates of *Geastrum* (Geastrales) in pure culture. Rev Argent Microbiol. 2016. http://dx.doi.org/10.1016/j.ram.2016.09.002

lipasa, mostrando el amplio rango de fuentes de carbono que pueden ser explotadas por estas especies. Ensayos cuantitativos verificaron por primera vez en este género la actividad manganeso peroxidasa (hasta 0,6 mU/g) en cultivos de 30 días, así como también β-glucosidasa y β-xilosidasa.

Fungal extracellular enzymes have been a subject of study not only because of their intrinsic interest, but also due to their biotechnological applications. For this reason, most studies have been based on fast growing organisms that produce high enzyme activities, and on model species whose genetics and physiology are well known.

The finding of lignin-modifying enzymes in litter or soil fungi[5] redirected the attention of many scientists to the adaptive role they may play in this environment. Recent studies based on genomic data provide invaluable information about the enzyme machinery of many fungal taxa in relation to their ecology[11] and phylogenetic placement[1]; however, to date, no studies of this kind have included members of the order Geastrales.

Since degradative fungal enzymes may have an important role in bioremediation scenarios, the study of enzyme activities is also relevant for soil and litter fungal species regardless of their possible industrial application. One of the problems in this field is the slow growth in culture of many species (*e.g.* ectomycorrhizal fungi), which is usually the main obstacle to their enzyme research. An example of this fact is the genus *Geastrum*, which, though entirely saprotrophic, shows an extremely slow growth rate. Many of its species are known to form extensive mycelial networks and cushions in all kinds of forests, tolerating inhibitory substrates such as *Eucalyptus* or Cupressaceae litter[10].

This genus has been the subject of exhaustive taxonomic and floristic studies. Sunhede[10] made a systematic revision and took thorough notes on its morphology, biology and ecology, however, at present just a few works make reference to its ecophysiology. Sunhede[10] describes the growth of the isolates as ''very slow, after six weeks varying from 3 to 22 mm.'' Ilko et al.[4] indicate that the colony of *Geastrum pouzarii* reached 3–4 mm after six weeks and Zamora et al.,[14] report isolates of *G. argentinum* achieving up to 4 mm after a week. This characteristic makes these species hard to isolate and to maintain in pure cultures, and this is the main reason why there are no reports of their exoenzyme activities. Sunhede[10] reports oxidase enzymatic activity in some species by observing metachromatic staining of mycelia with some laccase and tyrosinase substrates. Zamora et al.[13] carefully studied similar reactions in fresh basidiomes; however, no attempts to study *in vitro* enzymatic activity have been performed until now.

Four *Geastrum* species were isolated and studied, namely *G. argentinum*, *G. papinuttii*, *G. morganii*, and *G. plicatum*. Specimens were collected in the province of Buenos Aires and deposited in BAFC (Universidad de Buenos Aires), LPS (Universidad Nacional de La Plata) and MA-fungi (Real Jardín Botánico de Madrid) herbaria (Index Herbariorum, http://sweetgum.nybg.org/science/ih/)[12]. Isolates were obtained from the pseudoparenchymatous layer of the exoperidium in malt agar medium, and deposited in BAFC Culture Collection under the following numbers (herbarium collection numbers in parentheses): *G. argentinum* BAFC 3282 (LPS 48446), *G. plicatum* BAFC 3283 (MA-Fungi 83774), *G. morganii* BAFC 3284, *G. papinuttii* BAFC 3285 (MA-Fungi 83764). Three of the four species (*G. argentinum*, *G. plicatum*, and *G. papinuttii*) were molecularly characterized by Zamora et al.[15,16], and GenBank accession numbers are available there for ITS (Internal Transcribed Spacer) nrDNA (nuclear ribosomal DNA), LSU (Large Subunit) nrDNA, rpb1 (polymerase II largest subunit gene) and *atp6* (ATPase 6 gene).

An agarized medium containing glucose and glutamic acid (GG medium)[3] was utilized for the screening. Laccase (EC:1.10.3.2) activity was quantified according to Paszczynski et al.[8] and MnP (manganese peroxidase; EC:1.11.1.13) following Paszczynski et al.[9] with and without $MnCl_2$ 0.1 mM as inducer. In all cases, aliquots of supernatant were replaced by agar plugs (5 mm diameter) provided by 60-day malt agar cultures. β-xylosidase (EC:3.2.1.37) and β-glucosidase (EC:3.2.1.21) enzyme activities were determined by measuring the release of p-nitrophenol from p-nitrophenyl-β-D-xylopyranoside (pNPX) and p-nitrophenyl-β-D-glucopyranoside (pNPG). Agar plugs were incubated in 900 μl of 1 mM substrate at 50 °C for 30 min. The reaction was stopped by adding 2 ml of Clark and Lubs buffer pH 9.8. The release of p-nitrophenol was measured using a UV-vis spectrophotometer at λ 405 nm and 1 unit of β-xylosidase activity was defined as the amount of the enzyme releasing 1 μmol p-nitrophenol equivalent per minute under the assayed conditions. Hydrolytic enzyme activities were qualitatively evaluated according to Pardo and Forchiassin[7], replacing glucose in each assay by starch 0.1% (for amylase EC:3.2.1.1 activity), pectin 0.1% (for pectinase EC:3.2.1.15 activity), carboxymethyl-cellulose (CMC) 0.1% (for endoglucanase EC:3.2.1.4 activity) and xylan 0.1% (for xylanase EC:3.2.1.8 activity). Lipase (EC:3.1.1.3) qualitative assay was as indicated by Nikoleit et al.[6] using Tween 80 as a carbon source but replacing the medium by basal GG medium[3] without a carbon source. Laccase and LiP (lignin peroxidase) activity were also qualitatively evaluated in a GG medium by adding dimethoxyphenol 0.1 mM and Azure B. All data presented are the means of the results of triplicates and error bars indicate SE.

All isolates showed active growth in each assayed carbon source (xylan, cellulose, starch and pectin). The use of lipid esters as a carbon source was also demonstrated in *G. plicatum* through the active growth and degradation of Tween 80.

Please cite this article in press as: Kuhar F, et al. Detection of manganese peroxidase and other exoenzymes in four isolates of *Geastrum* (Geastrales) in pure culture. Rev Argent Microbiol. 2016. http://dx.doi.org/10.1016/j.ram.2016.09.002