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Carboxylesterase activity of fungi isolated from *Marchantia polymorpha*

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ABSTRACT

Twenty two (22) fungi associated with Marchantia polymorpha were tested for carboxyl esterase activity. Eleven were endophytic and the other eleven were epiphytic on the plant's thallus. Results show that all the isolates produced this enzyme measured through spectrophotometric means. The carboxylesterase activity was quantified in 1-naphthol equivalents (μg/mL). The least producer of the enzyme was Colletotrichum boninense (KP714268) at 33.37 μg/mL and the isolate that had the highest activity was Nodulisporium sp. (KP714282) at 64.69 μg/mL.

Keywords: *carboxyl esterase activity, endophytic fungi, Marchantia polymorpha*

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INTRODUCTION

Microorganisms are prolific sources of different compounds of importance. Enzymes from these organisms are one of such compounds that have multiple applications in today's world. Biotransformations mediated by enzymes are described as low cost and environmentally compatible (Romanoa et al.,2005). In contrast, more traditional chemical treatments are generally non- specific and not always easily controlled, and may create harsh pollutants. Industrial processes currently facilitated by enzymes are fast becoming to be mainstream as it offers better financial and ecological alternatives to chemical-physical and mechanical processes and applications (Binod et al., 2013). Currently, about 150 industrial processes use enzymes or whole microbial cell catalysts (Adrio and Demain, 2014).

Because of increasing commercial demand for these compounds, numerous biological sources have been explored in last few decades. It has been seen that fungi are one of those organisms that can become biofactories for these (Srilakshmi et al., 2014). Specifically, non-pathogenic filamentous fungi are attractive source organisms for industrially important enzymes for the reason that they are easily cultivated and that they can produce extracellular enzymes in high amounts (Guimaraes et al, 2006). Also, these organisms are said to possess special posttranscriptional modification machinery which allows for glycosylation and correct protein folding (Quintanilla et al., 2015).

One class of these industrially important enzymes is carboxylesterase (EC 3.1.1.1). Carboxylesterases are widely distributed in animals, plants, and microorganisms and catalyze the hydrolysis of various acetyl esters. Specifically, these enzymes are used to effect stereoselective esterification, inter-esterifications, and transesterification of substrates that produce products with potential applications as biodegradable surfactants, emulsifiers, and preservatives [7 Cui et al., 1999]. Carboxylesterases also has applications in the food industry and in the enzymatic production of lipophilic fine chemicals (Ay et al., 2011).

Researches on carboxylesterase in filamentous fungi are sparse. The few researches delved on the production of carboxylesterase of fungi in response to the presence of pyrethroid insecticides. Alvarenga et al. (2014) and Guo et al. (2009) studied the role of this enzyme in the biodegradation of parathion and pyrethroids respectively.

It is in the context of the dearth of researches on fungal carboxylesterases that this study was done. In this study, the fungal isolates from *Marchantia polymorpha* both endophytic and epiphytic to the thallus were investigated for their capacity to produce the carboxylesterase enzyme *in-vitro.* This research was performed with the intention of looking for interesting fungi that can be source organisms of this industrially important enzyme.

MATERIALS AND METHODS

Fungal isolates and Culture Conditions

The fungal isolates used in this study were previously isolated from the thalloid liverwort *Marchantia polymorpha*. Table 1 summarizes the identity and their accession numbers in the GenBank database. The isolates were revived by inoculating them onto potato dextrose agar (PDA) plates incubated at 30°C prior to the assay for carboxylase activity.

Carboxylesterase Activity Assay

The method of Avicor et al. (2012) was employed in the assay with slight modifications. Approximately 25 mm^2 of fungal mycelia was aseptically cut from a plate culture and placed into a test tube containing 2.8 ml of phosphate buffer solution (pH 7.0). 100 µl of the 30 mM 1-naphthyl acetate solution was pipetted into the test tube with the buffer incubated for 10 minutes in at 40°C in a water bath. After incubation, the reaction was stopped by adding 0.25 ml of stop solution. The stop solution consisted of 3.4% by mass SDS along with 0.8% Fast Blue B salt, dissolved in Type 1 water (Sree et al., 2015). The Fast Blue B dye formed a complex with 1-naphthol that resulted in color development. The absorbance of the mixture was read immediately at 600 nm. An increase in absorbance of the sample against a control indicates carboxylesterase activity. The negative control was the same set-up without fungal mycelia. All treatments and the control were in triplicates. Carboxylesterase activity was then calculated using a calibration curve previously prepared from a standard solution of 36µg/ml of 1-naphthol and its 1:1 serial dilutions ($y = 0.0416x + 0.0569$, $R^2 = 0.9937$).

Statistical Analysis of Assay Results

Statistical analysis was carried out using IBM SPSS version 21 using One-Way ANOVA ($p<0.05$). A Post hoc range test was done using Dunnet's post hoc criterion.

RESULTS AND DISCUSSION

Twenty- t w o fungi that were previously isolated from *Marchantia polymorpha* were tested for carboxylesterase activity. There were 11 isolates each for epiphytic and endophytic fungi. Two of the 22 were identified only up to their class level because of sequence

homology lesser than 95%. On the other hand 4 were identified up to the genus level only because sequence homology was lesser than 98%. A total of 16 isolates were identified to the species level. There were three classes that were represented in this study namely Eurotiomycetes, Dothideomycetes and Sordariomycetes (Table 1).

In the assay for carboxylesterase activity, 1-naphthyl acetate is used as the enzyme's substrate. Ester cleavage converts it into 1-naphthol and acetate. The 1-naphthol subsequently reacts with Fast Blue B salt to form a purple diazonium dye (Komsta 2013). The formation of a purple diazonium dye indicated a positive result for carboxylesterase activity. This was measured as the extent of absorbance of the solution at 600 nm. Analysis of variance showed that all isolates were significantly different from the control ($p<0.05$). The isolate with the least activity was *Colletotrichum boninense* (KP714268) whose mean absorbance was still three times the absorbance measured from the negative control. The computed carboxylesterase activity in terms of liberated 1-naphthol equivalent was 33.37 µg/mL. The isolate that had the highest carboxylesterase activity was *Nodulisporium* sp. (KP714282). The measured 1-naphthol equivalent was 64.69 µg/mL. This value was 6 times the value computed from the negative control and almost twice the amount produced by the least producer which was *Colletotrichum boninense* (Figure 1).

Table 1: Fungal isolates studied in this research and their identities together with their GenBank accession numbers.

Identity	Epiphytic/ Endophytic	GenBank accession number	Family	Class
Colletotrichum boninense	Endophytic	KP714268	Glomerellaceae	Sordariomycetes
Penicillium sclerotiorum	Epiphytic	KP714289	Trichomonaceae	Eurotiomycetes
Trichothecium roseum	Epiphytic	KP714306	*Incertaesedis*	Sordariomycetes
Penicillium meridianum	Epiphytic	KP714286	Trichomonaceae	Eurotiomycetes
Colletotrichum novae-zelandiae	Endophytic	KP714271	Glomerellaceae	Sordariomycetes
Unidentified Eurotiomycetes	Epiphytic	KP714307		Eurotiomycetes
Penicillium sp.	Epiphytic	KP714290	Trichomonaceae	Eurotiomycetes
Penicillium funiculosum	Epiphytic	KP714285	Trichomonaceae	Eurotiomycetes
Penicillium neomiczynskii	Epiphytic	KP714287	Trichomonaceae	Eurotiomycetes
Penicillium purpurogenum	Endophytic	KP714288	Trichomonaceae	Eurotiomycetes
Fusarium sp.	Endophytic	KP714276	Nectriaceae	Sordariomycetes
Phoma herbarum	Epiphytic	KP714296	Didymellaceae	Dothideomycete
Colletotrichum karstii	Endophytic	KP714270	Glomerellaceae	Sordariomycetes
Penicillium crustosum	Endophytic	KP714284	Trichomonaceae	Eurotiomycetes
Fusarium oxysporum	Endophytic	KP714275	Nectriaceae	Sordariomycetes
Lophiostoma sp.	Epiphytic	KP714278	Lophiostomataceae	Dothideomycetes
Pestalotiopsis microspora	Endophytic	KP714295	Amphisphaeriaceae	Sordariomycetes
Penicillium thomii	Epiphytic	KP714291	Trichomonaceae	Eurotiomycetes
Unidentified Sordariomycetes	Endophytic	KP714310		Sordariomycetes
Pestalotiopsis lespedezae	Epiphytic	KP714294	Amphisphaeriaceae	Sordariomycetes
Acremonium alternatum	Endophytic	KP714265	Hypocreaceae	Sordariomycetes
Nodulisporium sp.	Endophytic	KP714282	Xylariaceae	Sordariomycetes

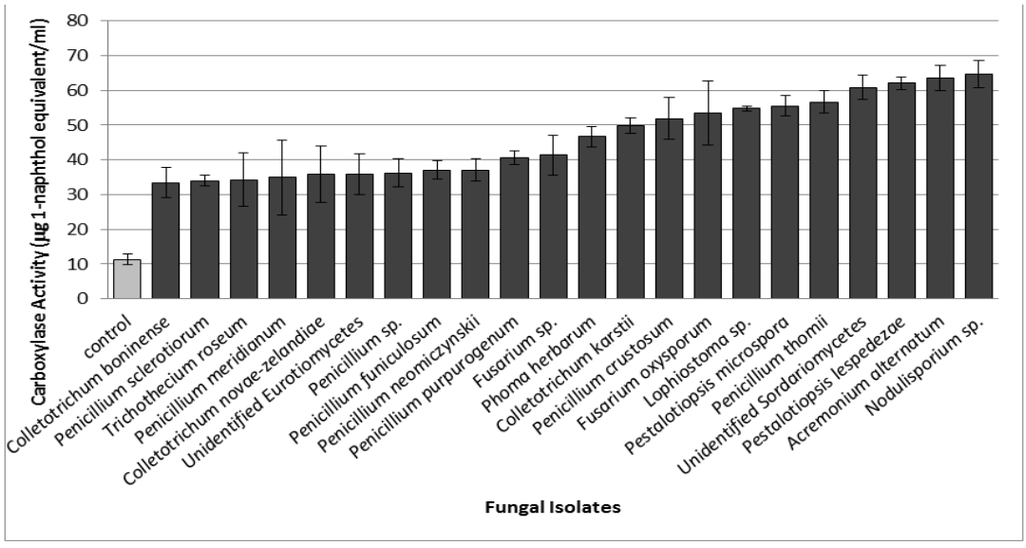

Figure 1: Mean carboxylesterase activity of fungi associated with the thallus of *Marchantia polymorpha* measured as 1-naphthol equivalents (µg/mL).

There are several observations from the results that may need further study in order to have a plausible explanation. One is that, of the top ten producers of carboxylesterase, 7 are endophytes and only 3 are epiphytes. On the other hand, of the 10 least producers of this enzyme, 7 are epiphytes and only 3 are endophytes. It may seem that endophytic fungi have evolved carboxylesterase activity that may have a purpose as they live within the tissues of their host plants. It is said that the organisms occupying the endosphere are not accidentally there but most probably have been selected for this niche by the plant because of the beneficial effects they offer their host (Backman and Sikora, 2008); Maor and Shirasu, 2005). It can be assumed therefore that possessing the ability to produce carboxylesterase benefits endophytes more than epiphytes as this enzyme may provide the endophytes access to compounds provided by the host plants as they provide some benefit to their host.

The UniProt database (uniprot.org) is a comprehensive, high-quality and freely accessible resource of protein sequence and functional information. In this database, only one of the isolates in this study was reported to possess carboxylesterase activity (*Fusarium oxysporum*). The genera *Colletotrichum, Penicillium, Pestalotiopsis* and *Acremonium* are also in the database with carboxylesterase activity. However, the specific species investigated in this study are not represented in the database. *Trichothecium, Phoma, Lophiostoma* and *Nodulisporium* are not found in the

database for the presence of the enzyme of interest. It may be inferred that this research may be the first instance where these fungal isolates are documented to have carboxylesterase activity.

The isolate with the highest measured carboxylesterase activity was *Nodulisporium* sp. This fungus has been found to occur as an endophyte of *Thelypteris angustifolia* found in Central America (Riyaz-Ul-Hassan et al., 2013). It was also found within the tissues of *Myroxylon balsamum* from the Ecuadorian amazon [16]. Representatives of this genus have also been found to produce interesting secondary metabolites such as volatile organic compounds (VOCs) that have both fuel and biological potential (Mends et al., 2012) and nodulisporic acids which are novel indole diterpenes that exhibit potent insecticidal properties(Maheshwari and Dubey, 2008). Along with all of these secondary metabolites that can have various uses, this fungus can be tapped for applications that require the activity of carboxylesterases. However, further research is necessary to purify and characterize this enzyme from *Nodulisporium* sp. The necessary conditions for its optimal activity need to be discovered so as to determine the possible applications it may have.

CONCLUSION

All of the twenty two fungal isolates investigated in this study were able to produce the carboxylesterase enzyme whose activity was measured as 1-naphthol equivalents (μg/mL). *Colletotrichum boninense* (KP714268) produced the least activity while *Nodulisporium* sp. (KP714282) produced the most. It is interesting to note that there were more endophytes that produced the higher amounts of the enzyme than epiphytes. Also, only *Fusarium oxysporum* is entered in the UniProt database as a fungus whose carboxylesterase enzyme is characterized. All the other 23 isolates in this study are not found in the database with characterized carboxylesterase. This study reveals that fungi associated with *Marchantia polymorpha* are producers of the industrially important enzyme carboxylesterase. It is recommended that further studies are to be done on the enzymes themselves (purification and characterization) in relation to optimal conditions of activity (i.e. pH, temperature, etc.) so as to ascertain possible industrial applications of these.

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