BEYOND CHITIN: UNVEILING NEW CARBON SOURCES FOR EFFICIENT ENZYME INDUCTION IN CUNNINGHAMELLA ECHINULA PA3S12MM

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ABSTRACT

Chitin is one of the most abundant biopolymers in nature, present in a large number of organisms under very different forms. This polymer can be hydrolyzed by Chitinases, extracellular enzymes produced by bacteria, fungi, viruses, and plants that break down the β-1,4 bonds in the chitin structure. Displaying a broad use in biotechnology and industry, Chitinases can lead to greener, energy-efficient, pollution-free, and economically sustainable processes. This work aimed to identify which carbon sources were best suited to the induction of chitinase in Cunninghamella echinulata PA3S12MM, isolated from soil samples collected in the nearby Nova Aurora, Parana State, Brazil. The fungus was cultivated in a liquid media, at 28ºC, for 10 days in static conditions along with 14 different carbon sources. The best carbon source inducer was Glucose 1% (4,45 U/ml) followed by tilapia schama 1% (3,54 U/ml). The present study suggests that Chitinases can be induced from other carbon sources than chitin and its derivates.

Keywords: Chitinase. Carbon source. Biotechnology. Fungus.

1 INTRODUCTION

The current progress in biotechnology has made the enzymology field captivating. Industrial scale procedures can find in the study of enzymes the answers to the production of high-value products, based on the knowledge of enzyme specificity, efficiency, and speed of metabolic reactions. Among the vast field of enzymes with a biotechnological approach, Chitinase is one pivotal in many biological systems, transpassing the boundaries of just chitin breakdown [1,2].

Chitinase (EC 3.2.1.14) is part of the glycoside hydrolase family (GH), found in viruses, bacteria, fungi, protozoa arthropods, and plants [3–5] that act by hydrolyzing the β-1,4 bonds in the chitin structure, releasing chitooligosaccharides (COS), later converted into N-acetylglucosamine by the action of chitobiases. Chitin can be found in nature under three different crystal forms, α, β, and γ chitin, being the β-chitin easier to hydrolyze or solubilize due to its less intermolecular tension, when compared to the other 2 crystal forms [6–8]. Considered one of the most ubiquitous polysaccharides spread throughout nature, Chitin can be found in cell walls of fungi, as a structural component of many organisms such as mollusks, crustaceans, algae, and marine invertebrates, composing the structure of shells and exoskeletons os these organisms [5,9].

Initially studied for their role in the natural recycling process, Chitinases soon began to draw more attention for their prominence in biotechnology in many different industries. Predominantly, Chitinases can be applied to organic waste control, increasing the degradation rates of sea waste like crab, lobster, and shrimp shells [10], as biocontrol agents with antifungal activity presented in bacteria and plants aiming the protection against fungal diseases [11], in insect control management as biopesticides, offering benefits such as low toxicity, biodegradability and selectivity towards the target pest [12], in food preservation, since they can break down the fungal cell wall and prevent spore germination [13]. Also, they can be used in several human health care applications, such as in the pharmaceutical industry, combining enzymes and antifungals to treat fungal infections [14].

Therefore, this study aimed to analyze which carbon source can induce chitinase production at a better rate in the conditions pre-established in preview work (data not shown) regarding the intention of optimizing the processes in its initial stages.

2 MATERIAL & METHODS

Cunninghamella echinulata PA3S12MM was isolated from soil samples collected in the nearby Nova Aurora, Parana State, Brazil. The routine maintenance of the fungal strain was carried out in test tubes containing 10mL of potato dextrose agar (PDA), incubated at 28ºC for seven days, then kept in a refrigerator for 30 days maximum.

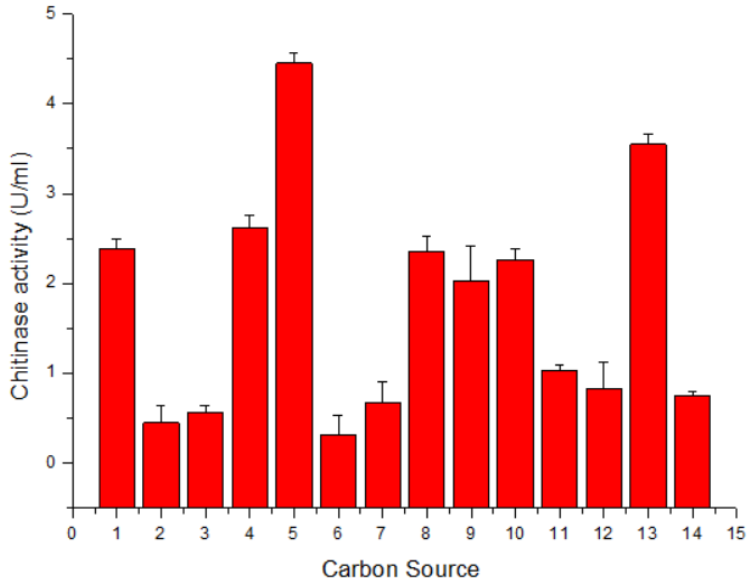
The fungus *C. Echinulata* was grown in a Czapeck liquid medium, supplemented with 1% of 14 different types of carbon sources to induce Chitinase activity (macerated pupa, chitosan, flower of shrimp shell, flower of shrimp shell + glucose, glucose, colloidal chitin, colloidal chitin + glucose, tomato peel, tomato peel + flaxseed flour, flaxseed flour, orange peel, whole pupa, tilapia schama and pieces of shrimp shell). 1,5mL of spore suspension ($2x10^5$ spore/mL) was inoculated in Erlenmeyer flasks (150mL), containing 25mL of Czapeck media. The cultures were incubated at 28ºC for 10 days in stationary conditions. After growth, the cultures were filtered, and the cell-free filtrates were used to determine enzyme activity.

The Chitinolytic activity was determined using a mixture of the substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide 0,25mg/mL (Sigma Aldrich®) mixed into 0,5 M sodium phosphate buffer, pH of 4,5. The reaction was carried out by adding 50µL of the enzyme extract + 50µL of the substrate p-nitrophenyl-N-acetyl-β-D-glucosaminide, incubated at 40ºC for 10 minutes. The reaction was stopped with 200µl of 0,4M Na_2CO_3 and the products were measured in a spectrophotometer at 405nm. The chitinase unit was defined as the amount of enzyme required to produce 1µmol of p-nitrophenol per minute of reaction. The determination of extracellular protein was measured using the Bradford method (1976) [15], using bovine serum albumin (BSA) as standard. The unit was defined as mg of protein per ml.

3 RESULTS & DISCUSSION

The influence of the carbon source in extracellular Chitinase production by the fungus *C. echinulata* PA3S12MM obtained from liquid media cultivation showed that Glucose 1% was the best inducer (4,45 U/ml) followed by tilapia schama 1% (3,54 U/ml). The rest of the tested carbon sources demonstrated an extracellular activity lower than 3 U/ml as shown in figure 1.

Figure 1 Carbon souces used as inducer to Chitinase extracellular production by *C. echinulata* PA3S12MM. The cultures were supplemented with 1% (w/v) of different carbon sources and incubated for 10 days under static conditions at 28ºC. The carbon sources are (1) macerated pupa, (2) chitosan, (3) powder of shrimp shell, (4) powder of shrimp shell + glucose, (5) glucose, (6) colloidal chitin, (7) colloidal chitin + glucose, (8) tomato peel, (9) tomato peel + flaxseed flour, (10) flaxseed flour, (11) orange peel, (12) whole pupa, (13) tilapia schama and (14) flakes of shrimp shell.



In the majority, carbon sources that present chitin are the most inducible to chitinase production [3,16,17] although the results found in this work have shown an alternative way to chitinase production, where Glucose (Figure 1, (5)), was capable of inducing higher values of chitinase activity in *C. echinulata* extracellular extracts. Likewise, a study conducted with *Myceliophthora thermophila* C1, using Glucose as a carbon source resulted in a 3,5 U/ml of chitinolytic activity [18], showing a considerable similarity to what was found in this study.

Regarding the other carbon sources, what we might consider as an interferent in Chitinase production is that since chitinases play different roles in different microorganisms, their induction is not always unified. As presented in a chitin inducer study using *Bacillus cereus* CH, the medium composition shows a significant role in the chitinase B (ChinB) production, showing the necessity of chito oligomers in the medium to induce Chin B. Thus, suggesting that acetylated chitin oligomers, rather than chitin itself, are inducers [19,20].

4 CONCLUSION

The fungus *Cunninghamella echinulata* PA3S12MM, isolated from soil samples, has shown an unexplored potential for Chitinase production using a common carbon source, being one of the very few studies that show fungal Chitinase production using carbon sources with a chitin-free composition. The use of Chitinase can change the way industries acquire substrates for many biotechnological reactions, increasing productivity and efficiency along with lower costs. We point out that this is an initial study with great prospects for future research.

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