



Detection of extracellular hydrolytic enzymes produced by two *Aspergillus* isolates.

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Abstract

Two *Aspergillus* isolates were evaluated for their ability to produce extracellular enzymes using minimum medium that was supplemented with specific carbon source substrate to produce extracellular enzymes (protease, cellulase and sucrase). Both *Aspergillus* isolates exhibited maximum proteolytic activity followed by sucrase activity and negligible cellulolytic activity.

Keywords: Extracellular enzymes, minimum media, protease, sucrase and cellulase.

Introduction

Among microbes inhabiting soil ecosystems fungi are ubiquitous and mainly responsible for breaking down of organic matter and releasing phosphorus, nitrogen and carbon into the atmosphere and soil. They also secrete a wide variety of enzymes, some of which are of great biotechnological interest (Zucconi et al., 2020).

Fungi can produce diverse extracellular enzymes that are used to hydrolyze complex macromolecules into simpler units that can be assimilated and used for growth and reproduction.

Enzymes are protein macromolecules that catalyze biochemical reaction in a living cell at a temperature compatible with normal functioning

of the cell. Enzymes are specific for their substrates, therefore for each type of chemical reactions that occurs in a cell, there is a certain enzyme catalyzing that reaction (Agrios, 2005., Tortora, 2012). For example, proteases (De Souza., 2015), sucrases (Reese et al.,1962) and cellulases are used for hydrolysis of proteins, sucrose and cellulose respectively.

Many fungi produce extracellular enzymes for degradation and transport of nutrients into the cell (Bateman and Basham., 1976). Investment in extracellular enzyme production may be an important element for the survival strategy of these fungi in their habitat. Extracellular enzymes are important tools in manufacturing many industrial products. They are used in food, chemical, textile and pharmaceutical industries

(Alkorta et al., 1998). Fungi are good producers of cellulolytic extracellular enzymes and are widely used in pharmaceutical, agricultural, food, paper, detergent and petroleum industrial processes (Fernandes et al., 2012).

Materials and Methods

Aspergillus isolates were isolated from garden soil samples. Two strains were used to evaluate their potential for the production of extracellular enzymes in solid media.

Culture conditions

Minimal media was prepared with sodium nitrate 0.2%, dipotassium hydrogen phosphate 0.1%, magnesium sulphate 0.05%, potassium chloride 0.05%, ferrous sulphate 0.001% and agar 1.5%. For completion of media, 1% of specific carbon source substrate viz. casein, sucrose and cellulose (for testing activity of protease, sucrase and cellulase respectively) was added to the medium and sterilized.

Plates containing specific media were inoculated with culture in center of plates. Plates were incubated at 25 C for at least 7 days.

The enzyme activity was determined by growth parameter and clear zone.

Results and Discussion

Protease, sucrase and cellulase enzyme activities were detected in media containing protein, sucrose and cellulose respectively. Ability of isolates to grow on solid media supplemented with suitable substrates provides a basis for detection and categorization of enzymatic activities (Lechuga et al.,2016).

Proteolytic Activity

Aspergillus isolate A showed higher proteolytic activity as compared to isolate B.



Aspergillus Isolate A

Aspergillus Isolate B

Growth of *Aspergillus* isolate A (colony diameter) is greater in a medium containing only casein as organic carbon source therefore it can be said that *Aspergillus* isolate A shows higher proteolytic activity.

Sucrased Activity



Aspergillus Isolate A

Aspergillus Isolate B

Sucrased activity was greater in isolate B as compared to isolate A because colony diameter of isolate B is greater in comparison to A.

Cellulase Activity



Aspergillus Isolate A

Aspergillus Isolate B

Both the isolates showed lesser cellulolytic activity but on comparing the two isolates, isolate B showed greater cellulase activity.

Since only one organic carbon source was present in the medium, therefore the growth of fungi will depend on the rate of hydrolysis of a particular substrate, its assimilation and utilization for energy purposes. Rate of hydrolysis of substrate is dependent on production of extracellular enzyme, so colony diameter can be taken as a parameter for assessing production of extracellular enzyme.

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