Screening of Moulds from Soil for Pectinase Production

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Abstract: Enzymes are increasingly being used in different industrial processes globally as a result of their extreme efficiency and highly specific bio-catalytic activities. Pectinases are among the most important industrial enzymes and their demand is increasing by the day hence the need to search for cheap and readily available sources of the enzyme. Therefore, this study was undertaken with the aim of isolating and screening mould species from soil for pectinase production. Isolation of pectinolytic moulds was carried out using the spread plate method. Screening of the isolates for their pectinolytic activity was done by culturing on Pectinase Screening Agar Medium (PSAM) and flooding with iodine-potassium iodide solution. Six (6) fungal species; *Aspergillus niger, Monilia sitophila, Sclerotium rolfsii, Penicillium* spp*, Cladiosporium* spp and *Curvularia* spp were isolated. *Sclerotium rolfsii*, had the highest pectin hydrolysis zone (35 mm) upon screening. It was concluded that *Sclerotium rolfsii* isolated from botanical garden in Ahmadu Bello University Zaria, Nigeria has potential for pectinase production

Keywords: Pectinolytic Moulds, Screening, Pectinase, Pectinolytic activity

INTRODUCTION

Pectinases are major enzymes responsible for the degradation of the long and complex molecules called pectin that occur as structural polysaccharides in the middle lamella and the primary cell walls of young plant cells, its inducer. Pectin contains high molecular weight, negatively charged, acidic in nature and complex glycosidic macromolecule (Oliyad, 2017). In the world market, pectinase accounts for about 10% of total enzyme production (Sangilimuthu *et al*., 2018). Microbial sources have occupied an important place in the production of pectinases, among microorganisms; fungi (especially moulds) as enzyme producers have many advantages since they are normally GRAS (generally regarded as safe) strains and the produced enzymes are extracellular which makes recovery of products from fermentation medium quite easy (Torimiro *et al*., 2018). The ability of filamentous fungi to secrete pectinase and other hydrolyzing enzymes into their culture media has led to the harvesting of these useful enzymes (Adeleke *et al*., 2012). Moulds are well-known for having the ability to produce many varieties of extracellular enzymes; these extracellular enzymes primarily serve to procure nutrients for the survival and growth of fungi (Steudler *et al.*, 2019). Pectinase (especially from fungal sources) is notably one of the most important industrial extracellular enzymes, it has a wide range of applications and accounts for about 25% of the world's food enzyme production and decreasing its production cost has become one of the most important targets (Bajpai, 2018). Over the years, pectinases have been used in several conventional industrial processes, such as textile, plant fiber processing, tea, coffee, oil extraction, treatment of industrial wastewater containing pectinacious material. They have also been reported to work on purification of viruses and in making of paper (Reddy and Sreeramulu, 2012). Hence, the development of high yielding strains and use of cheap raw materials as carbon and nitrogen sources will reduce the cost of enzyme production and make the enzyme available for applications in various industrial processes. Due to the need of pectinases in numerous conventional industrial processes, the present work demonstrates the isolation and screening of the best pectinase producers among soil filamentous moulds.

MATERIALS AND METHODS

Collection of samples

Ten (10) grams of soil samples was collected each from Ten (10) different spots in the botanical garden of Department of Biological Sciences, Ahmadu Bello University, Zaria.

The samples were collected from three (3) different spots at a distance of 200 m interval at a depth of 5-10 cm. Each sample was packaged in a sterile bottle. The samples were brought to the Industrial/Food Research Laboratory, Department of Microbiology, Ahmadu Bello University, Zaria for further analyses.

Preparation of soil sample and serial dilution

The soil samples from the collection site were thoroughly mixed together and then twenty (20) grams was taken out of it and put in 180 mL of sterile distilled water to make the stock solution from which serial dilutions of up to 1×10^{-5} were prepared.

Isolation and characterization of pectinolytic moulds

Isolation of pectinolytic moulds from the soil

For the isolation, 0.1mL aliquots of samples from appropriate dilutions of soil sample was aseptically inoculated using the spread plate method onto sterilized and solidified Potato Dextrose Agar (PDA) enriched with 1% Citrus pectin and incubated at ambient temperature for seven (7) days after which the plates were observed for fungal growth. All morphologically contrasting colonies were purified by repeated streaking and sub-culturing on separate PDA plates; this process was continued till pure mould cultures were obtained (Geetha *et al*., 2012).

Identification of pectinolytic mould isolates

Pure mould cultures were identified by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures; to achieve this; a sterile straight wire was used to tease out a small portion of the pure culture from the PDA plates onto a sterile glass slide, this was then stained using Lactophenol Cotton Blue dye, the slide was covered with a sterile cover slip and viewed under the 40x magnification of the light microscope. Identification was carried out by relating the microscopic features and the micrographs to "Atlas of Mycology" by Barnett and Hunter (1972).

Storage of Moulds on Potato Dextrose Agar (PDA)

Pure mould isolates were maintained on PDA slants as stock cultures. The PDA medium was prepared according to the manufacturer's instructions.

Screening of the mould species for pectinase production

The mould isolates were screened by sub-culturing them unto pectinase screening agar medium (PSAM) with the following composition: 0.3gm/100mL (NH_4) H_2PO_4, 0.3gm/100mL KH_2PO_4, 0.3gm/100mL K_2HPO_4, 0.01gm/100mL $MgSO_4$, 2.5gm/100mL Agar and 1gm/100mL Pectin; pH 6 and then incubated at 30^{o}C for 72 hours. After 72 hours, the plates were flooded with Potassium Iodide dye solution; (1.0 g Iodine, 5.0 g potassium-Iodide in 330 mL distilled water), after decanting the excess dye solution they were left to stand for one hour and then the plates were observed for zone of hydrolysis (Ketipally and Ram, 2018). Pectinolytic moulds produced clear zones after staining. The diameter of the clear zone observed during that span of time was measured in millimetres using a transparent meter ruler in order to select the isolate with highest pectinase activity; the highest value was determined as the highest pectinase activity.

RESULTS

Isolation and identification of Pectinolytic Moulds

A total of six (6) isolates were identified from soil samples obtained from the botanical garden of Ahmadu Bello University, Zaria. Based on cultural morphology on the selective growth media and microscopic characterization the isolates were all found to be filamentous fungi. Genus identification was done by examining both macroscopic and microscopic features of a seven day old pure culture. Colour, texture, nature of mycelia and/or spores produced, growth pattern in addition to microscopic features such as separation and spore shapes were examined.

Based on these characteristics *Aspergillus niger, Monilia, Sclerotium rolfsii, Penicillium, Cladiosporium* and *Curvularia* species were identified. Details of the cultural and microscopic characteristics are given in Table 1, Plates I and II respectively.

Pectinase production by Pectinolytic Moulds

The six isolates were screened and found to be capable of producing pectinase which was identified by zone of clearance signifying hydrolysis of pectin on agar plate. The isolate coded 3 (*Sclerotium rolfsii*) had the highest (35mm) zone of pectinase activity (Plate III) while isolate coded 1 (*Monilia sitophila*) had the least (11mm) zone of pectinase activity. Details of the pectinolytic activity of the six isolates are presented in Table 2.

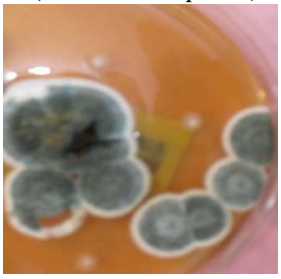
Table 1 Cultural characteristic of fungal isolates

Isolate Code	Colour	Surface features	Edge	Reverse colour	Identity
1	Whitish grey	Thin fluff	White, circular	Grey	*Monilia sitophila*
2	Black	Granular	Black, irregular	Cream	*Aspergillus niger*
3	White	Granular	White, irregular	Peach	*Sclerotium rolfsii*
4	Bluish green	Powdery	White irregular	Brownish red	*Penicillium* spp
5	Green	Cottony	Irregular	Black	*Cladosporium* spp
6	Grey	Woolly	Circular	Black	*Curvularia* spp

(*Monilia sitophila*)

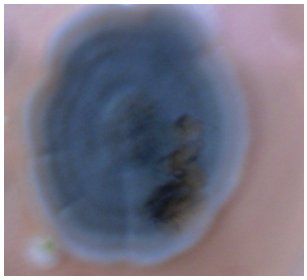
(*Aspergillus niger*)

(*Sclerotium rolfsii*)



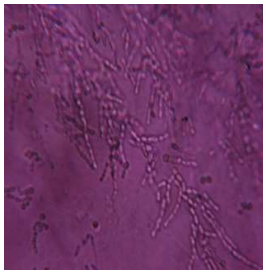
(*Penicillium* spp)

(*Cladosporium* spp)

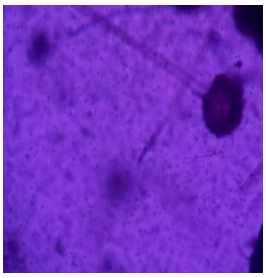


(*Curvularia* spp)

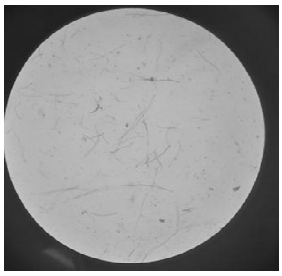
Plate I: Cultural morphology of the isolated moulds



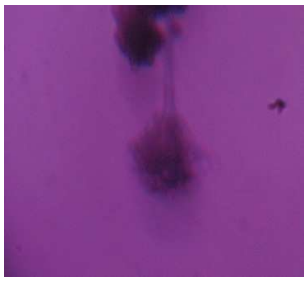
(*Monilia sitophila*)



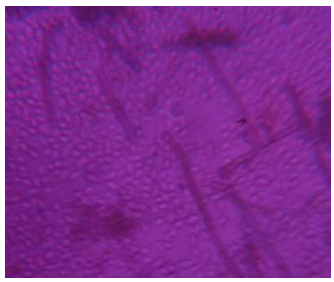
(*Aspergillus niger*)



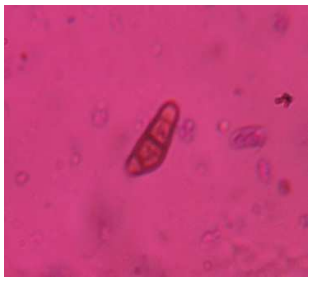
(*Sclerotium rolfsii*)



(*Penicillium* spp)



(*Cladosporium* spp)



(*Curvularia* spp)

Plate II: Microscopic characteristics of the isolated moulds (x40 magnifications)

Table 2. Pectinase production by Mould Isolates

Mould species	Diameter of zone of Pectin hydrolysis (mm)
Monilia sitophila	**11**
Aspergillus niger	**31**
Sclerotium rolfsii	**35**
Penicillium spp	**16**
Cladosporium spp	**15**
Curvularia spp	**12**

Plate III. Pectinolytic activity (Clear Zone of *Sclerotium rolfsii*)

DISCUSSION

A total of six (6) mould isolates were selectively isolated from the soil sample by enrichment method using a medium containing pectin as the sole carbon source. A total of six (6) isolates (*Aspergillus niger, Monilia* spp*, Sclerotium rolfsii, Penicillium* spp*, Cladiosporium* spp and *Curvularia* spp) were identified, their presence in the soil sample could be due to presence of abundant pertinacious plant materials such as fallen leaves in the soil. Similar techniques for isolation of pectinase producing microorganisms using pectin as sole carbon source were previously used by Ketipally and Ram (2018).

The isolates were subjected to plate agar screening the degradation was observed around fungal growth at varying diameters. Other Researchers such as Munir *et al*. (2019) and Ketipally and Ram (2018) also used the same plate agar screening method with evidence of hydrolysis around the colony indicating zone of clearance. Although, Souza *et al*. (2003) applied the cup-plate assay of isolation and screening of pectinolytic fungi and reported that all isolates were found to be pectinase enzyme producing.

Out of six (6) mould isolates identified and screened for pectinase production ability, *Sclerotium rolfsii* had the highest zone of pectin hydrolysis (35mm) whereas, the lowest zone of pectin hydrolysis (11mm) was observed with *Monilia sitophila*. This variation might be due to differences in the metabolic capabilities of the different isolates. This result is similar to the work of Sudeep *et al* (2020) who reported that *Aspergillus* spp Gm produced the maximum clearance zone (35 mm) around the colony. The occurrence of pectinolytic organisms in soil agrees with the earlier reports of Usha *et al* (2014) and Sangilimuthu *et al* (2018), where soil was described as a repository of pectinase producing organisms.

CONCLUSION

Pectinolytic moulds were isolated from garden soil. All the isolates obtained from this soil source have pectinase production capacity. From this present study, the isolated *Sclerotium rolfsii* showed the highest zone of hydrolysis of pectin (35 mm) and thus can be used for pectinase production.

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