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Isolation and screening of proteolytic fungal cultures from soil contaminated with abattoir waste

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ABSTRACT

In this study, proteolytic fungal cultures were isolated from soil contaminated with abattoir waste for protease production. The protease was produced under submerged fermentation using with various fermentation media. Among the fungal cultures isolated, in this study, fungal isolate (IS5) exhibited highest proteolytic activity and maximum enzyme with 0.277 U ml^{-1}. Minimum protease activity of 0.056U ml^{-1} was noticed with fungal isolate IS2 remaining are intermediate. The proteolytic property of fungal culture was screened through plate screening method. The potent proteolytic culture was identified as *Aspergillus spp.*

KEYWORDS

Abattoir waste;
Fungal cultures;
Plate screening;
Protease production.

INTRODUCTION

Proteases are an Industrial important enzymes which hydrolyze of peptide bonds of other proteins and have enormous applications in food, leather, pharmaceutical, detergent industries[1,2]. Proteases are classified as acid, neutral and alkaline proteases based upon pH at which they exhibit the maximum activity[3]. Microorganisms such as bacteria, fungi, and actinomycetes are protease producers. Among these, fungi produce higher protease compared to bacteria[4]. Fungal strains, *Aspergillus*, *Penicillium*, *Rhizopus*, and *Mucor* spp produce acid proteases and these are considered as generally regarded as safe[5]. Abattoir (Slaughter) waste is a protein rich waste which contains organic solids, inorganic matter[6,7]. In view of industrial applications of protease, the present investigation was carried out to isolate potent proteolytic fungal cultures from soil polluted with abattoir waste and to screen for proteolytic activity.

MATERIALS AND METHODS

Collection of soil

Soil polluted with abattoir waste (slaughter waste) was collected from slaughterhouse located in Tirupati, Chittoor district, Andhra Pradesh, India. Soil sample was collected from 0-3 inches with three increments

(0-1, 1-2, 2-3). The soil sample was placed in sterile polythene bags and was transported to the laboratory and then air dried at a temperature of 30 to 35°C. Further the soil samples was sieved through <2 mm sieves. The fractions <2 mm were stored in a refrigerator at 4°C for further studies.

Isolation of fungi

The fungal cultures were enumerated from soil contaminated with abattoir waste by serial dilution and spread plate techniques. The medium used for growth of the molds was PDA, contained (g/l): Potatoes infusion from 200.00g, dextrose 20.00g and agar 15.00 g and 30mg of streptomycin was added to avoid bacterial contamination. The medium was sterilized by autoclaving at 15 psi for 15 min at 121^{0}C. Then, the sterilized medium was cooled to 40 to 50^{0}C. Approximately 20 ml of warm medium was poured into the sterile plates and allowed to solidify at room temperature. The soil samples were suspended in water by vigorous vortexing and were serially diluted. Appropriate dilutions (10^{-3} to 10^{-5}) were plated into casein agar plates. Then, the plates were incubated at temperature of 30±2^{0}C for seven days. Different colonies developed on the solid medium were then repeatedly streaked on casein agar plates till the well separated and isolated colonies were obtained. These cultures were periodically sub cultured and maintained on potato dextrose agar slants at 4^{0}C.

Screening of fungi for protease production

In order to select the suitable strains for protease production, fungal isolates from soil were screened for proteolytic activity. Separate screening procedures carried out for the selection of strains for the enzyme production by the plate method and submerged fermentation respectively.

Submerged fermentation

For submerged fermentation, the basal media such as Luria broth, Sabouraud broth, and referred medium[8] were used. The referred medium contains following ingredients are: Soybean meal- 2.00g, Glucose-2.00g, Polypeptone- 1.00g, Yeast extract-0.20g, KH_2PO_4-0.20g, NaCl-0.20 g, Distilled water-100ml, pH-7.0. Five fungal isolates namely Isolate 1 – 5 were tested for protease production in five different media viz., Luria broth, Sabouraud broth, molasses (Dilution factor: 5), dairy industry effluents, and referred medium[8]. The fungal inoculum was prepared by addition of 10ml of 0.1% Triton X-100 solution to the 7th day old slant and was shaken well to obtain homogeneous spore suspension. Each flask was inoculated with 2ml of spore suspensions of fungal strains. Flasks were agitated with 150 rpm in incubator shaker at 30±2^{0}C for seven days. At the end of fermentation, the contents of flasks were filtered and then analyzed for extracellular acid protease activity[9].

Plate screening method

For this method, Casein - agar medium was prepared by dissolving one gram of casein and two grams of agar in 100milliliter of distilled water and sterilized in autoclave at 15psi for 15 min. Then the sterilized mixture, approximately 20 ml, was poured into the sterile petriplates and allowed to solidify at room temperature. Agar well of 0.5mm diameter was made by borer in the center of casein - agar plate and then it was filled with 100µl of spore suspension of isolate IS5. The plates were incubated at temperature of 30±2^{0}C for seven days. After incubation, the formation of zone of casein clearance was measured at the end of 72 hours.

RESULTS AND DISCUSSION

Microbial populations such as fungal flora in soil contaminated with abattoir soil was enumerated and counted. The fungal populations in contaminated soil were 19 ± 1.414 x 10^4 CFU/g soil. Higher fungal population in test soil could be due to the depositions of abattoir organic waste, higher potassium and phosphorous contents of in the soil. Similar observations were reported for the soil disposed with abattoir effluent[10]. Moreover, soil contaminated with other industrial effluents such as cotton ginning mill and sugar industry effluents improved the soil microbial populations[11,12].

Morphological study of fungal isolates

Macroscopic and microscopic observations of isolated fungal strains from test soil were studied which were compared with standard book[13]. TABLE 1 listed the details of characteristics of isolated fungal cultures.

Regular Paper

TABLE 1 Macroscopic characteristic of fungal isolates

Fungal Isolate	Colony colour	Colony Diameter (cm)	Reverse colour
IS1	Pure white	9.00	Yellow
IS2	Light grey	1.20	Orange
IS3	Green	1.50	White
IS4	Ash	0.80	White with yellow
IS5	Black	2.42	White

Screening of proteolytic fungi under submerged fermentation

It is common practice of converting industry effluents or waste to useful bio-products through fermentation. Hence sugar industry by-product (diluted cane molasses) and dairy industry effluents were used as fermentation media for enzyme production from above said five fungal cultures. In addition, commercially available media such as Sabouraud broth, Luria broth and the referenced medium[8] were used in this study. Figure 1 showed that all the isolated fungal cultures were capable of producing extracellular protease with above mentioned fermentation media with incubation time of 7 days at 30±2^{0}C. Maximum protease activity (0.277U ml^{-1}) was observed with IS5 with the diluted molasses as basal medium. However, the minimum activity of 0.056 U ml^{-1} was noticed with IS2 from the referenced medium[8] and with the fungal strain IS3 of 0.058 U ml^{-1} from Luria broth. Soil fungal isolate, IS5, was capable to utilize the sugars of cane molasses and secreted significant amount of protease enzyme than the remaining isolated strains under controlled fermentation conditions. The use of complex sugar substrates such as cane molasses, beet molasses is the common practice for industrial fermentations[14]. Similarly, another metabolite citric acid could be produced from untreated cane molasses from *Aspergillus niger* though the growth and product yield were less as compared with pretreated molasses[15]. In addition to these reports[16], also investigated the production of protease from *Bacillus subtilis* by using molasses-based complex medium. Recently[17], has reported that by-product in sugar industries, molasses, is an interesting raw material, it is rich in nutrients and minerals, cheap in price as well as it is present in plenty and can be used as a relatively inexpensive and economic alternative to synthetic medium for the production of protease. Hence the present study used the untreated, five times diluted cane molasses as the fermentation medium. Because of the low nutritive value of dairy industry effluent when compared to cane molasses, the low enzyme activities were observed with all the isolated strains.

Even though the nitrogen content of Luria broth (0.8 %) was higher than that of cane molasses (0.15 – 0.25 %) but the higher amount of sugars of molasses played a significant role in the protease enzyme production. Protease activity (0.13 U ml^{-1}) on Sabourad broth was as same as that on Luria broth[8]. investigated the protease production by *Pencillium chrysogenum* through SmF and their optimized medium was utilized in the present study as the referenced medium to investigate the potentiality of molasses for the growth and enzyme production.

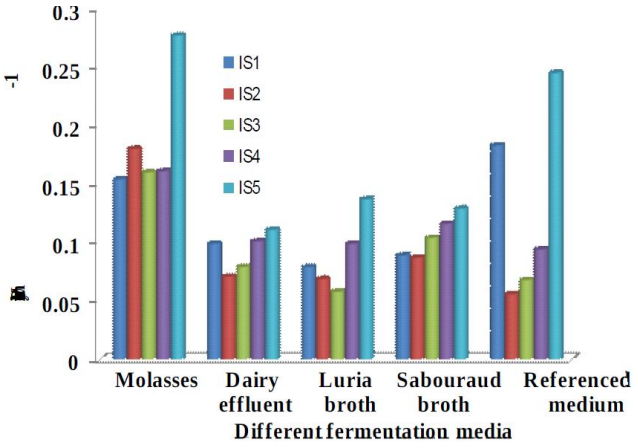

Figure 1: Production of protease from isolated fungal cultures on various media

From the above results, it was noticed that molasses is equally as effective as referenced medium[8]. Further, it was confirmed through plate screening method.

Screening of fungal isolate IS5 plate screening method

The effective fungal strain, IS5, was effective with zone of casein clearance of 49mm at the end of 72 hours of incubation at 30±2^{0}C among five isolated cultures (IS1 – IS5) from soil contaminated with abattoir waste (Figure 2). This indicated that IS5 was able to secrete significant amounts of protease enzyme among the isolated fungal cultures.

Identification of potent proteolytic fungi (IS5)

From the results of previous section, it could be

noticed that isolate IS5 was the potential proteolytic fungal strain. Hence the microscopic characteristics of proteolytic strain IS5 such as size of conidiophores, fruiting bodies and conidia were measured with a help of micrometer and shapes of spores were recorded. These characteristics of fungal strain IS5 were matched with those listed in standard reference book entitled "Compendium of soil Fungi"[13] and were identified as *Aspergillus* spp.

TABLE 2 : Macroscopic and microscopic characteristics of strain (IS5)

Macroscopic characteristics		Microscopic characteristics			
Colony Colour	Colony Diameter (cm)	Conidiophores (cm)	Conidia Diameter (µm)	Spore Size (µm)	Spore shape
Black	2.42	1.714	4.8	0.051 µm	Globulose

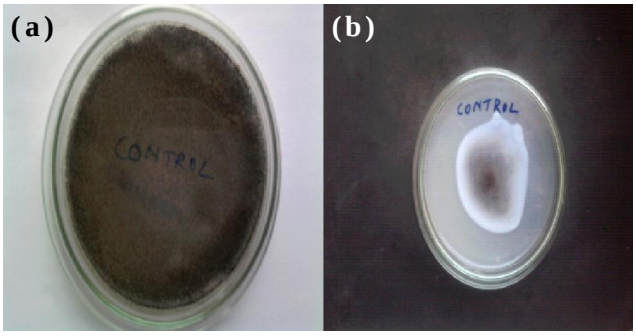

Figure 2: (a) *Aspergillus* spp (b) Casein clearance by *Aspergillus* spp

CONCLUSION

Proteolytic fungal cultures were isolated from soil contaminated with abattoir waste for protease production in the present investigation. All the isolated fungal strains were able to produce protease production in various fermentation media through submerged fermentation. The diluted molasses was proved to be a suitable fermentation medium for acid protease production by fungal isolate (IS5) which exhibited highest proteolytic activity and maximum enzyme production was observed in diluted molasses with an enzyme activity of 0.277 U ml^{-1}. The potent fungal strain IS5 was identified as *Aspergillus* spp.

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