OPTIMIZATION OF CULTURE CONDITIONS FOR PENECILLIUM ISOLATED FROM SOIL TO ENHANCE AMYLASE PRODUCTION POTENTIAL

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ABSTRACT: The objective of present study was to evaluate amylase production potential of indigenous *Penicillium sp*. Amylase producing specie was isolated from soil of livestock farms using starch agar and confirmed as *Penicillium* by macroscopic and microscopic features. Non-toxigenecity was evaluated by thin layer chromatography. Optimization experiments were carried out by submerged fermentation using standardized inoculum (10^6 spores/mL) at pH 4.5, 6.0 and 7.5, temperature 22, 28 and 37°C and substrates wheat bran, maize and rice husk with varying concentrations of 1, 3 and 5 percent for seven days of incubation period. The amylase production was measured in terms of enzyme activity (IU) by dinitro salicylic acid (DNS) method. The optimum temperature and pH were 37°C and 6, respectively with mean enzyme activity of 17.52±.56 IU. Wheat bran proved to be the best substrate for amylase production by *Penicillium sp.* with 76.7149±3.53 IU at five percent concentration. A direct relation was found between substrate concentration and amylase enzyme activity (IU). It was concluded that *Penicillium* isolate was a potential candidate for amylase production on industrial level.

Keywords: *Penicillium sp.*, amylase, wheat bran, maize and rice husk.

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INTRODUCTION

Penicillium is a filamentous fungus, comprises of almost 225 species (Pitt *et al.,* 2000). It is ubiquitous in nature and can be isolated from soil, air, feed stuff, vegetation and marine water (Park *et al.,* 2016; Kim *et al.,* 2014; Leitão, 2009; Suhail *et al.,* 2006). It is well known for plant diseases, decomposition of organic matter and production of a large number of biologically active molecules such as mycotoxins, antimicrobial agents, organic acids, natural pigments and hydrolytic enzymes (Ireneusz *et al*., 2017; An *et al.,* 2016; Park *et al.,* 2016; Dar *et al.,* 2015; Koolen *et al.,* 2012; Méndez *et al.,* 2011; Max *et al.,* 2010).

Starch degrading hydrolytic enzymes have potential application in starch-based industries including food, pharmaceutical, textile, paper, detergent, beverages and leather industry such as α amylase, β amylase, glucoamylase, etc. (Pandey *et al.,* 2000). These enzymes can be obtained from animal, plants and microorganisms. Microbial sources of amylases are preferred because of several reasons like easy cultivation, genetic manipulation, better strain selection and optimization of culture conditions for enhanced production of amylases (Konsoula and Liakopoulou-Kyriakides, 2007). Among microorganisms, filamentous fungi are selected as better source of industrial production of amylases because of it's generally regarded as safe (GRAS status). It is suitable for solid state fermentation due to presence of hyphae in its structure, ability to tolerate high osmotic pressure and low water activity (Singh *et al.,* 2014).

Agriculture waste is a cost-effective substrate which replaces the synthetic medium for industrial production of amylases. The amylase production depends upon various cultivation parameters including microbial strain, size of initial inoculum, incubation temperature, initial pH of medium, type of substrate, concentration of substrate and size of substrate particles (Poglayen *et al*., 2016; Saxena and Singh, 2011).

In present study agricultural waste; wheat bran, rice husk and maize flour were evaluated as substrate with varying temperature and pH using indigenous amylase producing *Penicillium* specie to determine optimum culture conditions for enhanced level of amylase production.

MATERIALS AND METHODS

Penicillium species (spp.) used in optimization experiment was isolated from soil of livestock farms. Effects of substrate, concentration of substrate, temperature and pH were explored on amylase production of *Penicillium sp*.

Isolation and identification: *Penicillium spp.* was isolated from soil of livestock farms. One gram of soil was dissolved in nine mL sterile normal saline (0.85% NaCl solution). The suspension was allowed to stand at room temperature for five minutes. One mL from this suspension was inoculated on sterile Sabouraud's dextrose agar (SDA) plates. The plates were incubated at 25 ºC for three days. The discrete colonies were purified by sub-culturing on SDA by spotting and incubating under similar conditions (Shi-Wei *et al*., 2017; Gugnani *et al.,* 2004). The purified isolates were observed for macroscopic features. Microscopic features were discerned by slide culturing (Hussain *et al*., 2016; Pitt and Hocking, 1997; Samson *et al*., 1996).

Screening for starch hydrolysis: Amylase production potential was screened by inoculating *Penicillium spp.* on starch agar. Followed by incubation at 25 ºC for three days, starch agar plates were flooded by iodine solution (1 percent iodine and 2 percent potassium iodide). Colony showing zone of hydrolysis around it was selected as candidate for amylase production (Singh *et al.,* 2014).

Screening of non-toxigenic specie: Forty-five days old cultures of *Penicillium spp.* were autoclaved (121 ºC, 15lbs and 15minutes), homogenized and processed for mycotoxin extraction. Organic solvents; chloroform (45mL) and Methanol (5mL) along with distilled water (5mL) and NaCl (2.5g) were mixed with 12.5g homogenized broth culture. The mixture was incubated at 37 ºC for 30 minutes followed by sieving using muslin cloth and filtered by Whatman filter paper. The filtrate was concentrated by evaporation and reconstituted in one mL chloroform. This was spotted (2µL) on silica coated aluminum sheet followed by immersion in mobile phase (95 mL chloroform and 5mL acetone). The plates were observed under 365nm wavelength in wooden lamp (Frisvad *et al.,* 1989).

Optimization of culture conditions for amylase production: Non-toxigenic *Penicillium sp.* was selected for optimization experiments by varying one factor at one time. Experiments were carried out in Erlenmeyer flasks(250mL) containing 50mL of inorganic broth (1g KH_2PO_4, 1g $NaNO_3$, 0.5g $MgSO_4.7H_2O$ and 0.01g $FeSO_4$). Three substrates including wheat bran, rice husk and maize flour were immersed in inorganic broth in concentration of one percent. Initial pH was adjusted 4.5, 6 and 7.5. One mL of standard inoculum of (10^6 spores) was inoculated and flasks were incubated at 37 ºC for seven days. Similarly, second experiment was conducted for temperature (22, 28 and 37 ºC) and substrate concentrations (1, 3 and 5 %). Followed by incubation, the broth culture was filtered using Whatman filter paper and filtrates were used as crude enzyme (Haq *et al.,* 2002).

Amylase activity determination: Amylase was quantified as international unit (IU). It was estimated by DNS (3, 5 dinitro salicylic acid) method as followed by Saxena and Singh (2011). One mL of crude enzyme was mixed with phosphate buffer having pH 6 (2 mL) and one percent starch solution (1mL). It was incubated at 37 ºC for 30 minutes. The DNS reagent was added to it and placed at boiling temperature till color of reagent changed. Three mL DNS reagent and seven mL distilled water were mixed, used as blank and optical density was recorded at 540 nm wavelength. Release of one µmole of glucose per minute (U/mL/min) under assay condition was defined as IU. A standard curve of glucose was used to determine concentration of released reducing sugar.

RESULTS

Non-toxigenic amylase producing *Penicillium:* Among (n=20) starch hydrolyzing filamentous fungi isolated from soil of livestock farms, one isolate was identified as *Penicillium sp.* The macroscopic and microscopic features of the isolate are presented in Fig-1. It was found non-toxigenic as it did not show any fluorescence on silica coated aluminum sheet when observed in wooden lamp.

Optimization of cultural conditions for amylase production: Among temperatures, the highest mean amylase activity (17.52±56IU) was observed at 37 ºC, followed by 13.63±59 IU at 28 ºC and 7.71±40IU at 22 ºC on one percent maize flour at pH 6. Amylase activities were recorded 10.01±0.46, 4.46±0.08 and 3.30±0.08IU at 37, 22 and 28 ºC, respectively for one percent wheat bran at pH 6. In case of one percent rice husk, less than 6 pH, the amylase units were recorded as 9.15±0.08, 5.01±0.03 and 0.1±0.00 IU at 22, 28 and 37ºC, respectively (Fig-2).

In optimization of pH, at 4.5 the amylase activity was 3.58±0.16, 4.08±0.16 and 3.80±0.13 for maize, wheat bran and rice husk, respectively at one percent concentration and 37 ºC. At same temperature and concentration, the amylase units were observed 17.53±0.56, 10.01±0.46 and 0.10±0.00 at pH 6 and 4.70±0.16, 4.49±0.09 and 8.75±0.19 at pH 7.5 for maize flour, wheat bran and rice husk, respectively (Fig-3).

Linear relation was found between substrate concentration and amylase activity. The amylase activities 17.52±56, 14.97±17 and 35.24±0IU at one, three and five percent of maize flour, 10.01±0.46 33.87±0.49 and 76.71±3.53 IU for wheat bran and 0.1±0.00[a]17.32±0.49 and 26.47±0.70 for rice husk were recorded (Fig-4). Highest amylase units produced by *Penicillium sp*. were 76.71±3.53 IU at five percent wheat bran, 37 ºC temperature and 6 pH.

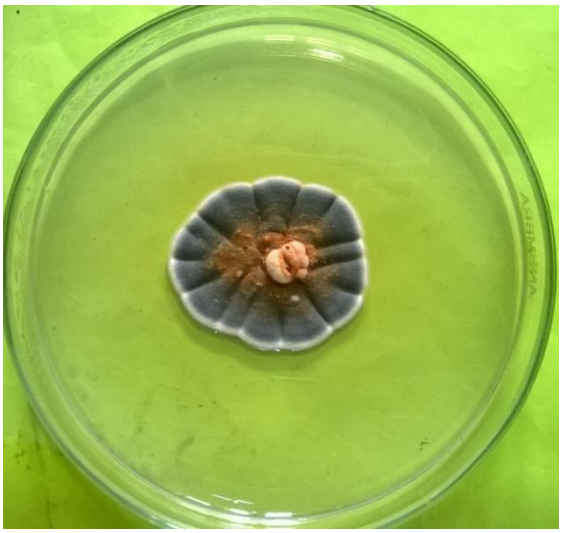

Figure -1a: Macroscopic view of *Penicillium sp*.

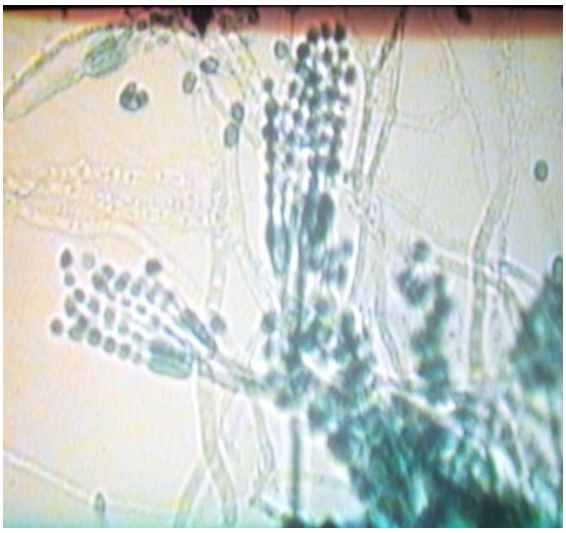

Figure-1b: Microscopic view of *Penicillium sp.*

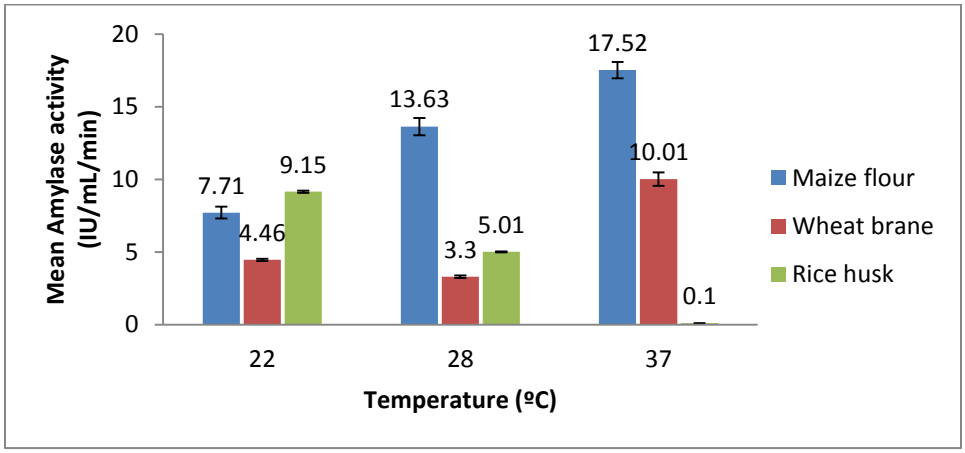

Figure-2: Effect of temperature on amylase production potential of *Penicillium sp*.

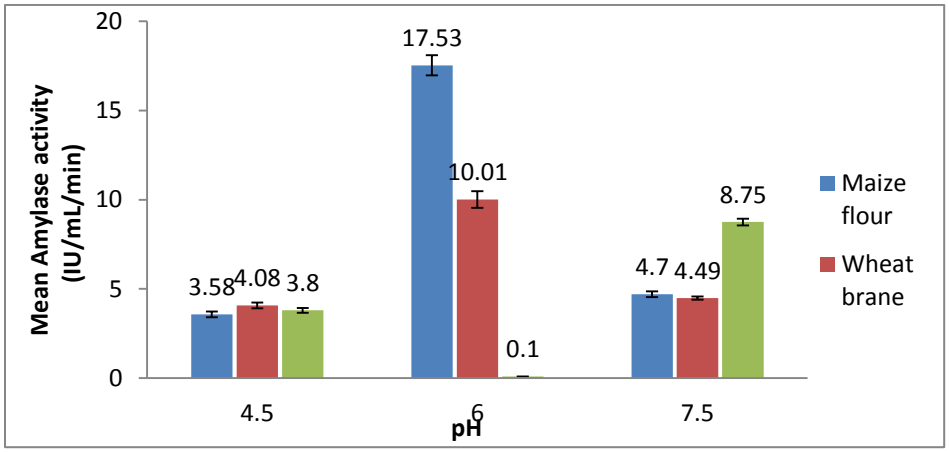

Figure-3: Effect of pH on amylase production potential of *Penicillium sp.*

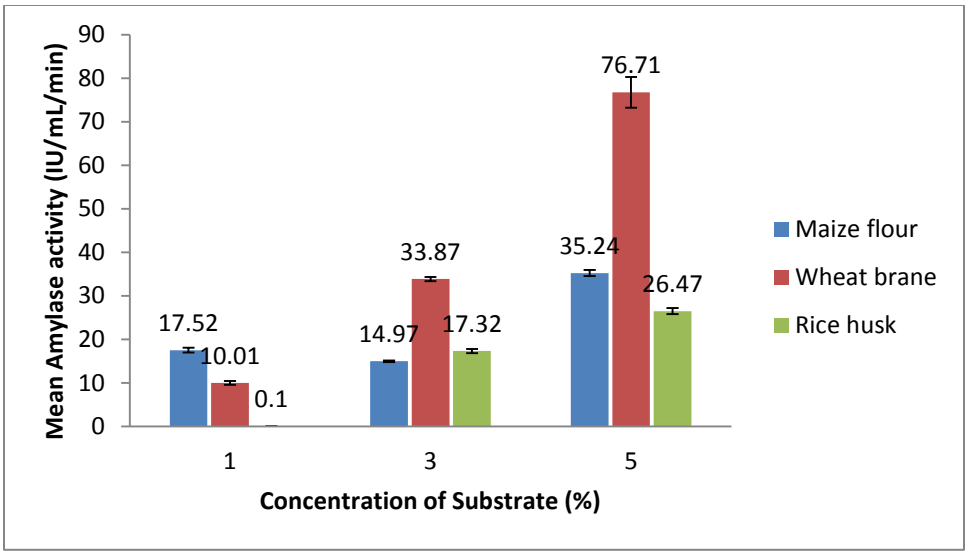

Figure-4: Effect of substrate concentration on amylase production potential of *Penicillium sp.*

DISCUSSION

A significant effect was recorded in amylase production by changing physicochemical cultural conditions. The optimum conditions for enhanced amylase production potential of *Penicillium sp*. were 37 ºC, 6 pH and wheat bran a better substrate at concentration of five percent. The findings were in contrast with Balkan and Ertan (2005). The amylase activity of 155 IU was recorded at 30ºC, pH 4-5 for incubation period of 6 to 8 days. Dar *et al.* (2015) found that *P. chrysogenum* produced 550 IU at 6 pH linseed oil cake as substrate for an incubation of six days. Silva *et al.* 2011 found *P. purpurogenum* to give best amylase production in Khanna medium at initial pH of 6 and temperature 30°C for 144 hrs.

Singh *et al.* (2014) found 341.7 IU at 35°C, initial pH 6 for incubation period of 6 days using *Aspergillus fumigatus* as amylase producing strain. *Trichothecium roseum* produced highest enzyme activity using wheat bran as substrate reported by Balkan *et al.*, 2011 strengthened the result of present study. However, the optimum temperature was observed 30 ºC opposite to present study. Prakasham *et al.* 2007 found optimum pH 4 and optimum temperature 31ºC amylase activity of *A. awamori*. Sunitha *et al.* 2012 carried out study on endophytic fungi isolated from plant and found 30°C temperature and pH 7 as optimum conditions for amylase production. Varalakshmi *et al.* (2009) used *A. niger* to optimize amylase production using agricultural waste and found best yield of amylase at 22°C and 7.5 pH.

Conclusion: It was concluded that indigenous *Penicillium sp*. had high amylase production potential which can be harnessed by optimizing cultural conditions for industrial scale production of amylases.

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