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SHORT COMMUNICATION

PRODUCTION OF ALPHA AMYLASE AND CELLULASE FROM SOLID STATE CULTURE OF *ASPERGILLUS OCHRACEUS*: A FEASIBILITY ANALYSIS

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ABSTRACT

The growing demand and application of industrially important enzyme necessitate the need to explore new sources with diverse enzymes ranging in their specificity and activities. Enzymes are safe alternatives to chemical synthesis due to minimum side effect and ease of manufacturing. Solid state fermentation (SSF) is a cost-effective alternative to submerged fermentation with agro-residues or waste, often being used as substrate for growing diverse organisms for production of metabolites. Current study is one of the scarce report on exploring alpha amylase and cellulase production ability *Aspergillus ochraceus* (MTCC 1877) using wheat bran as substrate at relative humidity of 90% and at 30 ºC, for 7 days. Result showed the potential of *Aspergillus ochraceus* (MTCC 1877), as potential source of the two enzymes. Results revealed comparatively higher alpha amylase activity in the SSF extract of *Aspergillus ochraceus* (MTCC 1877) in comparison to *Trichoderma longibrachiatum* (ITCC 7839). On the contrary, comparatively higher cellulase activity was observed in the SSF extract of *Trichoderma longibrachiatum* (ITCC 7839). The results showed the potential of *Aspergillus ochraceus* (MTCC 1877) as a source of the two enzymes. Variation in enzymes activity may be attributed to the experimental culture conditions and may be further optimized to enhance the enzymes yield.

Keywords: Enzymes, Solid state, *Aspergillus ochraceus*, *Trichoderma longibrachiatum*, cellulase, alpha amylase

INTRODUCTION

Solid state fermentation (SSF), is a well explored technology, used for production of value-added products, by growing bacterial and fungal strains on solid substrate (or support) with insignificant or no free water. The technique had been used for centuries, for developing fermented food **(Hyseni *et al.*, 2018; Pablo *et al.*, 2020)**. The 'GRAS' strains mediated development of fermented food, feed and pharmaceutical products had been well explored using SSF **(Barrios-González *et al.*, 1988; Ramachandran *et al.*, 2004; Sitanggang *et al.*, 2020)**. Enzymes are one of the most well explored and utilized biomolecules, in various industrially important sectors, and are economically produced using SSF **(Pandey *et al.*, 2008; Liu and Kokare, 2017)**. These biocatalysts are primarily proteins in nature and are produces from diverse range of organisms **(Liu and Kokare, 2017).** It is this diversity of biomolecules from different sources and their associated properties that obtrude them as a suitable safe alternative to chemical catalysts **(Vanacek *et al.*, 2018).** It is this diversity in the catalytic properties of enzymes that had been a boon for their application in various commercial sectors ranging from food, feed and pharmaceutics to paper and pulp, jute and textile sectors, environmental remediation, and others **(Koyani and Rajput, 2015; Raveendran *et al.*, 2018).**

Diversity in the habitats of different microorganisms is associated with the diversity in the reaction conditions variability of the enzyme **(Müller *et al.*, 2015; Garcia-Garcera and Rocha, 2020).** It is these diversities and wide acceptability of enzymes in different sectors that provide an impetus to explore new or novel organisms as source of enzymes.

The enzyme production from fungal strains on agro-residues as substrate, through SSF had been reported to be higher in yield and cheaper in cost, thereby making the overall process economical **(Pandey *et al.*, 2008; Agrawal *et al.*, 2013; Pablo López-Gómez *et al.*, 2020).**

Previous studies have shown the vast varieties of enzymes, that can be successfully produced using *Aspergillus sp.* **(Hu *et al.*, 2011; Lopes *et al.*, 2011; Shinkawa and Mitsuzawa, 2020).** The fungi, *Aspergillus ochraceus* is, well known for its mycotoxic effects on plant and chicks owing to the production of secondary metabolite, Ochratoxin A, extensively reported for its hazardous effects **(Doupnik, 1970; Hocking, 2006; Martínez-Rodríguez and Santiago, 2011; de Almeida *et al.*, 2019).** However, recent interest in *A. ochraceus* is primarily due to the therapeutic potential of other metabolites from the fungi. Studies by **Hu *et al.*, (2021)** had shown the anti-Parkinson's effects of alkaloids and other metabolites produced by *A. ochraceus*. In another study by **Aracri *et al.*, (2019),** the potential of *A. ochraceus*, as a potential source of enzyme Tannase had been reported.

Current study is the first feasibility analysis of the fungal strain, *Aspergillus ochraceus* MTCC 1877, as a potential source of industrially important enzymes (*viz.* alpha amylase and cellulase) on agro-residues, using solid state fermentation.

MATERIALS AND METHODS

Fungal strain and culture media

The current studies were performed using *Aspergillus ochraceus* (MTCC 1877), purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The fungi *Trichoderma longibrachiatum* (ITCC 7839), was used to compare the output of SSF among the two fungi. All further studies were performed using the two fungi, under similar experimental conditions. The growth and maintenance of the strain was done using Potato dextrose agar (HiMedia Laboratories Pvt. Ltd., India). The solid-state fermentation studies were performed using wheat bran as substrate. All other chemicals used in the studies were of analytical grade, unless specified.

Growth and maintenance of fungal strain

The fungi were revived from the cryostocks of fungi, preserved at – 20 ºC. Both the fungi, *Aspergillus ochraceus* (MTCC 1877) and *Trichoderma longibrachiatum* (ITCC 7839), were revived from the cryostock and propagated on potato dextrose agar (PDA) plate at 30 °C for 7 days in incubator (Kühner, Switzerland). After revival of the strains, the culture plates were stored at 4 °C.

Solid state fermentation studies

The humidity is an important component of solid-state fermentation and largely depends on nature of substrate. In current study, the change in relative humidity (RH) with increasing volume of water was measured using Winner Thermo – hygrometer TH-402 (Fig. 1). The wheat bran was autoclaved twice at 121 ºC for 30 minutes to reduce the microbial load. The plastic petri plate with 20 g wheat bran and RH of 90% was used for solid state fermentation studies. The plates were inoculated with two cuboidal pieces (1cm × 1 cm; length × width, height depends on thickness of agar plate) each, and the plates were incubated at 30 ºC for 7 days. After the 7 days of culture, the fermented solid materials were harvested in a muslin cloth that was used for enzyme extraction.

Extraction of Enzymes

The feasibility of SSF harvest as a source of enzymes, alpha amylase and cellulase, were analyzed by suspending 40 g harvest and tied in muslin cloth, in

200 ml distilled water at 270 rpm, 4 ° C for 2 hours. The liquid extract was used further for enzyme activity.

Enzyme assay for Alpha Amylase

The activity of α-amylase in the extract was analyzed using the alpha amylase assay method reported by **Luo *et al.*, (2019),** with modification. The assay method involves estimation of reducing sugars, produced on hydrolysis of starch using Dinitrosalicylic acid (DNS) reagent. The reaction mix for prepared by mixing 1 ml of 1`% (w/V) starch with 1 ml of extract. The reaction mixture was incubated for 10 min. Further 1 ml DNS reagent and 5 ml distilled water was added, and the solution was boiled. The solution was cooled and the absorbance at 540 nm was monitored. The enzyme activity was defined to be 1 U/ml when 1 mg of reducing sugar was liberated per unit time, per unit volume of enzyme under optimum conditions of temperature and pH. The quantity of reducing sugar was estimated using the standard plot, prepared using glucose as standard.

Enzyme Activity of Cellulase

The activity of Cellulase in the extract was estimated using the assay method suggested by **Lone *et al.*,(2012),** with some modifications. Briefly, 1ml of cellulose (1% microcrystalline in water) was mixed with 1 ml extracted and incubated at room temperature for 10 minutes. The mixture was further supplemented with 1 ml DNS Reagent and 5 ml water. The tubes were placed in boiling water bath at around 80 ºC – 100 ºC. The solution was cooled and the absorbance at 540 nm was monitored. The enzyme activity was defined to be 1 U/ml when 1 mg of reducing sugar was liberated per unit time, per unit volume of enzyme under optimum conditions of temperature and pH. The quantity of reducing sugar was estimated using the standard plot, prepared using glucose as standard.

RESULTS AND DISCUSSION

Cellulase and alpha amylase are widely used in food, feed and pharmaceutical sectors. The vast application of these industrial enzymes, obtrude them as highly demanded enzymes. Current study explored the potential of *Aspergillus ochraceus* MTCC 1877, as a suitable alternative source of the enzymes. The choice of using *Trichoderma longibrachiatum* ITCC 7839, in the study, for comparisons of results, was based on previous reports highlighting *Trichoderma longibrachiatum* to be the source of cellulase **(Tegl *et al.*, 2016)** and amylase **(Kovacs *et al.*, 2004)** enzymes. Results showed that the *Aspergillus ochraceus* MTCC 1877, had the potential of being a source of two industrially important enzymes, *viz.*, cellulase and alpha amylase. The comparative analysis of the results of the two fungi (Figure 1), showed comparatively higher activity of alpha amylase in the extract from *Aspergillus ochraceus* MTCC 1877. The results suggest the importance of culture conditions in the variation in yield of enzyme among the two fungal strains. Further, the analysis of cellulase activities among the two organisms (Figure 2), revealed the opposite response of two fungi. The cellulase activity after the SSF was observed to be higher in the extract of *Trichoderma longibrachiatum* ITCC 7839 than *Aspergillus ochraceus* MTCC 1877.

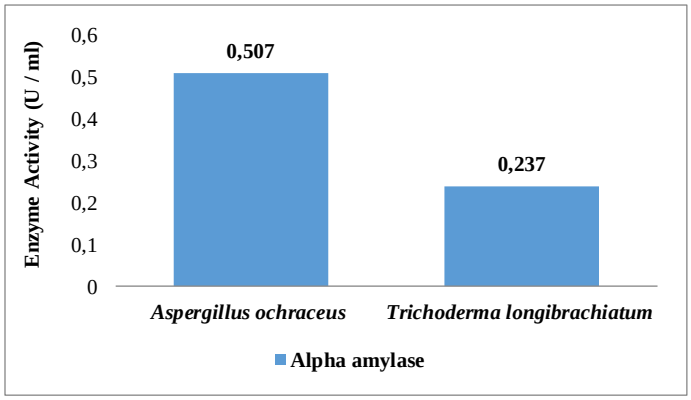

Figure 1 Comparison of the alpha amylase activity (U / ml) of *Aspergillus ochraceus* and *Trichoderma longibrachiatum* in solid state fermentation studies

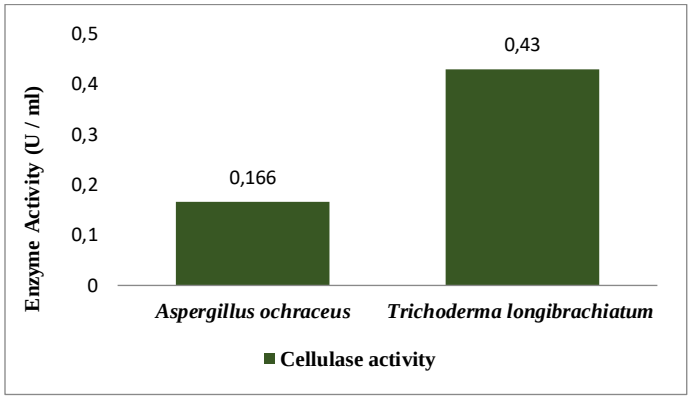

Figure 2 Comparison of the cellulase activity (U / ml) of *Aspergillus ochraceus* and *Trichoderma longibrachiatum* in solid state fermentation studies

The role of bioprocess parameters in the growth, metabolism and production of various primary and secondary metabolites from different industrially important organisms have been well explored **(Bailey and Ollis, 1986).** The organisms can produce both the enzymes. However, difference in enzyme activity of the two strains may be attributed to variation in the microenvironment around the growing fungal strains and their response to abiotic parameters (90% RH in wheat bran at 30 ºC after 7-day culture). The feasibility analysis suggests *Aspergillus ochraceus* MTCC 1877 to be a potential source of alpha amylase and cellulase.

CONCLUSION

Aspergillus ochraceus strain had been well explored for its mycotoxic effect and associated mycotoxins. The fungal metabolites had also shown some significant effect against neurological diseases. However, scarce reports are available on their potentials of being a source of enzymes. Current study explored the significance of co-production of alpha amylase and cellulase on the agro-residue, 'wheat bran', under controlled experimental conditions. The study will pave the way for exploration of other commercially important enzymes from the organisms and strategic approach to optimize culture conditions for improving the yield of enzymes.

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