

Assessment of Amylase Enzyme from Rhizospheric *Aspergillus oryzae*: An Enzymatic Study

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Abstract

Fungi are essential, fascinating and biotechnologically useful group of organisms with an incredible biotechnological potential for industrial exploitation. The knowledge about the overall diversity and uses of fungi is still fragmented and incomplete. Therefore, there are a lot of opportunities to fill the gaps in these areas. Fungi have been used to extract useful enzymes such as Amylase for industrial purposes and their bioactive compounds have shown significant biological properties. The current study was aimed at evaluation of amylase enzyme activity *A. oryzae* isolated from sugarcane field. Physiochemical analysis was performed to obtain the physiochemical characteristics of the soil. The isolated fungus was identified through microscopy, culture characteristics i.e., hyphal type, spore structure, and spore arrangement and scanning electron microscopy analysis (SEM) analysis. On the basis of these analysis and confirmation of spore structure via SEM analysis the specie was confirmed as *Aspergillus oryzae*. The amylase enzyme activity was performed through Di-Nitro Salicylic acid (DNSA) method and the protein was quantified through Lowry method of protein quantification. The amylase enzyme extracted from *A. oryzae* showed enzyme activity of 271.2 U/mg on the fourth day of incubation. It is concluded from current study that *A. oryzae* has a great potential of producing Amylase enzyme.

INTRODUCTION

The organisms in soil mainly play a variety of activities in soil, as soil is a complex structure having many components. There is a major role of soil microorganisms in the evaluation of soil conditions. Understanding the role of microbial communities and their association with plants during their growth, development, and extreme conditions in arid environments are of considerable interest to ecologists (Khan *et al.*, 2021). Soil properties like organic matter, pH and moisture content etc., affects the density and diversity of microbes in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. Microorganisms have been considered as a treasure of useful enzymes. In recent years, using microorganisms as biotechnological sources of enzymes that are industrially important has stimulated great interest in the exploration of extracellular enzymatic activity in many microorganisms. The first industrially produced enzyme was amylase from a fungal source in 1894, which was used for the digestive disorder treatment (Sheela *et al.*, 2024).

Amylases are commercially important enzymes and it represents about 25-33% of the worldwide enzyme market. Amylases are of various types, namely α , β , and glucoamylase α -amylases (endo-1,4- α -D-glucan glucohydrolase) are extracellular enzymes that randomly cleaves the 1,4- α -D-glycosidic bonds between adjacent glucose units in the linear amylase chain (Sundarram and Murthy, 2014). β -amylases (β -1,4-glucan maltohydrolase) are mostly of plant origin, but some microbial strains are also known to produce them. It is an exoacting enzyme that cleaves amylose at non-reducing ends, amylopectin, and glycogen molecule. Glucoamylase also called amyloglucosidase, gluconeogenic enzymes, starch glucogenase, and exo-1,4- α -D-glucan glucohydrolase. It hydrolyses single glucose units from the non-reducing ends of amylose and

also amylopectin in a stepwise manner (Singh and Kayastha, 2014).

Although many microorganisms are able to produce this enzyme, some of the most widely used for their industrial applications are *Bacillus licheniformis*, *Bacillus amyloliquefaciens*, *Aspergillus niger*, *Penicillium chrysogenum*. When compared to other microbial sources, the fungal amylases are preferred because of their more acceptable GRAS (Generally Recognized as Safe) status, the conditions such as hyphal mode of growth and good tolerance to low water activity (a_w) and high osmotic pressure makes fungal species most efficient for bioconversion of solid substrates and thus attracting more interest as source of amylolytic enzymes suitable for industrial applications (Sheela *et al.*, 2021).

Fungal species have several morphological characteristics that help these for distinct identification and one of the prominent features is development of various shapes and sizes of macro and micro asexual spores. These are also identified on the basis of growth rate on agar media and the pigmentations produced by them. Identification of the species is done through macro and microscopic analysis but the most reliable form of identification is through the information gathered via nucleotide sequencing from conserved gene regions which include Internal Transcribed Spacer (ITS). There are more than 172,000 fungal ITS sequences present in Genbank (Ferreira *et al.*, 2021).

Materials and method

Study Site and soil samples collection

This research work was carried out in the Microbiology Research laboratory (MRL) of Abasyn University, Peshawar from October, 2021 to July, 2022. The soil samples were collected in sterile polythene bags from 15cm depth of a sugarcane field, located in Mera Kachori, Peshawar, Pakistan. The soil samples were

brought to laboratory and kept at 4 degrees centigrade until further processing.

Physiochemical Analysis of Soil Samples

The analysis of soil samples was performed at Soil and Water Analysis laboratory of Agricultural Research Institute, Tarnab, Peshawar. Different physiochemical parameters were analyzed i.e. Organic Carbon (C), Nitrogen (N), Phosphorous (P), Potassium (K), pH and Soil Texture (Sand, Silt, Clay content and Textural class), The color and temperature of the samples were recorded at the time of collection. All of the analysis of the samples was done according to the manual of (Ryan *et al.*, 2001).

Isolation of Fungi from Soil Samples

Serial dilution of soil samples was performed to obtain diluted concentration of soil samples i.e. 10^2 , 10^3 , 10^4 , 10^5 and 10^6 . Then by pour plate method, the samples were streaked on Potato Dextrose Agar Plates (PDA). Chloramphenicol, broad spectrum antibiotic was poured into the media after autoclaving to avoid any bacterial growth in the medium. One ml of suspension from the second (10^2), fourth (10^4) and sixth (10^6) test tubes were separately poured onto four petri plates, each containing 25 ml PDA media. The plates were rotated clockwise and anticlockwise to spread the suspension evenly on the plates. The plates were sealed and kept in a static incubator at 28 degrees centigrade for 6 to 8 days and were observed daily. Sub culturing technique was used to obtain a pure culture of the fungi (Raja *et al.*, 2017).

Determination of Fungal Amylase activity

The isolated fungus species was first tested for amylase production by starch hydrolysis before further processing. The modified starch agar media (soluble starch-2g, peptone-2g, Yeast extract-1g, Agar Technical-2g, distilled water-100 ml at pH 6) was inoculated with the isolates and incubated for 2 days at 28 degrees centigrade in a

static incubator. After completion of the incubation period, the petri dishes were flooded with iodine solution and the zone of clearance formed around the fungal growth indicated the production of Amylase enzyme (Lakshmi *et al.*, 2020).

Spore Harvesting and Storage

After observing the amylase activity, the fungal specie was further processed by harvesting the spores. The spores were harvested after the culture had been incubated for 14 days. During this stage the culture was fully sporulated. The petri dish was flooded with a mixture of 0.5ml sterile distilled water and 5.0ml of 0.05% Tween-80, then rubbed the culture gently with a sterile bent glass rod. The spores were released and spore suspension was roughly filtered through sterilized glass wool positioned in the funnel into a 50ml Erlenmeyer flask. Spore suspensions were transferred directly to formerly sterilized 50ml screw cap tubes and centrifuged for 5mins at 1000rpm. After centrifugation, supernatant was removed and the pellet was re-suspended in 10ml sterile distilled water containing 0.1% (v/v) Tween-80, using vortex shaker for 30sec to suspend it completely. Then it was centrifuged once again to wash the spores free of debris. This process was repeated thrice and finally re-suspended in 5.0ml sterile distilled water containing 0.1% (v/v) Tween-80 and shaken for 30sec before storage. The spore suspension was stored at 4°C in darkness until its requirement (Lakshmi *et al.*, 2020).

Identification of the Soil Fungi

The identification of fungi was done by observing morphology, microscopic (compound and electron microscopy) observation of the fungal structures and molecular characterization of the isolated fungi. The morphology of the hyphae was observed by looking at the pigmentation (of spores and mycelium) and texture of the growth of fungi on PDA media.

Microscopic observation

Both Compound and Scanning electron microscopy (SEM) were performed to observe different structure of the isolates.

Smear Method

A drop of Lactophenol cotton blue stain (LPCB) was pipetted onto a sterile slide. A small hypha of fungus from the plate was transferred onto the slide with the help of needle. The fungal growth was carefully and slowly smeared and distributed onto the surface of slide. A sterile cover slip was carefully placed on the slide to avoid any bubble formation. The slide was then observed under 400X through a compound microscope.

Scanning electron Microscope (SEM) Analysis

Scanning electron microscopy was also performed to observe the structure of the fungus under high magnification. For SEM analysis, the sample of the isolated fungus was sent to the National Centre of excellence in geology, University of Peshawar.

Amylase production

For Amylase production, the isolated fungus was subjected to fermentation medium containing

The graph was constructed by carrying out the reactions, shown in the table 1 below.

Table 1: Standard Graph of Maltose

S.NO	Stock solution (Maltose) (ml)	Concentration of maltose ($\mu\text{g/ml}$)	Distilled water (ml)	DNSA reagent (ml)	Incubation
1	Blank	-	1.0	1	In boiling water bath for 10 minutes
2	0.1	100	0.9	1	
3	0.2	200	0.8	1	
4	0.3	300	0.7	1	
5	0.4	400	0.6	1	
6	0.5	500	0.5	1	
7	0.6	600	0.4	1	

(KH_2PO_4 -0.14g, NH_4NO_3 -1g, KCl -0.5g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.01g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -0.001g, soluble starch-2g, Distilled water-100 ml at pH 6.5). Ten Erlenmeyer flasks, out of which one was control and nine were the test samples (each containing 100 ml of the media) were autoclaved at 121°C for 15 min. The media was cooled to room temperature and spore suspension (0.5 ml) was added and was kept in a shaking incubator for 48 hours at 28°C . The broth obtained after incubation, was centrifuged at 7000 rpm for 30 minutes and the cell free supernatant was used for the estimation of amylase (Muthusamy *et al.*, 2017).

Determination of Enzyme Activity

Enzyme activity (EA) is the amount of product released or the amount of substrate converted into product per unit time. The unit is $\mu\text{mol/min}$ or U (units). In this study, DNSA method was used to calculate the amount of reducing sugar in the reaction mixture (Balakrishnan *et al.*, 2021). In the first step, standard graph of Maltose was constructed.

Standard Graph of Maltose

8	0.7	700	0.3	1	
9	0.8	800	0.2	1	
10	0.9	900	0.1	1	
11	1.0	1000	-	1	

Key *No stock solution in the blank tube.

The stock solution of maltose was prepared by suspending 1000µg of maltose per ml of water, DNSA reagent was prepared by taking 1 gram of DNSA (di nitro salicylic acid), 1N NaOH (20ml) and 40gram of Na-K Tartarate in 100ml of water. The whole process was carried out in test tubes. After completion of the incubation time, optical density of the reaction mixture (in a calorimeter tube) was recorded at 540nm in a colorimeter. The graph of absorbance (optical density) against

concentration of maltose was plotted in MS Excel and the slope was calculated.

Concentration of product (Maltose) in Reaction Mixture

Unknown concentration (µg) = Absorbance (O.D)

Slope

Unknown concentration of maltose in the reaction mixture was recorded by performing the reaction shown in the table 2 below.

Table 2: Unknown concentration of product

Reagents	Blank	Test sample
Starch 1%	1 ml	1 ml
Distilled water	0.5 ml	-
Crude enzyme	-	0.5 ml
Buffer (PH 7)	0.5 ml	0.5 ml
Incubation	At room Temperature for 10 min	
DNSA reagent	1 ml	1 ml
Incubation	In boiling Water Bath for 10 min	

Key: *No crude enzyme in the blank tube. *

*Incubation in boiling water was carried out to stop the reaction.

After incubation in boiling water, the reaction mixture was transferred from test tubes into the calorimeter tubes and optical density of the mixture was measured at 540 nm. That value was then divided by the slope of the standard graph of maltose and the concentration of reduced sugar (Maltose) per ml was recorded.

Calculation of Enzyme Activity

After getting the value of concentration of product released or amount of sugar (maltose) reduced by amylase enzyme present in the reaction mixture, the enzyme activity was calculated by using the equation:

$$\text{Enzyme activity } (\mu\text{mol}/\text{min}) = \frac{\mu\text{g of product released} \times 1000}{\text{Molecular weight of product} \times \text{Incubation time}}$$

Here the value (1000) is the conversion factor, molecular weight of maltose is 342.3 g/mol and incubation time is 10 minutes.

Specific Enzyme Activity

Specific enzyme activity is the amount of substrate converted into product per unit time per mg of protein. The unit of specific enzyme activity is U/mg of protein. To find the concentration of

protein, Lowry method was used (Sheela *et al.*, 2021). Firstly, the standard graph of protein was plotted by taking BSA (bovine serum albumin) as standard.

Standard graph of Protein

The graph was constructed by carrying out the reactions shown in the table 3 below:

Table 3: Standard Graph of Protein

	Stock solution BSA (1000µg/ml)	Concentration of BSA (µg/ml)	Distilled Water (ml)	Reagent C (ml)	Incubation	Fc Reagent (1:1) (ml)	Incubation
1	Blank	=	1	4	At room Temperature for 10 min	0.4	Incubation at room temperature for 15 min
2	0.1	100	0.9	4		0.4	
3	0.2	200	0.8	4		0.4	
4	0.3	300	0.7	4		0.4	
5	0.4	400	0.6	4		0.4	
6	0.5	500	0.5	4		0.4	
7	0.6	600	0.4	4		0.4	
8	0.7	700	0.3	4		0.4	
9	0.8	800	0.2	4		0.4	
10	0.9	900	0.1	4		0.4	

Key: *No BSA solution in blank

The stock solution of BSA was prepared by suspending 1000 µg of BSA per ml of water. Reagent C was prepared by mixing 50 ml of reagent A (2% Na_2CO_3 in 0.1% NaOH) and 1 ml of reagent B (0.1% CuSO_4 in 1% Na – K Tartarate. Fc (Folin ciocalteus phenol Reagent) was diluted to 1:1. After completion of the incubation time, the reaction mixture was transferred from test tubes into colorimeter tubes

and the optical density (Absorbance) was measured at 660 nm. The standard graph of optical density against concentration of BSA was plotted in MS Excel and slope was calculated.

Unknown concentration of Enzyme

Unknown concentration (µg/ml) = Absorbance (O.D)

Slope

The unknown concentration of enzyme (amylase) was calculated by performing the procedure, shown in the table 4 below:

Table 4: Unknown concentration of Enzyme

Reagents	Blank	Test sample
Crude enzyme	-	1 ml
Distilled water	1 ml	-
Reagent C	4 ml	4 ml
Incubation	At room temperature for 10 min	
Fc Reagent	0.4 ml	0.4 ml
Incubation	At room temperature for 15 min	

After completion of the incubation time, the reaction mixture was transferred into calorimeter tubes and the optical density was measured at 660 nm. The value of optical density was divided by the value of slope of the standard graph of BSA and the concentration of amylase per ml of reaction mixture was calculated.

Calculation of Specific Enzyme Activity

After calculating the concentration of Amylase in the reaction mixture, the value of the enzyme was divided by 1000 to convert the value from microgram to milligram. Then the specific enzyme activity was calculated by using the equation:

Specific Enzyme Activity (U/mg of protein =
Enzyme activity

mg of protein

The results of enzyme activity were taken for 2nd, 3rd, 4th and 5th day respectively.

Results

Physiochemical Analysis of Soil

Soil properties like organic matter, pH etc, affects the density and diversity of microbes in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. Some of the physicochemical characteristics of the soil from which the fungus was isolated are shown in the table 5.

Table 5. Physiochemical Characteristics of Soil

S.NO	Physiochemical Characteristics		Results
1	Clay	%	10
	Slit		72
	Sand		18
	Textural Class		Silty Loam
2	pH1:5		8.5

3	Organic Matter %		0.81
4	Nitrogen	mg/kg	0.041
	Phosphorus		28.6
	Potassium		112
5	Color		Brown
6	Smell		Normal

Isolation of Fungi

A total of five fungi species were isolated after serial dilution and those fungi species were frequently sub-cultured to obtain pure cultures of the fungi. The five fungal species were named as (FTTC1, FTTC2, FTTC3, FTTC4, FTTC5).

Determination of Amylolytic Activity

For further research work on the isolated fungi, the five species were subjected to a specialized media to find out their ability to breakdown starch into maltose (starch hydrolysis). The isolate (FTTC1) produced the maximum zone of starch hydrolysis and was selected for further study. The zone formed by FTTC1 in specialized starch media is shown in the figure 1.



Figure 1. Amylolytic Activity of Isolated Fungus

Identification of Fungi

Culture characteristics

The growth of isolate (FTTC1) on PDA media after 7 days of incubation was examined and the growth characteristics were observed. The isolate showed greenish-yellow growth of spores and mycelium on PDA medium. The mature spores and mycelia appeared green while the developing mycelia appeared yellowish with white borders.

Microscopic Observation

The lactophenol cotton blue staining (LPCB) technique was performed and different structures of the isolate (FTTC1) were observed. The slide culture technique proved to be more efficient in providing clear image of the fungus as compared to smear and scotch tape method. The isolate (FTTC1) appeared to have a conidiophore, a bunch of spores with single chain and a phialide from which the spores appeared to be rising upward. The structural characteristics are shown in table 6.

Table 6: Microscopic observation of isolated fungus

Parameters	Isolate
Spore producer	Conidiophore
Spores Arrangement	Bunch of spores with single chain
Vesicle	Round shaped

Scanning Electron Microscopy Analysis (SEM) Analysis

The SEM analysis provided further information about the structure of the fungus. The SEM analysis of the spores confirmed that the isolate (FTTC1) has oval shaped spores that are shown in figure 2.

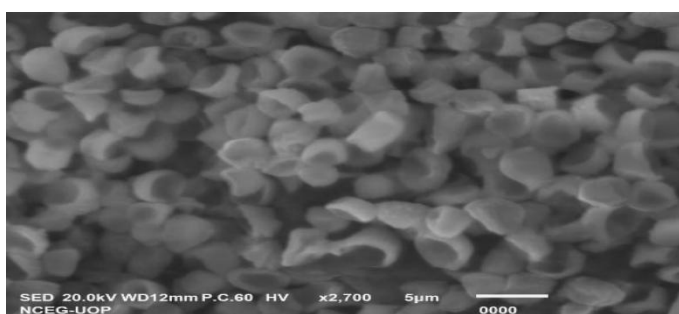


Figure 2: SEM Image of Spores of Isolated Fungus

Amylase Production

Enzyme Activity

After incubating the fungus in the enzyme media, the enzyme activity was calculated after 2nd, 3rd, 4th and 5th day of incubation respectively.

Standard Graph of maltose

Standard graph of maltose was constructed to obtain the slope value. The standard graph

constructed in MS Excel.

Concentration of Maltose in the Reaction Mixture

The unknown concentration of maltose in the sample was calculated in the reaction mixture for each day of incubation. The values of unknown concentration are shown in the table 7.

Table 7: Concentration of Maltose in Reaction mixture

Days of Incubation	Concentration of Maltose (µg/ml)
2 nd	163
3 rd	220
4 th	325
5 th	128

Enzyme Activity

After calculating the unknown concentration of Maltose in the reaction mixture, the enzyme

activity was calculated for each day of incubation. The values of Enzyme activity are illustrated in the table 8.

each day of incubation. The values of unknown concentration are shown in the table 9.

Table 9: Concentration of Maltose in Reaction mixture

Days of Incubation	Concentration of Maltose ($\mu\text{g/ml}$)
2 nd	163
3 rd	220
4 th	325
5 th	128

Enzyme Activity

After calculating the unknown concentration of Maltose in the reaction mixture, the enzyme

activity was calculated for each day of incubation. The values of Enzyme activity are illustrated in the table 10.

Table 10: Enzyme Activity after each day of Incubation

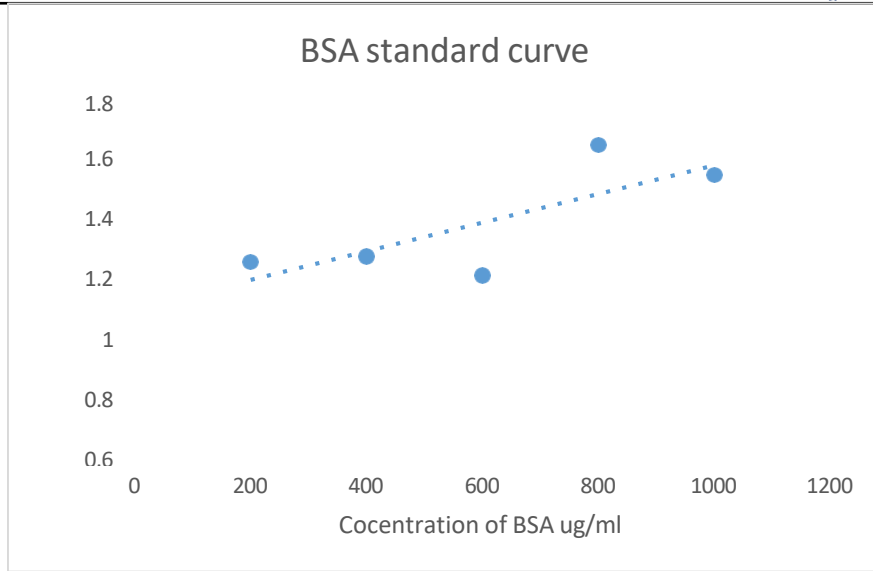
Days of Incubation	Enzyme Activity ($\mu\text{mol/min}$)
2 nd	47.6
3 rd	64.2
4 th	94.9
5 th	37.3

Specific Enzyme Activity

After calculating the enzyme activity, the specific enzyme activity was calculated as the enzyme

activity values are prerequisite for specific enzyme activity calculation.

Standard graph of Protein (Bovine Serum Albumin)



The standard graph of protein constructed in MS Excel is shown in the figure 4.

Figure 4: BSA Standard Curve



Absorbance at 660 nm				

Unknown concentration of Enzyme

The unknown concentration of enzyme in the reaction mixture for each day was calculated and the values are shown in the table 11.

Table 11: Unknown concentration of Enzyme

Days of Incubation	Unknown concentration of Enzyme (µg/ml)
2 nd	303
3 rd	270
4 th	355

5 th	220
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Specific Enzyme Activity

After calculating the unknown concentration of enzyme in the reaction mixture, the specific enzyme activity was calculated for each day. The

maximum specific enzyme activity was recorded on 4th day of incubation and the minimum specific enzyme activity was recorded on 2nd day of incubation.

Table 12: Specific Enzyme Activity

Days of Incubation	Specific Enzyme Activity (U/mg)
2 nd	158.7
3 rd	237.7
4 th	271.2
5 th	169.5

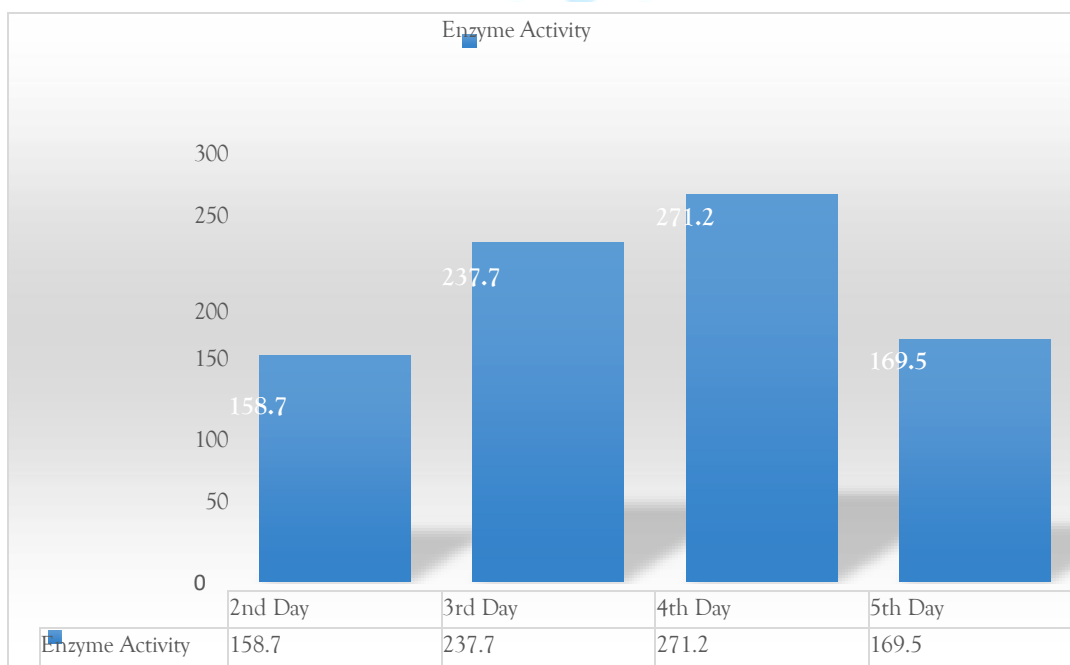


Figure 5: Graphical representation of Enzyme Activity

Discussion

Fungi along with bacteria, protozoa, small invertebrates, and plants play an essential and significant role in the soil ecosystem. Soil fungi were also considered as very important producers for secondary metabolites. Fungi produced several skeletally unique compounds that were used as pharmaceuticals. Numerous species of fungi including *Penicillium*, *Aspergillus*, *Fusarium*, *Cladosporium* and *Yeasts* are able to produce enzymes and secondary metabolites including antibiotics. Approximately 20% of antibiotics have been obtained from fungi isolated from soil. Those antibiotics, produced by fungi, including fusidic acid, cephalosporin and penicillin are widely used for treatment of many diseases and they are the most important source of potentially significant pharmaceutical drugs (Syal *et al.*, 2017). Alami *et al.*, (2020) reported that soil properties like organic matter, pH and moisture content etc., affects the density and diversity of microbes in the soil. Therefore, it is important to study the relation between soil physicochemical properties and abundance of indigenous microorganisms. Hence, in present study, we performed the soil analysis to find out the critical properties that the soil exhibit from which the fungi were isolated. Sara *et al.*, (2020) conducted a study that involve the antimicrobial activity of the *Aspergillus oryzae* strain isolated from saline soil (El-Baida marsh in Algeria). Whereas, in current study, we isolated the fungus *Aspergillus Oryzae* from the rhizospheric soil of a sugarcane field.

Ababutain *et al.*, (2021) in their study identified the fungus through morphological characteristics and 18s rRNA gene sequencing method. Similarly, in this study, we utilized LPCB staining and SEM analysis to observe the morphology of the *Aspergillus Oryzae* and 18s rRNA gene sequencing to identify the isolated fungus. Lakshmi *et al.*, (2014) used the fermentation medium that contained (KH_2PO_4 -0.14g, NH_4NO_3 -1g, KCl -0.5g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ -0.01g, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ -0.001g, soluble starch-2g, Distilled water-100ml at

pH 6.5) for the production of amylase enzyme. In current study, the same fermentation medium was used for the production of amylase enzyme Balakrishnan *et al.*, (2021) in their study utilized DNSA (dinitro salicylic acid) method for the calculation of reducing sugar in the reaction mixture of isolated amylase enzyme. Similarly, in current study, DNSA method was used to find out the amount of reducing sugar (maltose) in the reaction mixture. Sheela *et al.*, (2021) used the Lowry method of protein quantification to quantify the isolated amylase enzyme present in the reaction mixture. Therefore, in present study, Lowry method of protein quantification was used to quantify amylase enzyme by using pholin cicalteus reagent. UV-Visible spectroscopy was then used to obtain the optical density of the reaction mixture. Khadse (2018) represented the amylase activity in U/ml. While in this study the amylase activity was measured in U/mg of protein. This method of representing the amylase activity is also reported in the study of Lakshmi *et al.*, (2014).

Conclusion

It is concluded from current study that the Rhizosphere of sugarcane field inhabit an abundance of fungal species. The fungi showed excellent growth on PDA media at a temperature range of 28°C to 30°C. The best method for observing the morphology of fungi through LPCB staining is through the method of slide culture technique. The fungus *Aspergillus oryzae* showed significant amylase activity. The amylase activity showed minimum values during the first days of incubation and the activity increased as the incubation increased. But the activity started to decline as the incubation time exceeded 96 hours.

References

- 1) BALAKRISHNAN, M., JEEVARATHINAM, G., KUMAR, S. K. S., MUNIRAJ, I. & UTHANDI, S. 2021. Optimization and scale-up of α -amylase production by *Aspergillus oryzae* using solid-state fermentation of edible oil cakes. *BMC biotechnology*, 21, 33.
- 2) BRINK, B. 2010. Urease test protocol. *American society for microbiology*, 1-7.
- 3) BRÜCK, M., BENRA, F., DUGUMA, D. W., FISCHER, J., JIREN, T. S., LAW, E. A., PACHECO-ROMERO, M., SCHULTNER, J. & ABSON, D. J. 2024. A social-ecological approach to support equitable land use decision-making. *Ambio*, 1-16.
- 4) FERREIRA, A., CAHÚ, T., XU, J., BLENNOW, A. & BEZERRA, R. 2021. A highly stable raw starch digesting α -amylase from Nile tilapia (*Oreochromis niloticus*) viscera. *Food Chemistry*, 354, 129513.
- 5) JONES, W. P. & KINGHORN, A. D. 2012. Extraction of plant secondary metabolites. *Natural products isolation*, 341-366.
- 6) KATZ, D. S. 2010. Coagulase test protocol. *American society for microbiology*, 11.
- 7) KHAN, S. T., ADIL, S. F., SHAIK, M. R., ALKHATHLAN, H. Z., KHAN, M. & KHAN, M. 2021. Engineered nanomaterials in soil: their impact on soil microbiome and plant health. *Plants*, 11, 109.
- 8) LAKSHMI, S. S., MAHESH, C., GAYATRI, K., MANISHA, P. & AISHWARYA, K. 2020. Statistical optimization of amylase production and its purification from a palm wine isolate *Bacillus* sp., Q-164. *Biocatalysis and Agricultural Biotechnology*, 29, 101820.
- 9) LEHMAN, D. 2005. Triple sugar iron agar protocols. *American society for microbiology*, 1-7.
- 10) MACWILLIAMS, M. P. 2009. Citrate test protocol. *American Society for Microbiology*, 1-7.
- 11) MILLER, J. M. & WRIGHT, J. W. 1982. Spot indole test: evaluation of four reagents. *Journal of clinical microbiology*, 15, 589-592.
- 12) MURTHY, H. N., LEE, E.-J. & PAEK, K.-Y. 2014. Production of secondary metabolites from cell and organ cultures: strategies and approaches for biomass improvement and metabolite accumulation. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 118, 1-16.
- 13) MUTHUSAMY, B., SELVAN, L. D. N., NGUYEN, T. T., MANOJ, J., STAWISKI, E. W., JAISWAL, B. S., WANG, W., RAJA, R., RAMPRASAD, V. L. & GUPTA, R. 2017. Next-generation sequencing reveals novel mutations in X-linked intellectual disability. *Omics: a journal of integrative biology*, 21, 295-303.
- 14) RAJA, M., PRAVEENA, G. & WILLIAM, S. J. 2017. Isolation and identification of fungi from soil in Loyola college campus, Chennai, India. *International Journal of Current Microbiology and Applied Sciences*, 6, 1789-1795.
- 15) REINER, K. 2010. Catalase test protocol. *American society for microbiology*, 1, 1-9.
- 16) RYAN, J., ESTEFAN, G. & RASHID, A. 2001. *Soil and plant analysis laboratory manual*, ICARDA.
- 17) SHEELA, J., EBENEZAR, E., THERADIMANI, M., RAGUL, S., TAMILMALAR, M. P., ARAVINTHKUMAR, A., RAJINIMALA, N., PARAMASIVAN, M. & ARUMUGAMPILLAI, M. 2024. UNRAVELING THE PROTECTIVE POTENTIAL OF SPERMOSPHERE BACTERIA IN MITIGATING RICE BROWN SPOT (*Bipolaris oryzae* (Breda de Haan) Shoemaker) INFECTION. *JAPS: Journal of Animal & Plant Sciences*, 34.
- 18) SHEELA, J. M., DIVYA, K. & PREMINA, S. 2021. Amylase production by *aspergillus Niger* and *Penicillium* species by solid-state and submerged cultivation using two food industrial wastes. *Nature Environment and Pollution Technology*, 20, 1127-1135.

- 19) SHIELDS, P. & CATHCART, L. 2010. Oxidase test protocol. *American Society for Microbiology*, 4, 1-9.
- 20) SINGH, K. & KAYASTHA, A. M. 2014. α -Amylase from wheat (*Triticum aestivum*) seeds: its purification, biochemical attributes and active site studies. *Food chemistry*, 162, 1-9.
- 21) SUNDARRAM, A. & MURTHY, T. P. K. 2014. α -amylase production and applications: a review. *Journal of Applied & Environmental Microbiology*, 2, 166-175.
- 22) SYAL, K., MO, M., YU, H., IRIYA, R., JING, W., GUODONG, S., WANG, S., GRYS, T. E., HAYDEL, S. E. & TAO, N. 2017. Current and emerging techniques for antibiotic susceptibility tests. *Theranostics*, 7, 1795.
- 23) TRIPATHI, N. & SAPRA, A. 2020. Gram staining.

