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Enzyme Screening and Optimization from Coastal Soil Fungi of Dhanushkodi

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ABSTRACT

The screening of marine soils fungi was distinguished three different enzymes, namely cellulase, amylase and protease. The maximum zone of clearance was measured in the three potential fungi such as *A. niger, A. flavus* and *A. terreus.* Therefore, the subsequent investigations were examined solely utilizing specific fungi, namely *A. niger, A. flavus* and *A. terreus.* The maximum enzyme optimizes and production was recorded in the cellulase enzyme when compared with amylase and protease enzymes. The amylase that underwent dialysis without the presence of cells demonstrated specific protein activity, as well as yield and purification factor. Subsequently, the dialyzed a-amylase underwent additional purification. The crude and purified forms of the amylase were examined using SDS-PAGE. The gel images of the purified enzyme and zymogram provided confirmation of the elimination of various undesirable proteins during the purification.

Keywords: Soil fungi, amylase, *A. niger,* optimization, SDS-PAGE

INTRODUCTION

The study site Dhanushkodi is situated at eastern extremity of Rameswaram Island. The narrow strip of land is separated by a few meters and is encompassed on all other other sides by the sea. The tip of Dhanushkodi is known as Arichalmunai, which translates to the "eroded corner", where the deep and turbulent Indian Ocean meets the shallow and tranquil waters of the Bay of Bengal.

Amylases are a class of enzymes that catalyze the hydrolysis of complex sugars into simpler forms. Recent advancements in marine science and biotechnological research have led to the identification of a significant number of microorganisms inhabiting marine environments that are capable of producing amylase. In particularly the novel α-amylase has been isolated from the marine associated *Streptomyces* sp. Furthermore, the *Bacillus* sp. was isolated from the mangrove associated leaves decomposes, it have been found to exhibit maximum amylase activity, while displaying little to no activity towards cellulose (Chakraborty *et al*., 2009). These findings highlight the potential of marine microorganisms as a source of amylase enzymes for various industrial applications. Proteins are subject to degradation by a variety of hydrolytic enzymes, commonly referred to as proteases. Certain fungi, specifically those belonging to the genera *Penicillium* and *Aspergillus* have been identified as producers of proteases with significant commercial and biotechnological value (Kathiresan *et al.*, 2011).

Microbial enzymes possess a significant advantage in that they can be produces the high amount of quantity through fermentation method. The synthesis of enzymes is meticulously regulated the microbes by optimizations of parameters and enhance the productivity. The yield of cellulase is contingent upon a multifaceted relationship involving various factors such as pH, temperatures, incubation periods and carbon source. These parameters were achieved the successful

fermentations process, it is imperative to induce overproduction of the desired metabolite in the microorganism. A comprehensive investigation is therefore necessary to determine the optimal conditions for large scale productions of enzyme (Immanuel *et al.,* 2006; Bischoff *et al.,* 2006).

MATERIALS AND METHODS

Screening of potential fungi by enzyme assay

The potential fungi were screened based on the maximum amount of enzyme productions. Hence, the present investigation was carried out with the amylase, cellulase and protease enzyme plate assay.

Amylase Activity (Migahed, 2003)

The utilization of starch agar media was employed to assess the efficiency of amylase production. All the identified fungal species were centrally inoculated on the starch casein agar plates and stored at 28°C temperature. After the two days incubation period the inoculated plates were applied with iodine solution, then the zone of clearance was observed around the inoculated fungi. These zones are measured and recorded.

Optimization of enzyme productions (Goyal *et al.,* 2003)

Optimization of enzyme productions by potential fungi were carried out into the Erlenmeyer flasks. The different parameters such as pH, temperature, incubation period and nutrient sources were altered with basal medium of cellulase, amylase and protease enzyme from *A.flavus, A.niger* and *A.terreus*.

Production of amylase (Mandels *et al.,* 1976)

The starch casein broth was utilized for the production of amylase enzyme from potential fungi. The different parameters such as pH (4, 6 & 8), temperature (25, 30 & 35°C), incubation period (2, 4 & 6 days), Nutrient sources (carbon, nitrogen, phosphorus and potassium with 25, 50 & 75mg/g were treated on fungi inoculated medium. After the incubation the crude enzyme was read at 540nm by UV-vis spectrophotometer.

SDS-PAGE and activity staining (Mandels *et al.,* 1976)

The SDS-PAGE and activity staining were employed to determination of molecular weight of synthesized enzyme. This study was performed with 12% acrylamide gel and it was boiled for 5 minutes. In this regard the commercial enzyme was loaded into an electrophoresis without the boiling.

For activity staining, the proteins within the gel were renatured by incubated them with 1% Triton X-100 in a 100 mM sodium phosphate buffer (pH 6.0) for a duration of 30 minutes. The gel was then washed twice with sodium phosphate buffer (pH 6.0) after being rinsed with water. Subsequently, the gel was incubated at 60°C for 1 hour with a 1% soluble starch solution prepared in the same buffer. Upon addition of an iodine solution (consisting of 1% KI and 0.1% I2), a clear band was observed on a dark background, indicated the presence of amylolytic activity.

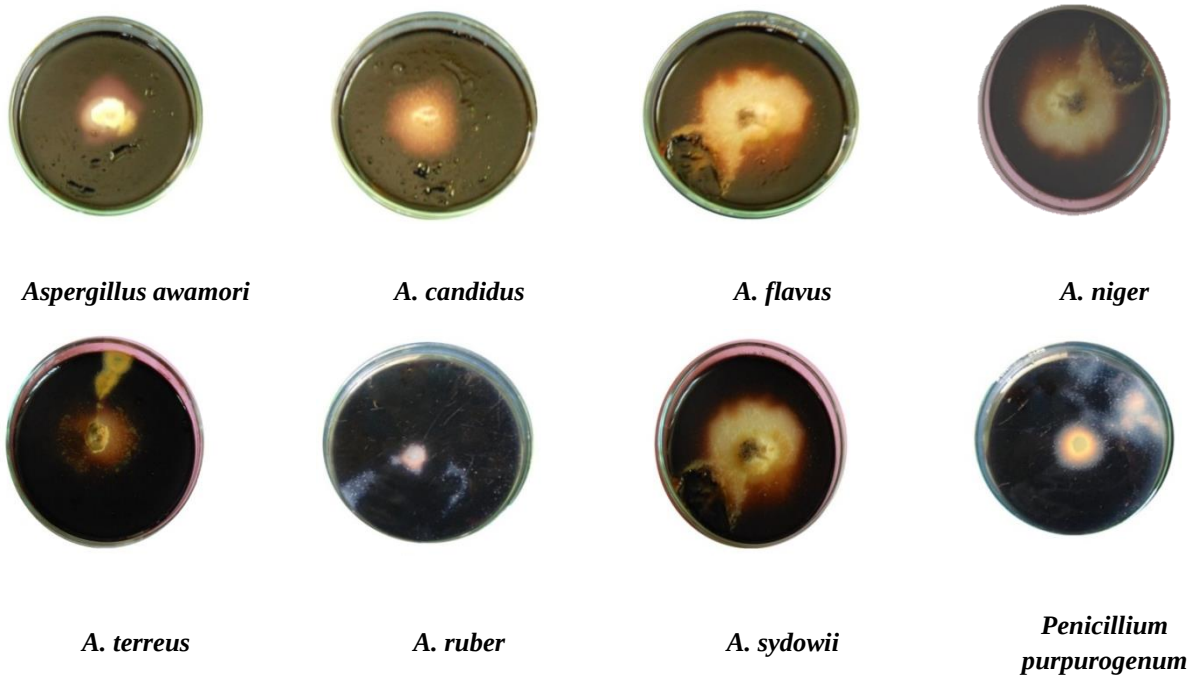
RESULTS

Primary screening of isolated potential soil fungi by using different enzymes

The screening of marine soil fungi was distinguished three different enzymes, namely cellulase, amylase and protease. The fungi were identified: *Aspergillus alliaceus, A. awamori, A. candidus, A. chevalieri, A. citrisporus, A. conicus, A. flavipes, A. flavus, A. fumigatus, A. fuscus, A. glaucus, A. nidulans, A. niger, A. ochraceus, A. ruber, A. spinulosum, A. sydowii, A. terreus, A. thomi, A. unguis, A. ustus, A. variegatus, A. versicolor, Absidia glauca, Acrocylindrium oryzae, Alternaria alternata, Chaetomium globosum, Curvularia geniculata, Fusarium moniliforme, F. equiseti, F. solani, F. oxysporum, Gliocladium* sp., *Helminthosporium* sp., *Humicola* sp., *Myrothecium verrucaria, Penicillium citrinum, P. janthinellum, P. purpurogenum, P. roqueforti, Rhizopus stolonifer, Syncephalastrum* sp., *Trichoderma polysporum* and *Verticillium* sp. The maximum zones measured in the three potential fungi such as *A.niger, A.flavus* and *A.terreus.* Therefore, the subsequent investigations were examined solely utilizing specific fungi, namely *A. niger, A. flavus* and *A. terreus* **(Table 1**; **Plate 1-3**).

Table1: Screening of fungi by using different enzymes

S. No.	Name of fungi	Zone of clearence (mm)		
		Cellulase	**Amylase**	**Protease**
1.	*Aspergillus alliaceus*	1	-	-
2.	*A.awamori*	-	2	-
3.	*A.candidus*	-	3	-
4.	*A.chevalieri*	1	-	-
5.	*A.flavipes*	2	-	1
6.	*A.flavus*	11	8	9
7.	*A.fumigatus*	3	-	1
8.	*A.niger*	8	16	5
9.	*A.ruber*	4	1	3
10.	*A.sydowii*	-	3	-
11.	*A.terreus*	11	5	7
12.	*A.versicolar*	-	-	1
13.	*Fusarium oxysporum*	1	-	3
14.	*F.solani*	1	-	-
15.	*Penicillium purpurogenum*	4	1	-
16.	*P.roqueforti*	2	-	2
17.	*P. citrinum*	1	-	-



Aspergillus awamori ***A. candidus*** ***A. flavus*** ***A. niger***

A. terreus ***A. ruber*** ***A. sydowii*** ***Penicillium purpurogenum***

Plate 1: Screening of isolated fungi using amylase enzyme

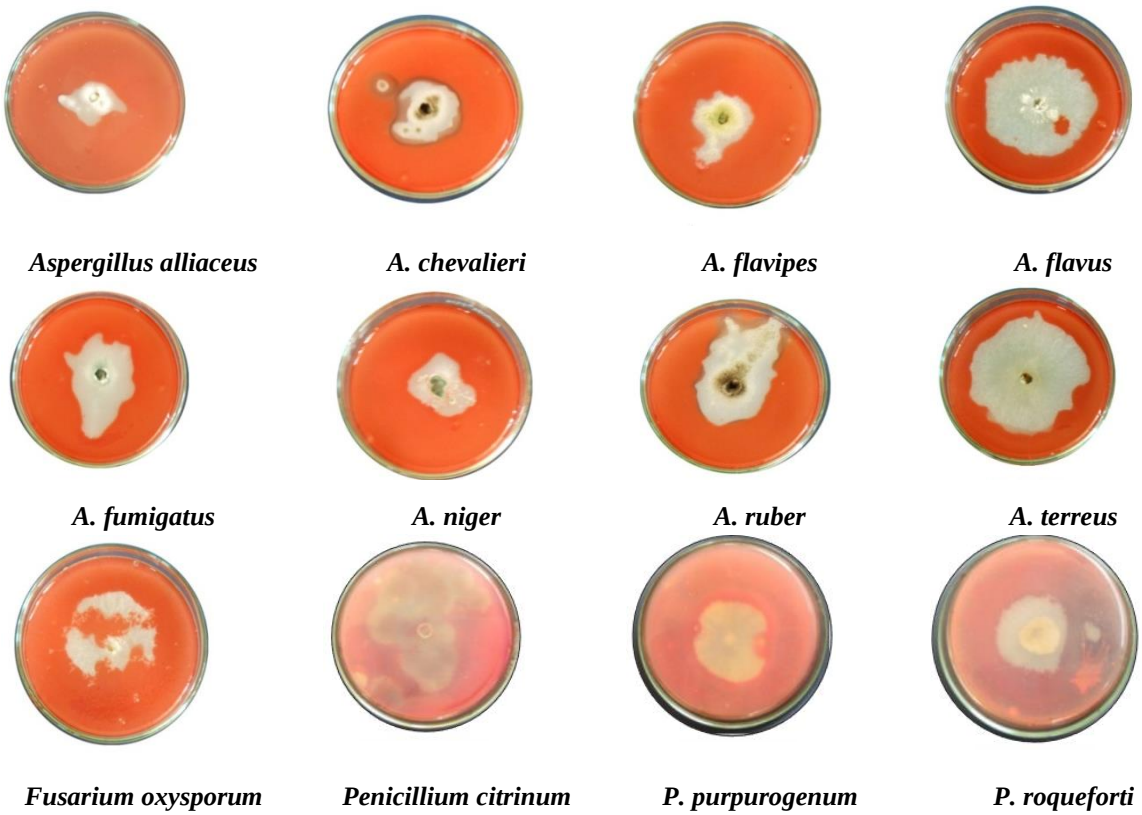


Plate 2: Screening of isolated fungi using cellulase enzyme

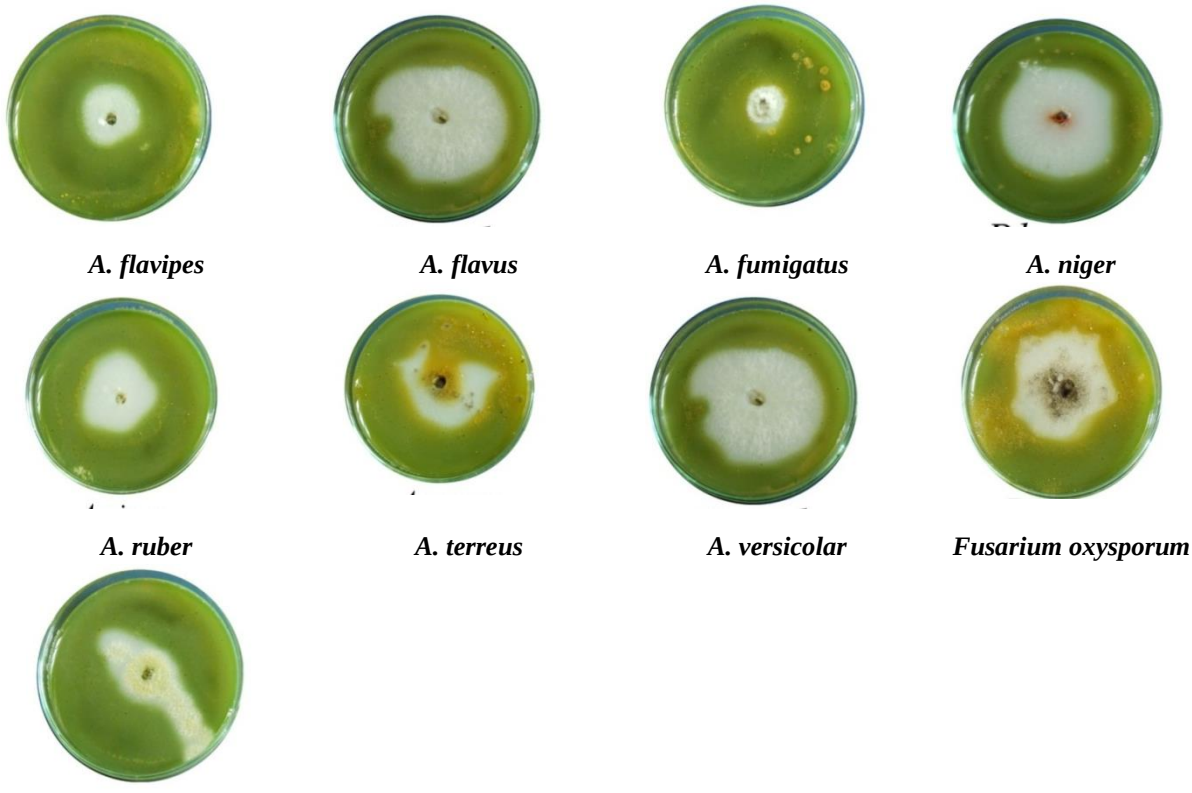


Plate 3: Screening of isolated fungi using protease enzyme

Optimization and production of enzymes from potential fungi

In accordance with the optimization and production of amylase, protease, cellulase enzymes with different substrates such as wheat bran, rice bran, coir pith, substrate from potential fungi, the impact of *A.niger, A.flavus* and *A.terreus* on the optimized enzymes were examined under various parameters. These parameters optimized pH in the range of 4, 6 and 8, temperatures of 25, 30 and 35ºC, incubation periods 2, 4, and 6 days, as well as carbon source, nitrogen source, phosphorus and potassium levels of 25, 50, and 75 mg/g quantity were altered for production of enzyme. The maximum enzyme optimize and production were recorded in the cellulase enzyme when compared with amylase and protease enzymes in all parameters with respectively (**Table 2**).

SDS page analysis of amylase enzyme

The cell-free dialyzed a-amylase displayed 381U and 15.24 Umg^{-1} protein specific activity, 36.95% yield and 2.305-fold purification. The dialized a-amylase was further purified by a column of Sephadex G100 and purified to a homogeneity with 9.28-fold, 27.35% yield, and a specific activity of 61.3Umg^{-1} using ammonium sulfate precipitation followed by Sephadex G-100 gel filtration chromatography giving one peak. The specific activity of a-amylase was increased from 6.6 to 61.30Umg^{-1} (10-fold) after purification. Before and after purification, the crude and purified a-amylase was analyzed by SDS-PAGE. Gel images of both purified enzyme and zymogram confirmed that various undesired proteins were eluted out during the series of purification. The crude supernatants of fermented sample of both LSSF and SSF were found to be rich in different types of proteins and exhibited 8 bands. The banding patterns were unique representing diversity in gene expression. The bands of the crude extracts of a- amylase were found to be in a range of 7–175kDa. However, the molecular weight of purified a-amylase was 15.3 kDa (**Figure 1**).

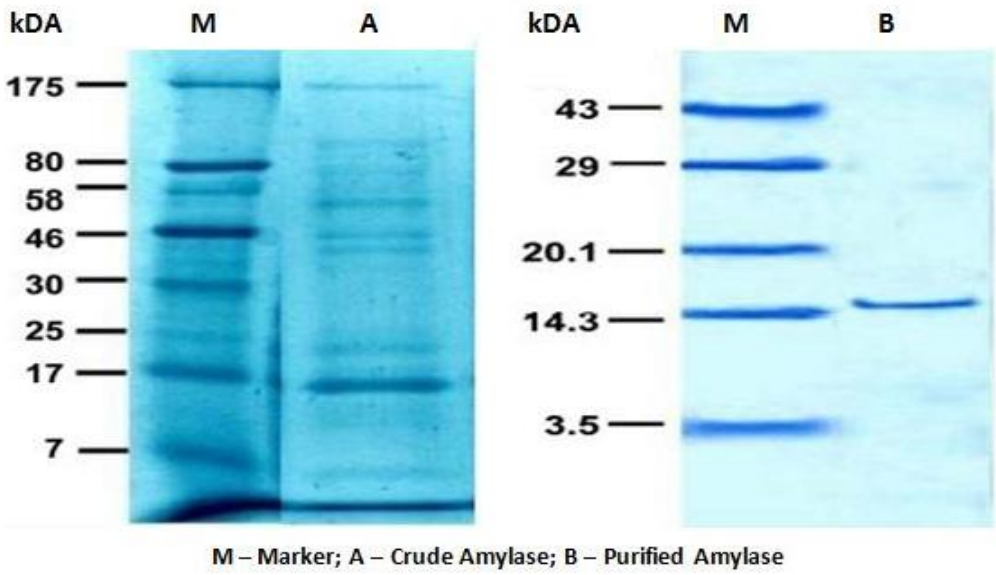


Figure 1: SDS PAGE analysis

DISCUSSION

The production of various extracellular enzymes were observed that a SR60 strain exhibited proteolytic, cellulolytic, aminolytic, and xylanolytic activity under conditions of high salinity ranging from 0 to 1.5 M NaCl. These enzymatic productions were discerned by the presence of zones of enzymatic hydrolysis (Bruno *et al.*, 2018). The protease enzyme productions of fungi were positively correlated with the duration of incubation. Notably, *A.flavus* exhibited the high amount of protease enzyme when compared to *A.fumigatus* (Oyeleke *et al.*, 2010). The observed variations in protease yield between the two fungal species may be attributed to their genetic heterogeneity (Ali, 1992). Similar, work has been performed with *M.indicus, P.variotii, M.thermophila, T.terrestris, B.dothidea, F.oxysporum, Fusarium* sp. and *F.verticillioides* for the production of protease enzyme.

Table 2: Optimization and production of Amylase enzyme from potential fungi

Parameters	Inference	Quantity (IU/ml) (Wheat bran)			Quantity (IU/ml) (Rice bran)			Quantity (IU/ml) (Coir pith)		
		A.niger	***A.flavus***	***A.terreus***	***A.niger***	***A.flavus***	***A.terreus***	***A.niger***	***A.flavus***	***A.terreus***
pH	4	1.51±0.00	4.66±0.00	3.51±0.00	1.55±0.00	2.58±0.03	1.52±0.02	1.97±0.03	1.68±0.03	1.35±0.03
	6	1.89±0.33	5.01±0.00	4.19±0.00	2.80±0.00	3.64±0.03	2.30±0.07	2.64±0.00	2.49±0.03	2.15±0.04
	8	2.63±0.08	7.13±0.00	4.55±0.00	2.99±0.05	4.00±0.03	2.31±0.02	2.80±0.03	2.98±0.03	2.93±0.04
Temperature (^{o}C)	25	5.59±0.05	6.96±0.00	6.64±0.05	6.60±0.03	7.69±0.03	6.61±0.01	4.80±0.00	3.46±0.05	5.40±0.00
	30	6.55±0.00	7.34±0.03	6.95±0.01	7.36±0.02	6.68±0.00	6.91±0.08	5.47±0.01	3.60±0.00	5.79±0.01
	35	7.51±0.08	7.80±0.05	7.43±0.00	6.99±0.05	7.69±0.02	7.21±0.08	5.76±0.00	4.45±0.00	6.29±0.05
Incubation period (days)	2	0.15±0.03	0.02±0.03	0.09±0.03	0.34±0.00	0.23±0.06	0.59±0.03	0.34±0.08	0.29±0.03	0.24±0.00
	4	0.26±0.03	0.47±0.03	0.53±0.06	0.47±0.05	0.29±0.03	1.36±0.02	0.42±0.00	0.30±0.03	0.47±0.00
	6	0.36±0.00	0.90±0.00	0.74±0.03	0.61±0.00	0.35±0.05	1.63±0.00	0.44±0.07	0.91±0.00	0.63±0.00
Nutrient content (Carbon) (mg/g)	25	1.03±0.20	1.23±0.06	0.95±0.06	0.89±0.02	0.79±0.05	0.80±0.02	0.69±0.05	0.89±0.05	0.96±0.02
	50	1.93±0.02	1.89±0.08	1.03±0.04	1.00±0.05	1.06±0.06	1.20±0.09	1.25±0.09	1.52±0.03	1.30±0.20
	75	2.30±0.09	2.05±0.06	1.66±0.02	1.85±0.06	1.58±0.09	1.70±0.07	1.97±0.08	2.00±0.06	1.98±0.05
Nitrogen (mg/g)	25	0.80±0.09	0.96±0.09	0.90±0.06	0.69±0.02	0.52±0.00	0.67±0.06	0.90±0.03	0.53±0.09	0.58±0.02
	50	1.14±0.06	1.25±0.06	1.05±0.09	1.08±0.05	1.43±0.06	1.52±0.05	1.30±0.01	1.60±0.05	1.20±0.03
	75	1.89±0.07	1.79±0.02	1.60±0.02	1.67±0.02	2.00±0.00	1.98±0.00	1.60±0.02	2.03±0.06	1.87±0.06
Phosphorus (mg/g)	25	0.90±0.03	0.59±0.06	0.78±0.08	0.54±0.00	0.96±0.00	0.97±0.03	0.52±0.01	0.66±0.00	0.69±0.06
	50	1.59±0.06	1.00±0.00	1.03±0.09	1.15±0.04	1.60±0.02	1.26±0.06	1.20±0.00	1.26±0.03	1.30±0.00
	75	2.03±0.05	1.96±0.05	1.82±0.02	1.99±0.00	2.03±0.02	1.90±0.05	1.87±0.02	1.59±0.05	1.75±0.03
Potassium (mg/g)	25	0.96±0.05	0.88±0.06	0.70±0.02	0.89±0.00	0.60±0.03	0.96±0.09	0.60±0.01	0.63±0.06	0.48±0.06
	50	1.05±0.09	1.20±0.03	1.30±0.05	1.02±0.06	1.56±0.06	1.59±0.05	1.63±0.00	1.59±0.06	0.98±0.00
	75	1.98±0.05	1.96±0.06	1.96±0.00	1.98±0.00	2.06±0.00	1.99±0.00	1.79±0.05	1.86±0.00	1.26±0.00

The values are expressed in terms of (Mean ± Standard deviation)

Among the all fungi, only the *F.oxysporum* showed significant zone of clearances (Peciulyte, 2007; Barek *et al.,* 2019; Saroj & Narasimhulu, 2018). The evaluation of fungal isolates for their ability to produce extracellular enzymes including amylase, protease, lipase, cellulase and pectinase. The results of the screening revealed that 67%, 56%, 49%, 39%, and 25% of the endophytic fungi were capable of producing lipase, cellulase, pectinase, protease and amylase enzymes respectively (Kathawut & Siriluck, 2020). The two facets of exploration capacity and specific enzyme activity of ECM fungi, which has rarely been attempted previously. This findings indicate that specific enzyme activities, rather than the root-tip surface area of ECM, better explain the variation in ground-area based enzymatic activities across study sites (Okada *et al.,* 2024).

Marine microorganisms are promising source of enzymes with industrial applications due to their immense genetic and biochemical diversity. Availability of enzymes, advancements in enzymology and enzyme technology have significantly contributed to the industrial application of enzymes and the rapid expansion of the enzyme market. In this context marine fungal enzymes assume greater attention recently owing to the great demand for novel and efficient biocatalysts for industrial applications and other services. This situation has warranted exploration of marine fungal biodiversity for new enzymes. The present review focus on bioprospecting of marine fungal enzymes produced by lesser studied fungi, identification of research gaps, challenges in pursuing research in harnessing the potentials of marine fungi, and the scope for future prospects. Role of fungal enzymes in biogeochemical processes in marine environments, bioremediation, and plastic degradation is discussed indicating marine fungi as source of industrial enzymes (Chandrasekaran and Kalaiselvam, 2023).

Most of the halophilic fungi belong to Ascomycota and Basidiomycota. Halophilic species of ascomycetes namely *Aspergillus sydowii, A.versicolor, Aureobasidium pullulans, Hortaea werneckii, Penicillium chrysogenum, Phaetothecatri angularis* and *Trimmatostromas alinum* are important (Śliżewska *et al.,* 2022).

The present investigation involved the initial evaluation of potential fungi isolated from marine soils samples in a medium conducive to the production of cellulase, amylase and protease. Specifically, the screening process encompassed the assessment of all three enzymes in three distinct fungi namely *Aspergillus niger*, *A. flavus* and *A. terreus*. Consequently, subsequent analyses were exclusively conducted utilizing the aforementioned selected fungi namely *Aspergillus niger*, *A. flavus* and *A. terreus*.

In the present investigation optimization of cellulase, amylase, and protease enzyme production using wheat bran as a substrate. This study investigates the impact of different parameters on the optimized protease enzyme production by three potential fungi such as *A.niger, A.flavus* and *A.terreus* respectively. These parameters include pH levels of 4, 6, and 8, temperatures of 25, 30 and 35ºC, incubation periods of 2, 4 and 6 days, as well as varying amounts of carbon source, nitrogen source, phosphorus, and potassium (25, 50, and 75 mg/g). In the present study maximum amylase enzyme production was recorded.

Several fungal species isolated from soils were found to possess amylolytic activity with *Aspergillus* sp. exhibiting the highest amylase activity due to the wheat brans, rice brans and black grams bran which are considered as carbon sources. The *A.niger* was more effective for the amylase enzyme productions when compared to the other fungal organisms (Deepali & Priya, 2018).

The impact of heat treatment on the enzyme activity of various fungi with a focus on the thermal stability temperature range. The temperature range examined in this study spanned from 30^oC to 100^oC. It is noted that the *A. flavus* exhibited the highest enzyme activity at 40^oC. However, this activity was completely lost when the temperature reached 100^oC. Conversely, *A. niger* demonstrated its highest enzyme activity at 30^oC. *Aspergillus fumigatus* exhibited its peak enzyme activity at 50^oC, with a value of $6.2x10^{-3}$U/ml/min. Hence, *Penicillum italicum* displayed its highest enzyme activity at 40^oC, with a value of $4.7x10^{-3}$U/ml/min. notably even at the extreme temperature of 100^oC, *Penicillum italicum* still retained a slight level of enzyme activity (Oseni, 2011).

The thermal stability of the production of amylase, cellulase and protease enzymes from diverse substrates including black gram dust, sawdust, sugarcane bagasse, wheat bran, rice bran and coir pith, employing potential fungi such as *A. niger*, *A. flavus* and *A. terreus* with different temperatures like 30, 40, 50, 60 and 70°C, the amylase enzyme exhibited the highest degree of thermal stability.

Production from *A. niger* and *A. flavus* using black gram dust and sugarcane bagasse substrates respectively, at a substrate quantity of 1gm and a time interval of 96 hours. Similarly, the highest cellulase enzyme production was observed from *A. niger* using saw dust substrate at a substrate quantity of 1gm and a time interval of 72 hours. Protease enzyme production was found to be highest from *A. terreus* using wheat bran substrate at a substrate quantity of 1gm and a time interval of 48 hours. These findings suggest that amylase enzyme production, cellulase and protease enzyme can be optimized by selecting appropriate substrates and fungal strains as well as by controlling the time interval of enzyme production.

This study investigated the thermal stability of amylase, cellulase, and protease enzymes produced from various substrates, of black gram dust, saw dust, sugarcane bagasse, wheat bran, rice bran and coir pith, using potential fungi such as *A. niger*, *A. flavus* and *A. terreus*. Different concentrations of substrates specifically 5.0, 10.0, and 15.0 g/ml, were utilized. The findings of the study indicated that the highest level of enzyme production was observed in *A. terreus* when utilizing black gram dust, saw dust, rice bran and coir pith waste. Conversely, *A. niger* demonstrated the greatest enzyme production when utilizing sugarcane bagasse.

The a-amylase enzyme was isolated through a series of purification techniques including ammonium-sulfate precipitation, Sephacryl S-200 gel filtration of chromatography and Q-sepharose fast flow ion exchange chromatography. The partial purified enzymes were exhibited the single band in SDS-PAGE which is indicated that it exists as a monomer with a molecular mass of approximately 57 kDa. The specific activity of the purified enzyme was determined to be 4071 $IUmg^{-1}$, representing a four-fold increase in purification (Balu Jancy & Shunmugiah, 2014).

The results of this study indicate that cellulase enzymes can be produced via the submerged fermentation of rice straw incorporated with *T. reesei* or *A. awamori* very quickly and without any nutrient supply. *T. reesei* is better for the production of cellulase enzyme (27.04 mg/0.5 mL) as compared with *A. awamori* (15.19 mg/0.5 mL). Among the three incubation times of 3, 5, and 7 days, 5 days of incubation was the best time for the production of a higher amount of cellulase enzyme using *T. reesei*, while for *A. awamori*, 3 days of incubation time was the best. In addition, using rice straw for cellulase production could reduce national rice straw waste, and it could benefit the enzyme industry in terms of the effective cost (Nather *et al.*, 2021).

CONCLUSION

It was concluded that, the present review focuses on detergent compatible fungal amylase, examine their productions, characteristics and stabilities of other detergents. The investigation focused on the characteristics of the protease derived from *A. terreus.* The enzyme was identified as a thermostable alkaline serine protease, exhibiting a strong affinity for a range of protein substrates. Additionally, it demonstrated remarkable stability and compatibility when combined with surfactants, bleaches, oxidizing agents and local powder detergents. Through the process of immobilization, the properties of amylases can be enhanced to effectively contribute to the advancements in contemporary biotechnology industries.

The findings of significance as they demonstrated that the enzymes remained active even in the presence of elevated temperatures and pH levels, making it suitable for use in demanding conditions encountered during textile wet processing. Additionally, the crude amylase preparation contains valuable industrial accessory enzymes, further enhancing its usefulness for industrial applications. Utilizing a single enzyme preparation across multiple stages of industrial processing has the potential to enhance the economic efficiency of the overall process. However the, research will focus on applying the crude enzyme from *Aspergillus terreus* in various stages of textile wet processing, exploring

cost-effective enzyme utilization. A microfungus like *Aspergillus terreus* was suitable for industrial production of enzymes.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

REFERENCES

Ali, G.A.O. 1992. Formation of protease by *Aspergillus fumigatus* and *Penicillium* spp. *Journal of King Saudi University Science*, **4(2)**:127.

Balu Jancy, K. and Shunmugiah, K. 2014. Halotolerant, acid-alkali stable, chelator resistant and raw starch digesting a-amylase from a marine bacterium *Bacillus subtilis* S8–18. *Journal of Basic Microbiology*, **54**:802-811.

Barek, M., Taidi, H.N., Smaoui, B., *et al.*, 2019. A. Isolation, screening and identification of ligno-cellulolytic fungi from northern central Morocco. *Biotechnology, Agronomy, Society and Environment*, **23**:207-217.

Bischoff, K.M., Rooney, A.P., Li, X.L., *et al.*, 2006. Purification and characterization of a family 5 endoglucanase from a moderately thermophilic strain of *Bacillus licheniformis*. *Biotechnology Letters*, **28(21)**:1761-1765.

Bruno, O., Yago, Q., Katharina, M., *et al.*, 2018. Screening of protease, cellulase, amylase and xylanase from the salt-tolerant and thermostable marine *Bacillus subtilis* strain SR60. *F1000Research*, 7(1704): 1-7.

Chakraborty, S., Khopade, A., Kokare, C., Mahadik, K. & Chopade, B. 2009. Isolation and characterization of novel α-amylase from marine *Streptomyces* sp. *D1*. *Journal of Molecular Catalysis B: Enzymatic*, **58**:17-23.

Chandrasekaran Muthusami and Kalaiselvam Murugaiyan 2023. Bioprospecting marine fungasl enzymes scope and challenges. *Kavaka*, **5(1)**:33-47.

Deepali, J. and Priya, K. 2018. Optimization of gluco-amylase production from *Aspergillus* Spp. for Its use in Saccharification of liquefied corn starch. *Biotechnology*, **8(2)**:101-108.

Goyal, N., Jian, S.C., and Banerjee, U.C. 2003. Comparative studies on the microbial adsorption of heavy metals. *Advances in Environmental Research*, **7**:311-319.

Immanuel, G., Dhanusha, R., Prema, P. *et al.*, 2006. Effect of different growth parameters on endoglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environmental Science and Technology*, **3(1)**:25-34.

Kathawut, S. and Siriluck, I. 2020. Isolation and screening of extracellular enzymatic activity of endophytic fungi isolated from Thai orchids. *South African Journal of Botany*, **134:**273279.

Kathiresan, K., Saravanakumar, K., Anburaj, R., *et al.*, 2011. Microbial enzyme activity in decomposing leaves of Mangroves. *International Journal of Advanced Biotechnology and Research*, **2**:382-389.

Mandels, M., Andreotti, R., and Roche, C. 1976. Measurement of Saccharifying Cellulase. *Biotechnolnology and Bioengineering Symposium*, **6**:21-33.

Migahed, F.F. 2003. Distribution of fungi in the sandy soil of Egyptian beaches. *Mycobiology.* **31(2)**:61-67.

Naher, L., Fatin, S.N., Sheikh, M.A.H., *et al.*, 2021. Cellulase Enzyme Production from

Filamentous Fungi *Trichoderma reesei* and *Aspergillus awamori* in Submerged Fermentation with Rice Straw. **7**:868.

Okada, K.I., Yokoyama,D., Aiba, S.I. *et al*., 2024. Exploration capacity versus specific enzymatic activity of ectomycorrhizas in response to primary productivity and soil phosphorus availability in Bornean tropical rainforests. *Scientific Reports*, **14**:2842.

Oseni, O.A. 2011. Production of Microbial Protease from Selected Soil Fungal Isolates. *Nigerian Journal of Biotechnology*, **23**:28-34.

Oyeleke, S.B., Egwim, E.C., and Auta, S.H. 2010. Screening of *Aspergillus flavus* and *Aspergillus fumigatus* strains for extracellular protease enzyme production. *Journal of Microbiology and Antimicrobials*, **2(7)**:83-87.

Peciulyte, D. 2007. Isolation of cellulolytic fungi from waste paper gradual recycling materials. *Ekologija*, **53**:11-18.

Saroj, P.P.M. and Narasimhulu, K. 2018. Characterization of thermophilic fungi producing extracellular lignocellulolytic enzymes for lignocellulosic hydrolysis under solid-state fermentation. *Bioresources and Bioprocessing*, **5**:31.

Slizewska, W., Swita, S.K., and Milkolajczyk, O. 2022. Metabolic potential of halophilic filamentous fungi current perspective. *International Journal of Molecular Sciences*, **23**:4189.