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Screening and Partial Purification of Enzymes from Marine Fungi of Thiruvallur and Chennai District

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Abstract

The natural products like enzymes in micro fungi from marine soil. In the current study, screening and partial purification of enzymes at marine soil fungi from Chennai and Thiruvallur district. The fungi were screened for the synthesis of amylase, protease, lipase and chitinase enzymes. The screening result showed that the isolated marine soil fungi like *Aspergillus niger, A. flavus, A. terreus, A. candidus, A. awamori, A. fumigatus, Pencillium citrinum, P. longibrachiatum, P. lanosum, Pencillium* sp*, Fusarium* sp*, F. solani, Cunninghamella vitricillate, Neurospora* sp and *N. crassa* has the ability to produce all the four enzymes. The *Aspergillus niger, A. terreus* and *A. fumigatus* were screened based on quantum of enzymes. The maximum amylase activity was observed in *Aspergillus niger* (3.601 IU/ml) followed by *A. terreus* (3.743 IU/ml) and *A. fumigatus* (3.905 IU/ml). The four enzymes from soil fungi showed a significant difference (P≤0.05). The fungi which is observed with maximum lipase activity has been taken for partial purification process. Among them, the lipase enzyme in *A. fumigatus* is preceeding with maximum purification fold (3.6) in the dialysis experiment. It was concluded that, the exceptional ability to produce extracellular proteins in the isolated soil fungi are the main source of industrial enzymes.

***Key words*:** Marine soil, Fungi, Enzymes, Screening, Partial purification

The enzymes are biocatalysts, which depending on the biological reaction's nature, catalyze particular biochemical reactions. Eco-friendly products are being developed by biotechnological industries using natural resources. Environmental pollution is reduced by replacing chemical products with biological factors like enzymes which decreases the formation of effluent. The production of various enzymes which are highly demanded in industries for a variety of purposes heavily depends on microorganisms. The bacteria, fungi and yeast which synthesize economically significant enzymes in microorganisms. The enzymes such as amylase, protease, lipase and chitinase are commonly isolated from microorganisms [1]. Starch serves as the substrate for the hydrolytic enzyme known as amylase. The food industry uses amylase extensively for a variety of procedures such as syrup making and fruit juice shelf-life improvement [2]. Pharmaceuticals, the paper industry, clinical research, starch analytical chemistry and medicinal chemistry are just a few of the several fields in which amylase is used. To release fatty acids and glycerol, lipase hydrolyzes the acylglycerols and glycerol esters. Long chain triglycerides are converted into diglycerides, monoglycerides, fatty acids and glycerol [3]. Lipases are used widely in the food, cosmetic and pharmaceutical industries as well as in the synthesis and degradation of fatty acid and glycerol esters. Proteases are a type of hydrolase enzyme that share content the polypeptide chain connecting the amino acids found in proteins. Proteases play an important role in commercial businesses [4]. The minimum cultivation requirements and ease of genetic manipulation, the commercially available protease is of microbial origin as compared to that of plants and animals [5]. Proteases are more important among these enzymes due to their numerous biotechnological applications in the detergent, pharmaceutical, dehairing process in the leather industry, brewing and cheese making, textile, laundry, and waste processing industries [6]. The therapeutic usage of protease includes the treatment of skin ulcers as a healing agent [7]. They are important enzymes used in the industries that process starch to help it break down into simple sugars [8-10]. The first major use of microbial enzymes in the food industry started with the use of starch-degrading enzymes [11-12]. Protease, the enzyme that performs proteolysis, accounts for over 60% of all enzyme markets [13-15]. This enzyme is important for a wide range of application including waste treatment [14], [16], the formulation of pharmaceuticals, detergents and the leather industry [17]. For order to search for microorganisms, [18] a variety of techniques can be used to find extracellular lipases. using inducer substrates including vegetable oils,

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standard triglycerides (tributirin, triolein), Tween 80 and dyes in a solid medium [19, 20]. Chitinases also play a significant role [21-22] in the production of single cell proteins, growth factors, mosquito control, a biocontrol agent of fungal pathogens, and isolation of fungal protoplasts [23-24]. Due to its high demand for stopping the chemical hydrolysis of starch during the starch liquefaction process, amylase holds a significant part (about 25%) of the global enzyme market [25-26]. Additionally, it has been used in the paper pulp, food, brewing, textile and other industries [27-29]. Being widely distributed, fungi survive in nature because to their remarkable physiologic versatility and ability to release a variety of enzymes that aid in the breakdown of complicated polymers [30]. They have a powerful extracellular enzymatic complex, able to depolymerize this aromatic polymer into lower molecular weight compounds [31]. Their application has been expanded into many other fields including as analytical and medicinal chemistry [32]. In the food industry, a number of starch hydrolysates have been used as sweeteners. Amylases have also been used in the brewing industry to transform grain starch into fermentable sugars for yeast strains [33]. Amylase enzymes are used by the paper and detergent industries [34-38]. Enzymes are among the most important products obtained for human needs through plants, animals and microbial sources. Nowadays, the use of enzymes in industrial sector is increasing due to increase in the number of industries especially food, beverages, textile, leather and paper industries [39-40]. There have been some interesting research on metabolites and extracellular enzymes that may break down the polymers in cell walls [41]. Enzymes are biological catalysts that aid in the occurrence of chemical reactions under a variety of physical conditions. Although all enzymes are proteins by nature and each one plays a distinct performance function [42]. The aim of this study to screening and partial purification of enzymes at marine soil fungi from Chennai and Thiruvallur district.

MATERIALS AND METHODS

Sample collection

Marina sea and Fore shore estate from Chennai district and Pazhaverkadu from Thiruvallur district selected for three marine soil sample collections. Soil samples were collected from the top 10-15 cm of the soil using a sterile spatula and placed in sterilized polythene bags [43]. These samples were brought to the lab and kept there at 4°C until processing commenced. A unique sample code was received to each sample.

Screening of enzymes

The isolated marine soil fungal culture was screened for the presence of enzymes amylase, protease, lipase and chitinase.

Amylase assay

Starch was used as the substrate in a method described by [44] using DNS to determine enzyme activity. In a total volume of 2 ml, the reaction mixture contained the following compounds: After 30 minutes of incubation at 40 °C in a water bath with 1 ml of enzyme extract added, 1 ml of 1% soluble starch in citrate phosphate buffer (pH 6.5), the reaction was stopped by adding 1 ml of DNS reagent (3, 5-dinitro salicylic acid, Sigma-Aldrich Spruce Street, St. Louis, USA) and boiling for 5 min at 80 °C. The tubes were cooled after the reaction was stopped, and the absorbance was measured at 540 nm. By using glucose as the reducing sugar in the standard plot, the amount of glucose produced was calculated. The amount of enzyme that releases 1 mol of glucose per minute under the assay conditions is regarded one unit of amylase activity.

Protease assay

With a few minor modifications and using casein as the substrate, the proteolytic activity in culture supernatant was evaluated in accordance with the method described by Keay and Wildi [45]. It was mixed with 1 mL of culture supernatant and 1 mL of 1% (w/v) casein in 0.1 M sodium phosphate buffer (pH 7.0). The mixture was then incubated for 10 minutes at 30 °C and 2 mL of 0.4 M trichloro acetic acid (TCA) was added to stop the reaction. The culture supernatant-containing mixture was then incubated at room temperature for 30 min before being centrifuged at 1000 rpm for 5 min. After 10 minutes, 1 mL of the Folin reagent was mixed to the resulting supernatant (1 mL), which had been combined with 5 mL of 0.4 M Na_2 CO_3. The absorbance at 660 nm was measured after allowing the tubes to stand at 30 °C for 30 minutes. The control was set up in a similar method. But casein was not being added until the reaction had already stopped. Tyrosine at a level of 0–60 g/mL was used to create a standard curve. The amount of enzyme needed to release one microgram (1 g/mL) of tyrosine under the assay conditions described was used to define one unit of protease activity. The assays were all repeated twice.

Lipase assay

TBA medium-grown cultures that were 5 days old culture were used for inoculation. For the production of lipase, two plugs of a fungal culture were incorporated into 100 cc of production medium. For five days, cultures were incubated at 30°C and 100 rpm. The enzyme assay was carried out using the procedure described by [46]. Every 24 hours, the culture filtrate was removed out of each flask and centrifuged at 10,000 rpm for 10 minutes at 40 °C. Enzyme assays were performed using supernatants. A spectrophotometric assay was used to measure lipase production using the substrate p-nitro phenyl palmitate (p-NPP). Lipase hydrolyzed p-NPP to give p-NP which gave yellow color and absorbance of which was measured spectrophotometrically at 410 nm against blank.

Chitinase assay

Colloidal chitin was selected as the substrate for the chitinase enzyme assay. One hour was kept incubating the reaction mixture, which included 0.5 ml of 1% w/v colloidal chitin and 0.5 ml of enzyme solution. The reaction was then stopped by adding 3 ml of the 3, 5-dinitrosalicylic acid reagent which was then heated at 100 ˚C for 5 min. After centrifugation, the modified method of [47] was used to determine the amount of reducing sugar in the supernatant. Absorbance was measured at 530 nm using UV spectrophotometer along with substrate and blanks. N-acetylglucosamine serial dilutions (from 0 to 50 mM) were prepared to determine the enzyme unit. For determination of enzyme unit, serial dilutions of N-acetylglucosamine (from 0 to 50 mM) were prepared. The amount of enzyme required to release 1 mmol of N-acetylglucosamine (as a standard) from chitin per minute is defined as one unit (U) of the chitinase activity.

Partial purification of enzymes

After the culture filtrates had been incubated for 12 days, the crude enzyme was precipitated with varying concentrations of ammonium sulphate, keeping the mixture under a magnetic stirrer at 4°C for 24 hours. The protein precipitate was then collected by cooling the mixture and centrifuging it at 10,000 rpm for 10 minutes. The pellet was dialyzed against 2-3 changes of buffer during the process under a magnetic stirrer at 4°C for

24 hours while being suspended in a sodium acetate buffer with a pH of 4.6 [48]. Proteins that were only partially purified were measured (U/mL) in an aliquot.

Purification profile

Enzyme activity

By using casein as a standard and ammonium sulphate precipitation and dialysis to purify the samples, the enzymes activity in the raw materials was determined [49].

Estimation of protein content

Using bovine serum albumin as a standard, the amounts of protein in the crude extract, ammonium sulphate precipitated and dialyzed enzyme samples were calculated [50].

Specific activity

The following formula was used to calculate the specific activity of the crude extract, ammonium sulphate precipitated and dialyzed sample.

$$\text{Specific activity (U/mg)} = \frac{\text{Total activity (Units/mL)}}{\text{Total protein (mg/mL)}}$$

Purification fold

The specific activity of the sample and the crude extract were used and calculated the purification fold for the ammonium sulphate precipitated sample and the dialyzed. The formula used as follows:

$$\text{Purification fold} = \frac{\text{Specific activity of the sample}}{\text{Specific activity of the crude extract}}$$

Statistical analysis

The enzymatic activity assay was performed in triplicate with three separate repetitions. Along with beneficial useful resource of the Statistical Package for Social Sciences (version 22), one-way ANOVA at the 95% level ($P \leq 0.05$) was used to statistically evaluate for significant differences between mean values.

RESULTS AND DISCUSSION

Screening of enzymes from marine soil fungi

In the current study, totally 15 fungi were isolated from three different marine soil samples were collected from Chennai and Thiruvallur district. The marine soil fungi was isolated such as *Aspergillus niger, A. flavus, A. terreus, A. candidus, A. awamori, A. fumigatus, Pencillium citrinum, P. longibrachiatum, P. lanosum, Pencillium* sp, *Fusarium* sp, *F. solani*, *Cunninghamella vitricillate, Neurospora* sp and *N. crassa* were screened for their ability to produce enzymes like amylase, protease, lipase and chitinase. In amylase enzyme, the maximum amount of activity was screened by the soil fungi were represented as *Aspergillus niger, A. terreus, A. fumigatus, Pencillium* sp, *P. longibrachiatum* and *Cunninghamella vitricillate*. In protease enzyme, the soil fungi were presented as exhibiting the highest level of activity when it comes to screening as the *A. niger, A. terreus, A. fumigatus, P.citrinum* and *Neurospora crassa*. In lipase enzyme, the higher amount of activity was screened as *Aspergillus niger, A. flavus, A. terreus, A. fumigatus, A. candidus* and *Neurospora* species. In chitinase enzyme, the maximum amount screened by *A. niger, A. terreus, A. fumigatus, P. lanosum* and *Fusarium solani*. When compared with all enzymes, the chitinase enzyme has been minimum amount was screened by the activity (Table 1).

Table 1 Screening of fungi by various enzymes

Name of the marine soil fungi	Quantity (IU/ml)			
	Amylase	Protease	Lipase	Chitinase
Aspergillus niger	3.601 ± 0.21^{k}	2.715 ± 0.12^{i}	2.903 ± 0.23^{j}	1.051 ± 0.39^{f}
Aspergillus flavus	1.521 ± 0.10^{b}	0.857 ± 0.34^{a}	3.936 ± 0.17^{l}	0.258 ± 0.11^{e}
Aspergillus terreus	3.743 ± 0.54^{l}	2.755 ± 0.77^{i}	3.023 ± 0.54^{k}	1.470 ± 0.85^{i}
Aspergillus candidus	1.660 ± 0.61^{d}	1.411 ± 0.49^{d}	2.822 ± 0.22^{i}	0.165 ± 0.16^{d}
Aspergillus awamori	2.272 ± 0.33^{f}	1.760 ± 0.61^{f}	1.843 ± 0.44^{d}	$0.028\pm0.49^{**}$
Aspergillus fumigatus	3.905 ± 0.27^{m}	2.937 ± 0.04^{j}	3.064 ± 0.87^{k}	1.036 ± 0.15^{f}
Pencillium citrinum	2.122 ± 0.28^{e}	2.168 ± 0.58^{g}	1.672 ± 0.41^{b}	0.060 ± 0.47^{a}
Pencillium longibrachiatum	3.298 ± 0.09^{i}	1.640 ± 0.26^{e}	2.605 ± 0.19^{g}	$0.045\pm0.10^{**}$
Pencillium lanosum	2.167 ± 0.17^{e}	0.980 ± 0.09^{b}	1.035 ± 0.08^{a}	1.226 ± 0.34^{h}
Pencillium sp	2.924 ± 0.05^{h}	1.465 ± 0.61^{d}	1.945 ± 0.74^{e}	0.147 ± 0.12^{d}
Fusarium sp	2.761 ± 0.37^{g}	1.399 ± 0.15^{c}	1.772 ± 0.10^{c}	0.096 ± 0.45^{c}
Fusarium solani	1.281 ± 0.41^{a}	1.414 ± 0.57^{d}	1.682 ± 0.12^{b}	1.112 ± 0.18^{g}
Cunninghamella vitricillate	3.481 ± 0.11^{j}	1.326 ± 0.16^{c}	2.012 ± 0.04^{f}	$0.011\pm0.49^{**}$
Neurospora crassa	1.577 ± 0.24^{c}	2.597 ± 0.37^{h}	1.824 ± 0.19^{d}	0.073 ± 0.56^{b}
Neurospora sp	1.527 ± 0.29^{b}	1.740 ± 0.08^{f}	2.796 ± 0.23^{h}	$0.035\pm0.28^{**}$

The values are expressed in terms of (Mean ± Standard deviation)
Mean values within the column followed by the letters 'a' to 'm' are not significantly different ($P \geq 0.05$) and
**are significantly different ($P \leq 0.05$)

The enzymes are mostly screened by the marine soil fungi and when compared to all isolated soil fungi, all enzymes are maximum screened by the fungi such as *Aspergillus niger, A. terreus* and *A. fumigatus*. In amylase enzyme, the maximum production of quantity in 3.601±0.21 IU/ml, 3.743±0.54 IU/ml and 3.905±0.27 IU/ml was recorded. In protease enzyme, the production of quantity in 2.715±0.12 IU/ml, 2.755±0.77 IU/ml and 2.937±0.04 IU/ml was observed. In lipase enzyme, the amount of activity is obtained in 2.903±0.23 IU/ml, 3.023±0.54 IU/ml and 3.064±0.87 IU/ml was found to be recorded respectively. In chitinase enzyme, the maximum amount of production is 1.051±0.39 IU/ml, 1.470±0.85 IU/ml and 1.036±0.15 IU/ml was recorded. The screening of lipase enzyme production quantity was recorded in *Aspergillus flavus* (3.936±0.17 IU/ml) are highly presented and the chitinase enzyme minimum quantity was recorded in *Cunninghamella vitricillate* (0.011±0.49 IU/ml). The amylase and lipase enzyme are almost screened and recorded when compared with protease

and chitinase enzyme represented respectively. The amylase, protease, lipase and chitinase screened from different marine soil fungi, showed (Table 1) are noted with letters 'a' to 'm' are not significantly different (P≤0.05) and four soil fungi screening of chitinase enzyme of such as *Aspergillus awamori*, *Pencillium longibrachiatum*, *Cunninghamella vitricillate* and *Neurospora* sp are significantly different (P≥0.05) as shown in (Fig 1).

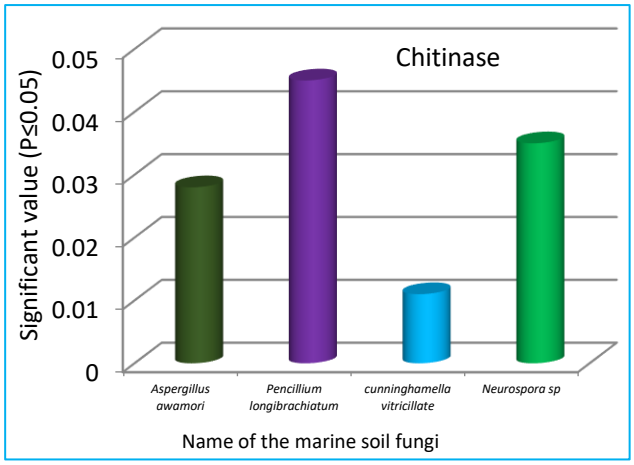

Fig 1 Quantification of Chitinase enzyme from three soil fungi

Partial purification of enzymes

In the present study, the partial purification of enzymes from selected and maximum production of marine soil fungi such as *Aspergillus niger*, *A. terreus* and *A. fumigatus* for all enzymes. The crude enzyme extract was subjected to purification by ammonium sulphate precipitation and dialysis in order to increase the specific activity of all enzymes. When compared with crude enzyme extract and dialysis, ammonium sulphate fractionation revealed that all enzymes and protein are also present in higher concentrations. After dialysis, all enzymes exhibited an increase in the specific activity of the enzyme with a purification fold (Fig 2-4).

Purification of enzymes from Aspergillus niger

In amylase enzyme, the crude amylase that total activity of 43.5 IU/ml and 49.6 IU/mg specific activity. When increase the specific activity of amylase, the crude extract was subjected to purification by ammonium sulphate precipitation and dialysis was found to be total activity of 41.9 IU/ml and 36.3 IU/ml and specific activity of 60.2 IU/mg and 75.1 IU/mg. Then protein are also higher content was presented by ammonium sulphate fractionation 38.7 IU/ml when compared with crude amylase and dialysis. After dialysis, the specific activity of the enzyme increased with 3.2 purification fold (Fig 1). When the crude extract was purified by ammonium sulphate precipitation and dialysis to increase the specific activity of protease, it was revealed to have total activity of 39.7 IU/ml and 32.5 IU/ml and specific activity of 59.6 IU/mg and 74.6 IU/mg. When compared to crude protease and dialysis, the ammonium sulphate fractionation 37.8 IU/ml protein content was also increased. The enzymes specific activity increased 2.6 purification fold after dialysis (Fig 1). In lipase enzyme, the crude lipase had a total activity of 45.6 IU/ml and a specific activity of 50.6 IU/mg. The crude extract showed total activity of 42.1 IU/ml and 35.4 IU/ml, and specific activity of 65.8 IU/mg and 76.2 IU/mg when it was purified by ammonium sulphate precipitation and dialysis to increase the specific activity of lipase. The ammonium sulphate fractionation 36.7 IU/ml protein content was similarly higher when compared to crude lipase and dialysis. Following dialysis, the enzymes specific activity increased 3.5 fold in terms of purification (Fig 1). In chitinase enzyme, the crude chitinase specific activity was 46.1 IU/mg and its total activity was 33.2 IU/ml. When the crude extract was purified using ammonium sulphate precipitation and dialysis to increase the chitinase specific activity, it showed total activity of 37.0 IU/ml and 32.4 IU/ml, and also specific activity of 60.3 IU/mg and 70.3 IU/mg. Observing the protein content of the ammonium sulphate fractionation compared to that of crude chitinase and dialysis, it was found to be higher at 35.9 IU/ml. The specific activity of the enzymes increased 2.3 fold in respect of purification after dialysis (Fig 1).

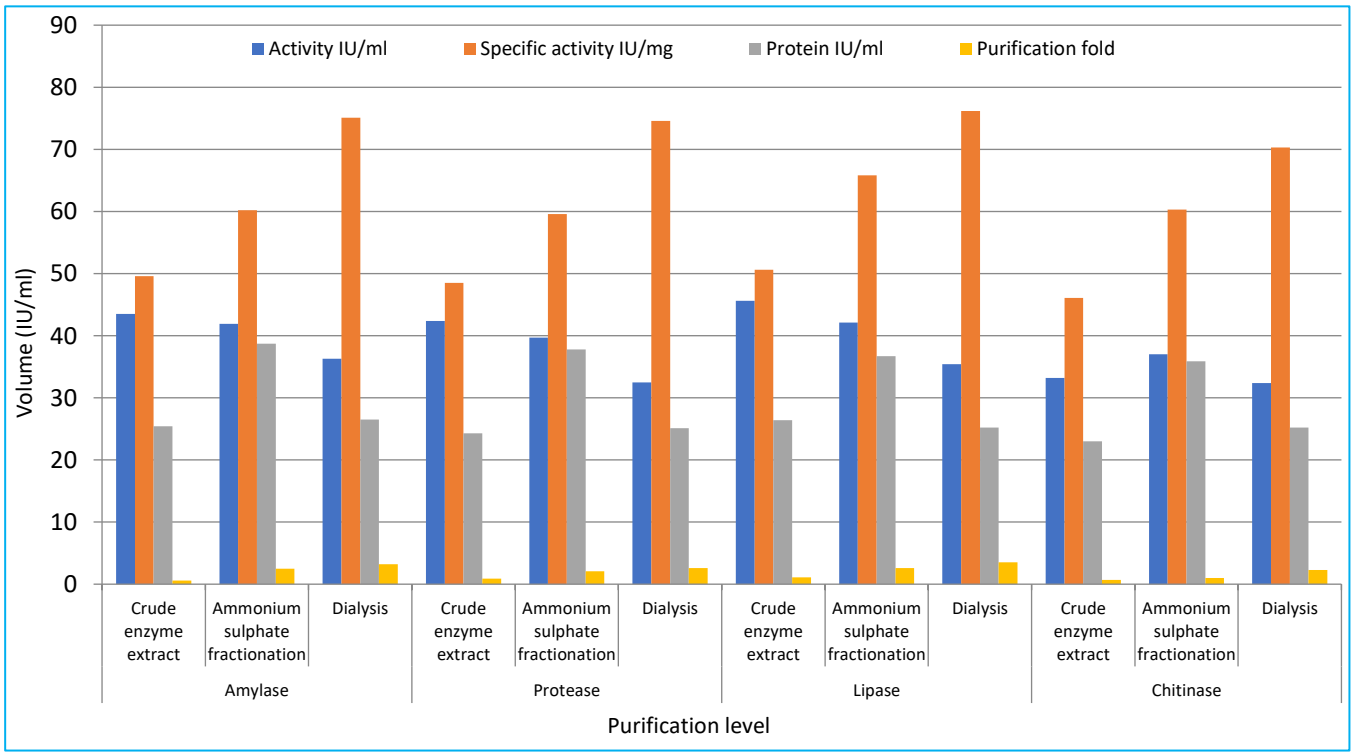

Fig 2 Partial purification of enzymes from *Aspergillus niger*

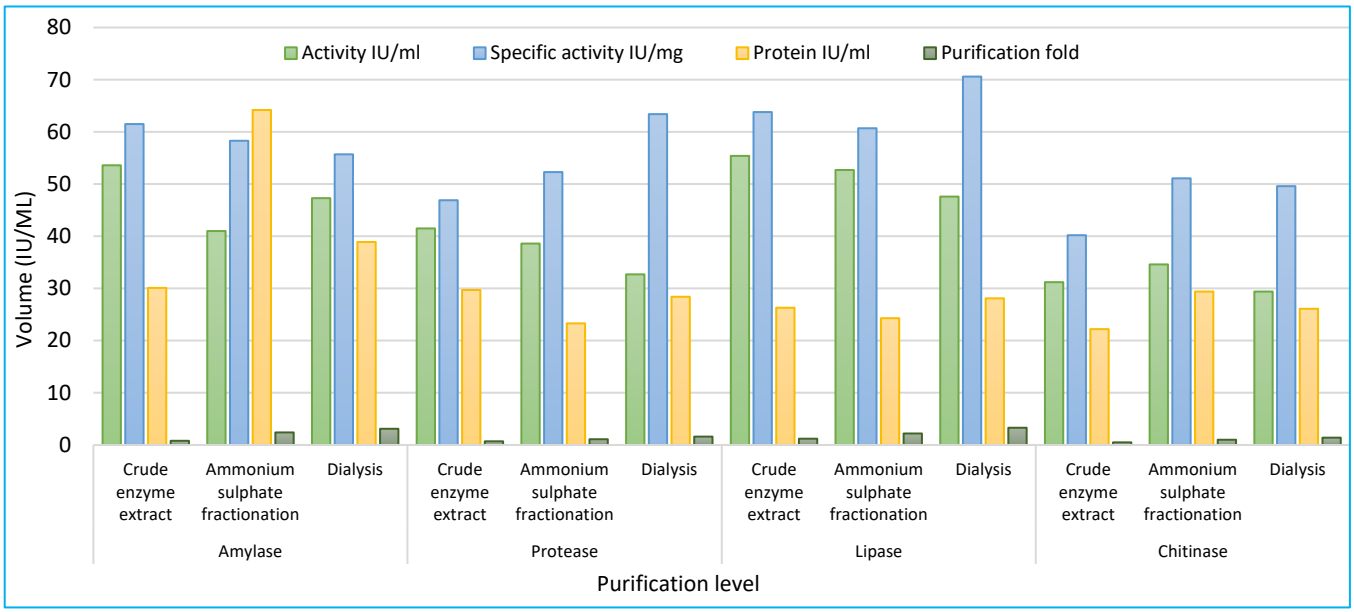

Fig 3 Partial purification of enzymes from *Aspergillus terreus*

Purification of enzymes from Aspergillus terreus

The crude amylase showed that the total activity of 53.6 IU/ml and 61.5 IU/mg specific activity. As increase the specific activity, the purification by ammonium sulphate precipitation and dialysis was found to be total activity of 41.0 IU/ml and 47.3 IU/ml and specific activity of 58.3 IU/mg and 55.7 IU/mg. Then protein content was also higher presented by ammonium sulphate fractionation 64.2 IU/ml. After dialysis, the specific activity of the enzyme increased with 3.1 purification fold (Fig 2). The crude protease exhibited 41.5 IU/ml of total activity and 46.9 IU/mg of specific activity. Purification of ammonium sulphate precipitation and dialysis to increase the specific activity, it was revealed to have total activity of 38.6 IU/ml and 32.7 IU/ml and specific activity of 52.3 IU/mg and 63.4 IU/mg. The ammonium sulphate fractionation 29.7 IU/ml protein content was also increased. The enzymes specific activity increased 1.6 purification fold after dialysis (Fig 2). The crude lipase had a total activity of 55.4 IU/ml and a specific activity of 63.8IU/mg. the total activity of 52.7 IU/ml and 47.6 IU/ml, and specific activity of 60.7 IU/mg and 70.6 IU/mg when it was purified by ammonium sulphate precipitation and dialysis to increase the specific activity. The ammonium sulphate fractionation 28.1 IU/ml protein content was higher. Following dialysis, the enzymes specific activity increased 3.3 fold in terms of purification (Fig 2). The crude chitinase specific activity was 40.2 IU/mg and its total activity was 31.2 IU/ml. When the crude extract was purified using ammonium sulphate precipitation and dialysis to increase the specific activity, it showed total activity of 34.6 IU/ml and 29.4 IU/ml, and also specific activity of 51.1 IU/mg and 49.6 IU/mg. Observing the protein content of the ammonium sulphate fractionation, it was found to be higher at 29.4 IU/ml. The enzymes increased 1.4 fold in respect of purification after dialysis (Fig 2).

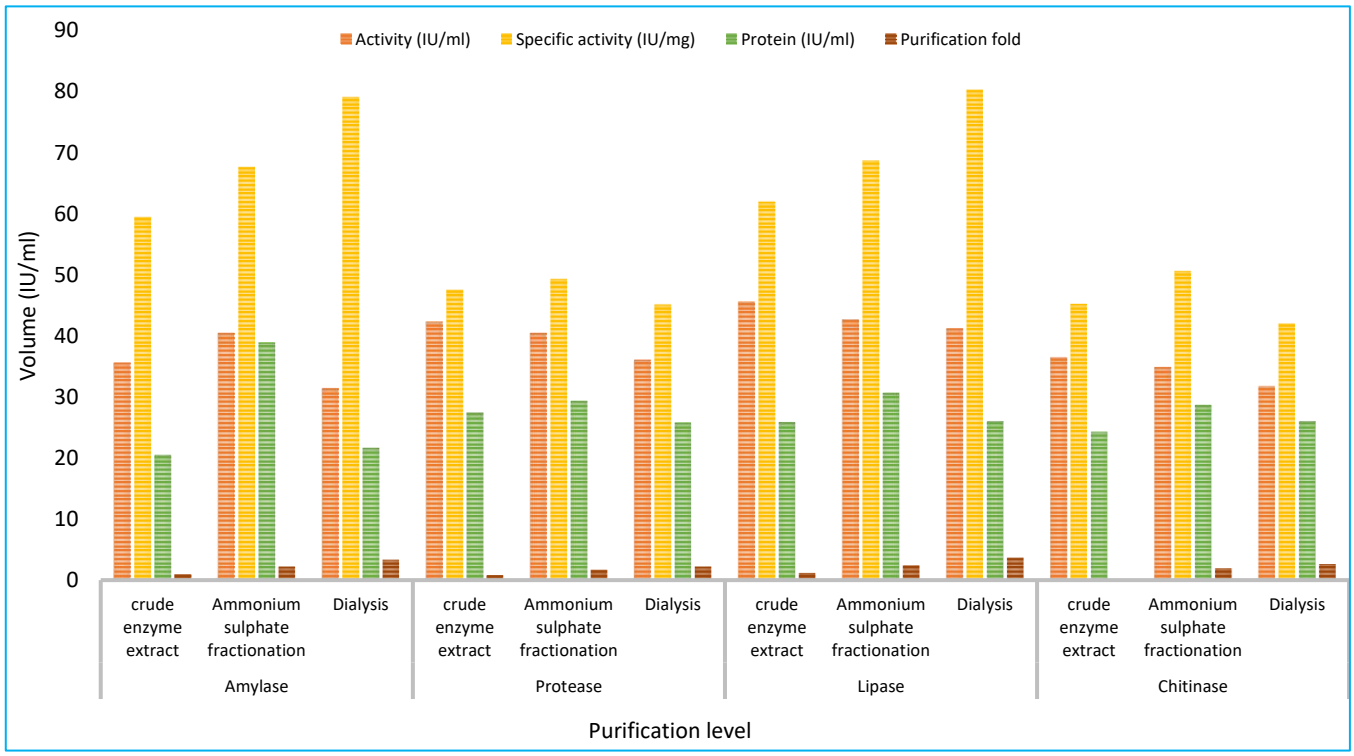

Fig 4 Partial purification of enzymes from *Aspergillus fumigatus*

Purification of enzymes from A. fumigatus

A total activity of 35.6 IU/ml and a specific activity of 59.4 IU/mg were obtained for the crude amylase enzyme. The specific activity increased after purification by ammonium sulphate precipitation and dialysis, with total activities of 40.5 IU/ml and 31.4 IU/ml and specific activities of 67.6 IU/mg and 79.0 IU/mg recorded respectively. Then, the protein content of ammonium sulphate fractionation 38.9 IU/ml was higher. The specific activity of the enzymes increased to 3.3 purification fold after dialysis (Fig 3). The overall activity of crude protease was 42.3 IU/ml, and the specific activity was 47.5 IU/mg. The specific activity was found to be 49.3 IU/mg and 45.1 IU/mg after purification of the ammonium sulphate precipitation and dialysis to increase the total activity. The total activity was 40.5 IU/ml and 36.1 IU/ml. Similarly, the protein content of the ammonium sulphate fractionation was 29.4 IU/ml. After dialysis, the enzymes specific activity increased by 2.2 fold in terms of purification (Fig 3). The crude extracts total activity for lipase enzyme were 45.6 IU/ml and its specific activity was 48.9 IU/mg. When it was purified using ammonium sulphate precipitation and dialysis to increase the specific activity, it had total activities of 42.7 IU/ml and 41.2 IU/ml and 53.6 IU/mg and 60.2 IU/mg of specific activity respectively. The protein level of the ammonium sulphate fractionation was 30.7 IU/ml greater. In terms of purification, the enzymes specific activity increased 3.6 fold after dialysis (Fig 3). The crude specific activity of chitinase enzyme was 45.2IU/mg, and its total activity was 36.5 IU/ml. The crude extract had total activity of 34.9 IU/ml and 31.8 IU/ml in addition to specific activity of 50.6 IU/mg and 42.0 IU/mg when it was purified using ammonium sulphate precipitation and dialysis to increase the specific activity. The ammonium sulphate fractionation's protein content was observed to be higher at 28.7 IU/ml. Following dialysis, the enzymes increased by 2.6 fold in terms of purification (Fig 3).

In most of the time, marine microorganisms are used to produce hydrolytic enzymes including amylase, lipase, protease, chitinase, etc. [51]. The soil is known to be a repository of amylase producers, the occurrence of amylolytic organisms in the soil especially at starch processing sites [52]. The incubation time for achieving the maximal enzyme level was governed by the characteristics of the culture and based on the growth rate and enzyme production [53]. Earlier researchers also looked into how metal ions affected the activity of amylase and discovered that the enzyme didn't must have a particular ion for catalytic activity [54]. Whenever proteins were digested using multiple protease enzymes produced by isolates and sources of protein from skim milk were added to the mixture to determine the generation of protease by strains, the resulting quantity demonstrated greater digestibility and higher activity [55]. In contrast, they are powerful lipase makers of major industrial importance. The manufacturing medium added with oil promoted fungal growth. On the fourth day of submerged fermentation, high levels of fungal mycelium, biomass and maximum crude lipase production were obtained. These results are comparable to those of other research on *Aspergillus* sp. strains [56]. *Aspergillus* species that produce lipase were more frequently found in and isolated from composts made from palm oil mill effluent [57]. *Fusarium solani* was found to produce lipase when using cotton oil as a carbon source while submerged in an aquatic environment where leaves were decomposition [58]. Due to its natural origin, chitin is intrinsically variable and occurs in various forms, each of which has unique properties [59-60].

Using ammonium sulphate precipitation, [61] purified the *Aspergillus flavus* amylase enzyme. In protease enzyme, the crude protease exhibited 42.4 IU/ml of total activity and 48.5 IU/mg of specific activity. The enzyme activity in the other fractions was significant. According to [62], protease isolated from *Bacillus* sp. reaches its maximum activity at a 75–80% ammonium sulphate saturation. The highest total protease activity is also shown by *Penicillium janthinellum* and *Neurospora crassa* protease at a concentration of 70% ammonium sulphate [63]. According to [64], a purified lipase was able to increase its specific activity by 3.93 fold, from 5.29 to 20.8 Umg-1, using a two-step purification method. With further purification, including dialysis, the purification fold increased to 1.75 and the specific activity to 251.13 Uml-1 [65]. Using a starch adsorption method, [66] also purified extracellular amylase from the same species. Similar to this, [67] used ammonium sulphate fractionation to purification a thermostable amylase enzyme and obtain a homogenous product. A protease isolated from *Bacillus cereus* with a purification fold of 3.05 was reported [68]. The protease isolated from *Aspergillus flavus* was reported by [69] with a purification fold of 2.53. The [70] research on partially purified lipase revealed a 4.10-fold increase in activity compared to the corresponding crude extract. At 80% ammonium sulphate saturation, [64] partial purification of the chitinase enzyme from the supernatant showed the best protein recovery with the highest activity (143.68 UmL-1). At 30˚C, amylase had such a specific activity of 3.5 mol/ml. [71] An increase in a specific activity, partial purification rises 3 fold more than crude extract. At 30˚C, amylase used to have a specific activity of 4.16 mol/ml. Ammonium sulphate fractionation was used to separate the protease from *Bacillus* sp. Compared to crude extract, it had a 1.32-fold increase in purification [72]. According to [73], 80% saturation of $(NH4)_2SO_4$ resulted to a 2-fold increase in alkaline protease purification from the initial crude extract. These observations are consistent with the purification techniques used by [74] to precipitate lipase from Pseudomonas sp. G6. Among the various saturations that were examined, found that 60% saturation was an effective concentration with 6.53 fold specific activities. Using colloidal chitin, [75] determined the Km (6.74 mg/ml) and Vmax (61.3 U/mg) of pure chitinases made by a *Streptomyces* sp. According to [76], colloidal chitin Km and Vmax values for the purified chitinase generated by *Streptomyces griseus* were 400 mg and 180 IU mL-1 found to be recorded respectively.

CONCLUSION

The present study was carried out with the screening and partial purification of enzymes at marine soil fungi from Chennai and Thiruvallur district. The marine soil fungi namely *Aspergillus niger, A. flavus, A. terreus, A. candidus, A. awamori, A. fumigatus, Pencillium citrinum, P. longibrachiatum, P. lanosum, Pencillium* sp, *Fusarium* sp, *F. solani, Cunninghamella vitricillate, Neurospora* sp and *N. crassa* was screened for the synthesis of four types of enzymes such as amylase, protease, lipase and chitinase by assay method. The marine soil fungi such as *Aspergillus niger, A. terreus* and *A. fumigatus* were more screened for all enzymes and maximum screening of enzymes were observed amylase enzyme when compared with other enzymes. But in the present investigation, all the enzymes were selected for further purification studies. All the enzymes were subjected for partial purification and carried out by dialysis followed by ammonium sulphate precipitation method and maximum partial purification of lipase enzyme were recorded respectively. When compared with crude enzyme extract and dialysis, ammonium sulphate fractionation revealed that all enzymes and protein are also

present in higher concentrations. After dialysis, the enzymes are exhibited an increase in the specific activity of the enzyme with a purification fold. Nearly 400 common consumer and commercial products are produced and improved using enzymes. They are used to prepare food and beverages, feed animals, make fabrics, clean homes, fuel cars, and create electricity. Chitin is a flexible and promising biopolymer with a wide range of commercial, medical, and industrial applications. The majority of industrial enzymes currently in use are hydrolases, which include proteases and lipases. These enzymes are widely used in the detergent, dairy, and chemical industries. One of the most often employed amylase enzymes in the industry. It hydrolyzes starch and employed in industry to produce sugar syrups made of glucose, maltose, and higher oligosaccharides from starch. It can try to compensate for young animals lack of endogenous amylase, protease, lipase and chitinase are the main enzymes in a commercial sense and they can be produced by a wide range of microorganism genera, including fungal strains of *Aspergillus* sp. Lipases are the favoured enzymes for prospective uses in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries due to their versatility. The food, detergent, and pharmaceutics have mostly used lipases in significant industrial applications. The essential role of lipase is to transform lipids into fatty acids and glycerol which can be transported in water-based fluids like blood and lymph. In biotechnology applications, enzyme lipases function as biocatalysts and have a significant and essential function to perform. Applications of lipases in biotechnology include the generation of biopolymers, biodiesel, pure pharmaceutical compounds, agrochemicals, and flavour compounds. Hence, the screening and partial purification of enzymes are very useful in this world.

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Conflicts of Interest

The authors declare that they have no conflicting interests.

LITERATURE CITED

1. Gayathri R, Shobana A, Sujatha A, Subash A. 2020. Screening and partial purification of protease produced by bacteria isolated from termite soil. *Asian Jr. of Microbial. Biotech. Env. Science* 22(1): 133-139.
2. Karnwal A, Nigam, V. 2013. Production of amylase enzyme by isolated microorganisms and its application. *International Journal of Pharmacy and Biological Sciences* 3(4): 354-460.
3. Veerapagu M, Narayanan SA, Ponmurugan K, Jeya KR. 2013. Screening, selection, identification, production and optimization of bacterial lipase from oil spilled soil. *Asian Journal of Pharmaceutical and Clinical Research* 6(3): 62-67.
4. Chudasama CJ, Parmar SR, Patel HN. 2015. Isolation and characterization of metallo-alkaline serine protease from *Bacillus subtilis* strain CC2. *Journal of Cell and Tissue Research* 15(1): 4913-4919.
5. Singhal P, Nigam VP, Vidyarthi AS. 2012. Studies on production, characterization and applications of microbial alkaline proteases. *International Journal of Advanced Biotechnology and Research* 3(3): 653-669.
6. Vikramathithan M, Dhandapani R. 2014. Isolation and identification of protease producing halophilic bacteria. *International Journal of Advanced Science and Engineering* 1(2): 33-36.
7. Asker MMS, Mahmoud MG, Shebwy KE, Aziz MSA. 2013. Purification and characterization of two thermostable protease fractions from *Bacillus megaterium. Journal of Genetic Engineering and Biotechnology* 11: 103-109.
8. Abu EA, Ado SA, James DB. 2005. Raw starch degrading amylase production by mixed culture of *Aspergillus niger* and *Saccharomyces cerevisae* grown on *Sorghum pomace*. *Afr. Jr. Biotechnology* 4: 785-790.
9. Alva S, Anupama J, Salva J, Chiu YY, Vyshali P, Shruthi M, Yogeetha BS, Bhavya D, Purvi J, Ruchi K, Kumudini BS, Varalakshmi KN. 2007. Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp. JGI 12 in solid state culture. *Afr. Jr. Biotechnology* 6: 576-581.
10. George-Okafor UO, Tasie FO, Anyamene NC. 2013. Studies on low-cost substrates and other cultural conditions for optimal amylase yield from *Aspergillus oryzae*-SR2. *Am. Jr. Food Technology* 8(1): 54-64.
11. Pandey A, Soccol CR, Mitchell D. 2000. New developments in solid-state fermentation: bioprocesses and products. *Proc. Biochemistry* 35: 1153-1169.
12. Abe J, Bergmann FW, Obeta K, Hizukuri S. 1988. Production of the raw starch digesting amylase of *Aspergillus* sp. K-27. *Appl. Microbiol. Biotechnology* 27: 447-450.
13. Dubey R, Adhikary S, Kumar J, Sinha N. 2010. Isolation, Production, purification, assay and characterization of alkaline protease enzyme from *Aspergillus niger* and its compatibility with commercial detergents. *Developmental Microbiology and Molecular Biology* 1(1): 75-94.
14. Sawant R, Nagendran S. 2014. Protease: An enzyme with multiple industrial applications. *World Journal of Pharmacy and Pharmaceutical Sciences* 3(6): 56879.
15. Kumar L, Jain SK. 2017. A review on environmental pollution mitigation by fungal proteases. *Research and Reviews*: *A Journal of Microbiology and Virology* 7(3): 32-37.
16. Souza PM, Bittencourt ML, Caprara CC, Freitas MD, Almeida RP, Silveira D, Fonseca YM, Ferreira Filho EX, Pessoa Junior A, Magalhães PO. 2015. A biotechnology perspective of fungal proteases. *Brazilian Journal of Microbiology* 46(2): 337-346.
17. Paul T, Jana A, Mandal AK, Mandal A, Mohpatra PK, Mondal KC. 2016. Bacterial keratinolytic protease, imminent starter for NextGen leather and detergent industries. *Sustainable Chemistry and Pharmacy* 3: 8-22.
18. Ko WH, Wang IT, Ann PJ. 2005. A simple method for detection of lipolytic microorganisms in soils. *Soil Biology and Biochemistry* 37: 597-599.
19. Cardenas F, Alvarez E, Castro-Alvarez MS, Sanchez-Montero JM, Valmaseda M, Elson SW. 2001. Screening and catalytic activity in organic synthesis of novel fungal and yeast lipases. *Journal of Molecular Catalysis: B, Enzymatic* 14: 111-123.

20. Wang Y, Srivastava KC, Shen GJ, Wang HY. 1995. Thermostable alkaline lipase from a newly isolated thermophilic Bacillus, strain A30-1 (ATCC 53841). *Journal of Fermentation and Bioengineering* 79: 433-438.
21. Ferrer J, Paez G, Marmol Z, Ramones E, Garcia H, Forster CF. 1996. Acid hydrolysis of shrimp shell waste and the production of single cell protein from the hydrolysate. *Bioresource Technology* 57(1): 55-60.
22. Felse PA, Panda T. 2000. Production of microbial chitinases- A revisit. *Bioprocess Engineering* 23(2): 127-134.
23. Prabavathy VR, Mathivanan N, Sagadevan E, Murugesan K, Lalithakumari D. 2006. Intra-strain protoplast fusion enhances carboxymethyl cellulase activity in *Trichoderma reesei. Enzyme Microbial Technology* 38(5): 719-723.
24. Chang WT, Chen YC, Jao CL. 2007. Antifungal activity and enhancement of plant growth by *Bacillus cereus* grown on shellfish chitin wastes. *Bioresource Technology* 98(6): 1224-1230.
25. Rajagopalan G, Krishnan C. 2008. *α*-Amylase production from catabolite derepressed *Bacillus subtilis* KCC103 utilizing sugarcane bagasse hydrolysate. *Bioresource Technology* 99(8): 3044-3050.
26. Reddy NS, Nimmagadda A, Rao KRSS. 2003. An overview of the microbial *α*-amylase family. *African Journal of Biotechnology* 2(12): 645-648.
27. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. 2003. Microbial *α*-amylases: a biotechnological perspective. *Process Biochemistry* 38(11): 1599-1616.
28. Kandra L. 2003. *α*-Amylases of medical and industrial importance. *Journal of Molecular Structure: TheoChem*. 666/667: 487-498.
29. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R. 2000. Advances in microbial amylases. *Biotechnology and Applied Biochemistry* 31(2): 135-152.
30. Carlsen M, Nielsen J. 2001. Influence of carbon source on α-amylase production by *Aspergillus oryzae*. *Appl. Microbiol. Biotechnology* 57(3): 346-349.
31. Bajpai P. 2004. Biological bleaching of chemical pulps. *Crit. Rev. Biotechnology* 24(1): 1-58.
32. Pandey A, Nigam P, Soccol VT, Singh D, Mohan R. 2000. Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 31: 135-152.
33. Kennedy JF, Cabalda VM, White CA. 1988. Enzymatic starch utilization and genetic engineering. *Tibtech* 6: 184-189.
34. Béguin P, Aubert JP. 1994. The biological degradation of cellulose. *FEMS Microbiol. Rev*. 13: 25-58.
35. Borchert TV, Lassen SF, Svedsen A, Frantzen HB. 1995. Oxidation stable amylases for detergent. *In*: (Eds) S. B. Petersen, B. Svensson, and S. Pedersen. Progress in Biotechnology. Carbohydrate Bioengineering. Elsevier, The Netherlands. 10: 175-179.
36. Lange NK. 1993. Application of cellulases in the textile industry. *In*: (Eds) P. Suominen and T. Reinikainen. *Trichoderma reesei* Cellulases and Other Hydrolases. *Foundation for Biotechnical and Industrial Fermentation Research*, Helsinki. pp 263-272.
37. Pommier JC, Goma G, Fuentes JL, Rousset C, Jokinen O. 1990. Using enzymes to improve the process and the product quality in the recycled paper industry. Part 2: Industrial applications. *Tappi. Journal* 73: 197-202.
38. Prasad DY, Heitmann JA, Joyce TW. 1993. Enzymatic deinking of colored offset newsprint. *Nordic Pulp Paper Res. Journal* 8: 284-286.
39. Reddy NS, Nimmagadda A, Rao KRS. 2003. Production and characterization of fungal amylase enzyme isolated from *Aspergillus* sp. JGI 12 in solid state culture. *Afr. Jr. Biotechnology* 2(12): 645-648.
40. Romero MD, Aguado J, Gonz´alez LLM. 1999. Cellulase production by *Neurospora crassa* on wheat straw. *Enzyme Microbial Technology* 25: 244-250.
41. Hutapea NEB, Sibero MT, Ayuningtyas EP, Frederick EH, Wijayanti DP, Sabdono A, Pringgenies D, Radjasa OK, Zilda DS, Murwani R. 2021. Seaweed-associated fungi from Sepanjang Beach, Gunung Kidul, Yogyakarta as potential source of marine polysaccharides-degrading enzymes. IOP Conf. Ser.: *Earth Environ. Sci.* 750 012007.
42. Haile S, Masi C, Tafesse M. 2022. Isolation and characterization of pectinase-producing bacteria (*Serratia marcescens*) from avocado peel waste for juice clarification. *BMC Microbiology* 22: 145.
43. Singh BK, Walker A, Morgan JAW, Wright DJ. 2003. Effect of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos- degrading bacterium. *Applied Environmental Microbiology* 69: 5198-5206.
44. Mandels M, Andreotti R, Roche R. 1976. Measurement of saccharifying cellulase. *Bioteh. Bioeng. Symp*. 6: 17-37.
45. Keay L, Wildi B. 1970. Proteases of the genus *Bacillus*, neutral proteases. *Biotechnology and Bio-Engineering* 12(2): 179-212.
46. Patel BG, Shah RK. 2018. Biodegradation of cotton seed soap stocks by novel indigenous Bacillus species. *Bioscience Biotechnology Research Communications* 11(3): 505-511.
47. Miller GL. 1959. Use of dinitro salicylic acid reagent for determination of reducing sugar *Analytical chemistry* 31: 426-428.
48. Hara T, Lim J Y, Fujio Y, Ueda S. 1984. Purification and some properties of exopolygalacturonase from *Aspergillus niger* cultured in the medium containing *Satsuna mandarin* peel. 4th Conference on Recent Technologies in Agriculture. 31: 581-586.
49. Dam P, Das MP, Rebecca JL, Sharmila S. 2013. Production and optimization of extracellular proteases from *Bacillus sp.* isolated from marine soil. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 4(2): 1-8.
50. Lowry OH, Resebrough NJ, Farr AJ, Randell RJ. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.
51. Parmar FA, Patel JN, Upasani VN. 2021. Screening of microorganisms for production of therapeutic enzymes. *European Journal of Biotechnology and Bioscience* 9(3): 82-88.
52. Omemu AM, Akpan I, Bankole MO, Teniola OD. 2005. Hydrolysis of raw starches by amylase of *Aspergillus niger* AM07 isolated from soil. *Afr. Jr. Biotechnology* 4: 19-25.
53. Kunamneni A. 2005. Amylase production by solid state fermentation by the thermophilic fungus *Thermomyces lanuginosus*. *Jr. Biosci. Bioengineering* 100: 168-171.
54. Nahas E, Waldermarin MM. 2002. Control of amylase production and growth characteristics of *Aspergillus ochraceus*. *Microbiologia* 44: 5-10.

55. Cappuccino JG, Sherman N. 2008. *Microbiology: A laboratory Manual*. (Tenth Edition). Boston: Pearson Education.
56. Cihangir N, Sarikaya E. 2004. Investigation of lipase production by a new isolate of *Aspergillus* sp. *World Journal of Microbiology and Biotechnology* 20(2): 193-197.
57. Nwuche CO, Ogbonna JC. 2011. Isolation of lipase producing fungi from palm oil mill effluent (POME) dump sites at Nsukka. *Brazilian Archives of Biology and Technology* 54(1): 113-116.
58. Mendes DB, Silva FFD, Guarda PM, Almeida AF, de Oliveira DP, Morais PB. 2019. Lipolytic enzymes with hydrolytic and esterification activities produced by filamentous fungi isolated from decomposition leaves in an aquatic environment. *Enzyme Research* 1-13. 2019: 8182425. doi: 10.1155/2019/8182425.
59. Aranaz I, Mengíbar M, Harris R, Paños I, Miralles B, Acosta N, Galed G, Heras Á. 2009. Functional characterization of chitin and chitosan. *Current Chemical Biology* 3: 203-230.
60. Kathleen Van de Velde K, Kiekens P. 2004. Structure analysis and degree of substitution of chitin, chitosan and dibutyrylchitin by FT-IR spectroscopy and solid state 13C NMR. *Carbohydrate Polymers* 58: 409-416.
61. Khoo SL, Amirul AA, Kamaruzaman M, Nazalan M, Azizan MN. 1994. Purification and characterization of alphaamylase from *Aspergillus flavus*. *Folia Microbiology* 39: 392-398.
62. Aqel H. 2012. Phenotypic and protease purification of two different thermophilic *Bacillus* strains HUTBS71 and HUTBS62. *Annals of Biological Research* 3(4): 1747-1756.
63. Abirami V, Meenakshi SA, Kanthymathy K, Bharathidasan R, Mahalingam R, Panneerselvam A. 2011. Partial purification and characterization of an extracellular protease from *Penicillium janthinellum* and *Neurospora crassa. Eur. Jr. Exp. Biology* 1: 114-123.
64. Kareem SO, Adio OQ, Osho MB. 2014. Immobilization of *Aspergillus niger* F7-02 lipase in polysaccharide hydrogel beads of *Irvingia gabonensis* matrix. *Enzyme Research* 2014: 967056. doi: 10.1155/2014/967056.
65. Hungund BS, Tennalli GB, Achappa S, Divate MN. 2022. Production and partial purification of thermostable chitinase from *Bacillus cereus* BSH-4 and its antifungal activity. *Jr. Biochem. Technology* 13(2): 46-53.
66. Abou-Zeid AM. 1997. Production, purification and characterization of an extracellular alpha-amylase enzyme isolated from *Aspergillus flavus*. *Microbios* 89: 55-66.
67. Chakraborty K, Bhattacharyya BK, Sen SK. 2000. Purification and characterization of a thermostable α-amylase from *Bacillus stearothermophilus*. *Folia Microbiology* 45: 207-210.
68. Umayaparvathi S, Meenakhi S, Arumugam M, Balasubramanian T. 2013. Purification and characterization of protease from *Bacillus cereus* SU12 from oyster *Saccostrea cucullate*. *African Journal of Biotechnology* 12(40): 5897-5908.
69. Muthulakshmi C, Gomathi D, Kumar DG, Ravikumar G, Kalaiselvi M, Uma C. 2011. Production, purification and characterization of protease by *Aspergillus flavus* under solid state fermentation. *Jordan Journal of Biological Sciences* 4(3): 137-148.
70. Shafei MS, Allam RF. 2010. Production and immobilization of partially purified lipase from *Penicillium chrysogenum*. *Malaysian Journal of Microbiology* 6(2): 196-202.
71. Sanghvi, Gaurav V, Koyani RD, Rajput KS. 2011. Isolation, optimization and partial purification of amylase from *Chrysosporium asperatum* by submerged fermentation. *Jr. Microbial. Biotechnology* 21(5): 470-476.
72. Josephine FS, Ramya VS, Devi N, Ganapa SB, Siddalingeshwara KG, Venugopla N, Vishwanatha T. 2012, Isolation, production and characterization of protease from *Bacillus sp* isolated from soil sample. *Journal of Microbiology and Biotechnology Research* 2(1): 163-168.
73. Kumar L, Jain SK. 2017. A review on environmental pollution mitigation by fungal proteases. *Research and Reviews*: *A Journal of Microbiology and Virology* 7(3): 32-37.
74. Kanwar L, Goswami P. 2002. Isolation of a *Pseudomonas lipase* produced in pure hydrocarbon substrate and its applications in the synthesis of isoamyl acetate using membrane-immobilized lipase. *Enzyme Microb. Technology* 31: 727-735.
75. Karthik N, Binod P, Pandey A. 2015. Purification and characterisation of an acidic and antifungal chitinase produced by a *Streptomyces sp*. *Bioresource Technology* 88: 195-201.
76. Rabeeth M, Anitha AB, Srikanth GC. 2011. Purification of an antifungal endochitinase from a potential biocontrol agent *Streptomyces griseus*. *Pak. Jr. Biol. Science* 14: 788-797.