ISOLATION AND SCREENING OF AMYLASE AND PROTEASE PRODUCING BACTERIA FROM MARINE SEDIMENT SAMPLES

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Abstract
Marine sediments are attracting attention for exploring industrially useful enzymes**.** Marine microorganisms are a valuable source of novel enzymes with ideal characteristics because of the halophilic nature of the marine bacteria. Microbial enzymes are gaining more importance in the modern industrial world which can degrade complex compounds into simpler substances, and play an important role in industries. The aim of the present study is the isolation and screening of the marine bacterial strains which produce industrially important enzymes like amylase and protease. Marine sediment samples were collected and a total of seven bacterial strains were isolated and two of these five strains were showed to be amylase producers. Among the six isolates, only one isolate showed maximum proteolytic activity with a clear zone in skim milk agar. Whereas the other five isolates exhibited poor proteolytic activity . Morphological and biochemical characterestics of amylase and protease producing organisms were done. The study concludes that marine bacteria can be a source of amylase and protease for industrial purposes.

Key words-Marine Sediment, Amylase, Protease, Screening, Starch agar, Skim Milk Agar

I.INTRODUCTION

Marine ecosystems are rich source of both chemical and biological diversity. The actual diversity is still unknown to us from relatively small number of microbes, almost 12,000 novel chemicals are isolated [1]. Hence the potential for the diversity of novel molecules from yet-to-be discovered marine organisms is very high.Marine ecosystem is an immense source of novel enzyme [2-4].

Marine microorganisms, including bacteria and viruses, constitute about 70% of the total biomass. Marine organisms represent around 50% of the worldwide biodiversity in addition to their chemical and genetic diversities, they represent a potential source of broad spectrums of commercially valuable and diverse product, such as polysaccharides, enzymes, peptides, lipids, steroids and terpenoids [5-6].Microorganisms are the most abundant organism in the oceans. Marine microorganisms are increasingly being studied and becoming a hot spot in the search for industrially important biomolecules. It is estimated that the biological diversity in marine ecosystem is much higher than in the tropical rain forests [7].

Marine enzymes are produced from many organisms including plants and animal with potential industrial applications [8].Marine enzymes have great biotechnological and industrial applications in areas such as pharmaceuticals, foods, textile, beverage product, agricultural, chemical and biomedical sectors [9-10]. Marine microbial enzymes have wide applications in bioindustries. Microbial enzymes have a great number of usage in food, pharmaceutical, textile, paper, leather and other industries [11].

Amylase is one among such enzymes that are vital in the field of biotechnology. Apparently, the primary enzyme produced industrially was amylase from fungal basis in 1984. It was used as a pharmaceutical acid for the treatment of digestive disorders [12]. Amylase are a category of enzymes that used widely in industrial process and represent approximately 25 -30% of the global market of enzymes. There have been great advances in the use of amylases in industrial sectors as well. A wide range of industries such as food industries, textiles and beverages industries along with medicinal and clinical chemistry are amylase to manufacture their products [13].

Proteases are one of the largest group of industrial enzymes that catalyze the hydrolytic reactions by cleaving peptide bonds in protein. Proteases may be classified as two major groups; exopeptidase and endopeptidase based on their ability to degrade N-or C-terminal peptide bond. Endopeptidases, which have more potent industrial applications than exo-peptidases and can be divided into four types (aspartic, cysteine, metallo and serine protease) on the basis of their active site and sensitivity to various inhibitors [14].Protease

have been used in processing of various foods such as calf rennet or chymosin in cheese making, in which chymosin hydrolyze the specific peptide bond (the Phe-15-Met 106 bond) to generate para-k-casein and macro peptides [15]. The commercial applications of these enzymes are very wide, including their usage in detergent, leather, food & pharmaceutical industries. In pharmaceutical industry, they offer a gentle and debridment, supporting the natural healing process in the successfully local management of skin ulcerations by the efficient removal of necrotic material. The commercial applications of these enzymes are very wide, including their usage in detergent, leather, food & pharmaceutical industries [16].

For the above reason, marine sediment samples are chosen for the emergent studies and production of novel enzymes amylase and protease. Marine source of microbes are entirely different from other resources. In the present study we aimed to isolate novel source of amylase and protease from the marine sediment samples. The sediment samples were collected at 30 feet from sea coast and have high salt concentration than coastal area. Hence the present study is carried out to screen and produce amylase and protease enzyme by venturing into marine ecosystem and their industrial application being attracted with great attention. After screening morphological and biochemical characteristics were determined.

II. MATERIALS AND METHODS

2.1.Sample collection

Marine sediment samples were collected from Kovilthottam, Chavara located in Kollam district, Kerala. The place were located at 6 km distance from Neendakara fishing harbour area and at 1 km distance from KMML chemical factory. The sediment samples were collected in sterile bottles and brought to the laboratory, stored in room temperature at 37 ^{0} C for further analysis.

2.2.Isolation of marine bacteria

The collected marine sediment samples (1 ml) were serially diluted up to 10^{-9} with distilled water. Isolation of microbes were done by pour plate method [17-18]. The dilutions taken were 10^{-3}, 10^{-5}, 10^{-7} and 10^{-9}. The medium used for the growth of bacterial culture was zobell marine agar medium. The media was sterilized by autoclaving at 121 °C (15 lbs pressure) for 15 minutes. The plates were incubated at 37 °C for 24 hrs. Isolated bacterial strains were streaked in zobell marine agar slant.

2.3 Screening for amylase producing bacteria

All isolated bacteria were tested for amylase production by starch hydrolysis. The pure culture colonies were picked up from each slant and streaked on starch agar plates. The plates were incubated at 37º C for 24 hours. After incubation plates were flooded with iodine solution (0.5%). Amylase positive strains were determined by the presence of a clear zone of starch hydrolysis around the colony on the starch plates [19]. Colonies having a clear zone around them were selected for further investigation.

2.4 Screening for protease producing bacteria

The isolated bacterial strains were screened for the presence of protease enzyme .For screening , bacterial culture were streaked on skim milk agar plates .The plates are incubated at 37 ^{0} C for 5 days. Positive cultures were selected by observing the clearing zone around the growth [20].

2.5 .Morphological and biochemical characterestics

Gram staining, Motility, Indole production, Methyl red, Vogues-proskauers's, Citrate utilization, Catalase, Oxidase, Nitrate reduction, Urease, Starch hydrolysis, Casein hydrolysis were carried out. The potential bacterial strains was biochemically identified using Bergey's Manual of Determinative Bacteriology [21].

III. RESULTS AND DISCUSSION

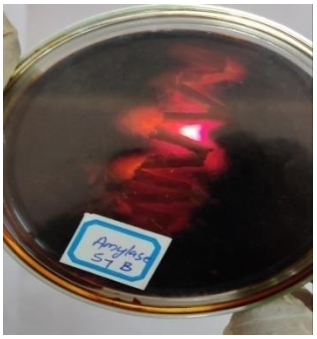
3.1 Isolation and screening of amylase producing microorganisms

In the present study, about seven bacterial strains were isolated from marine samples. From this, five isolates were screened for amylase producing ability on starch agar plate method. The bacterial strains were named AM S7B, AM S1A, AM S18, AM S1B, AM S4A .Out of five isolates, two bacterial strains (AMS7B and AMS1A) showed positive result with the zone of clearance and three showed negative results (Table 1). Therefore the efficient amylase producing isolates, AM S7B and AMS1A were selected for further experimental studies and biochemical test (Fig 1).

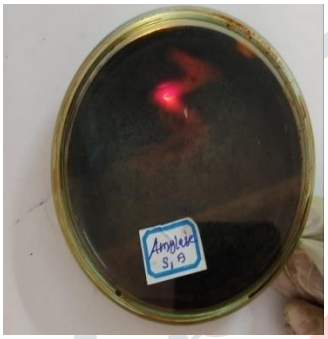
Table 1 : Amylase activity of various strains

SL No	Bacterial strain	Amylase activity(Qualitative)
1	AM S7B	Positive
2	AM S1A	Positive
3	AM S18	Negaitive
4	AM S1B	Negative
5	AM S4A	Negative

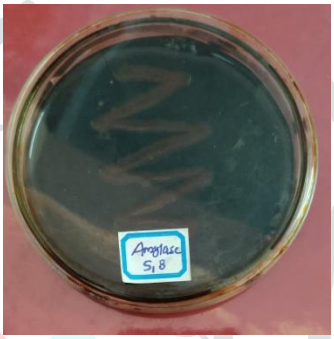
Fig 1: Screening of microorganisms for amylolytic activity (iodine test)



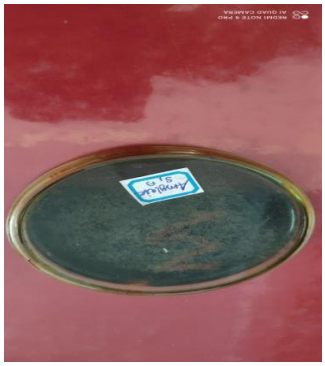
AMS7B showing positive result



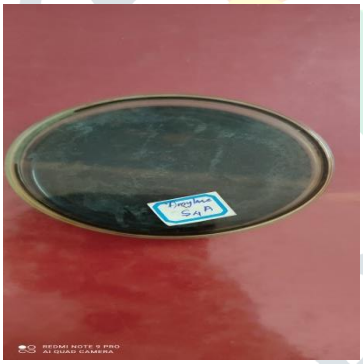
AMS1A showing positive result



AMS18 showing negative result



AMS1B showing negative result



AMS4A showing negative result

3.2 Isolation and screening of protease producing microorganisms

About seven bacterial strains were isolated from marine samples. From this, six isolates were screened for protease producing ability on skim milk agar. The zone formation around the bacterial growth was identified as the positive protease producers which may be due to hydrolysis of casein. Among the six isolates (PR S1C, PR S8A, PR S7C, PR S4A, PR S1B, PR S1A), only one isolate showed maximum proteolytic activity with a clear zone . Whereas the other five isolates exhibited poor proteolytic activity (Table:2).Therefore the efficient protease producing isolates PRS1C were selected for further experimental studies .(Fig 2)

Table 2: Protease activities of various strains

SL No.	Bacterial strains	Protease activity (Qualitative)
1	PR S1C	Positive
2	PR S8A	Negative
3	PR S7C	Negative
4	PR S4A	Negative
5	PR S1B	Negative
6	PR S1A	Negative

Fig 2 Screening of microorganisms for proteolytic activity in skim milk agar

PR S1C showing positive result (Casein hydrolysis)

PR S7C showing negative result

PR S8 A Showing negative result

PE S1B, PE S1A, PE S4A showing negative result

3.3 Morphological and Biochemical characteristics of amylase producing bacterial strain

Bacteria were characterized with reference to Bergy's Manual of determinative bacteriology were depicted in Table 3. According to this, morphological and biochemical characteristics of amylase producing isolates, AM S7B and AM S1A were identified.

Table 3 : Morphological and Biochemical characteristics of amylase producing isolates

Morphological characterestics	Bacterial strain	
	AM S7B	AM S1A
Gram's staining	-	-
Morphology	Rod	Rod
Motility	-	-
	Biochemical test	
Indole(I)	Posiive	Positive
Methyl red(MR)	Positive	Positive
Vogues Proskauer's (VP)	Positive	Positive
Citrate utilization	Positive	Negative
Nitrate reduction	Positive	Positive
Urease test	Positive	Positive
Oxidase	Negative	Negative
Catalase	Negative	Negative

3.4 Morphological and Biochemical characteristics of protease producing bacterial strain

Morphological and biochemical characterestics of the selected strain PRS1C were characterized with reference to Bergey's Manual of systematic bacteriology were depicted in Table 4. According to this morphological and biochemical characteristics of protease producing bacterial isolate, PR S1C were identified.

Table 4: Morphological and Biochemical characteristics of protease producing isolates

Morphological characteristics	Bacterial strain
	PR S1C
Grams staining	-
Morphology	Rod
Motility	-
Biochemical Test	
Indole (I)	Positive
Methyl red (MR)	Positive
Vogues Proskauer's(VP)	Positive
Citrate utilization	Positive
Nitrate reduction	Positive
Urea hydrolysis	Positive
Catalase	Negative
Oxidase	Negative
Casein hydrolysis	Positive

Marine sediment samples that produces industrially useful enzymes amylase and protease. Marine organisms that provide biotechnological applications in enzyme industry and pharmaceutical sector. Researchers explore and exploit the marine reservoirs [22]. The location of the marine sediment samples collected for the study showed the significance of the industrial area which is nearer to KMML factory and also Neendakara harbour. So the research findings provide an insight in to ecology of microbes in the sediment samples. The study also reveals the potential of microbes producing industrially useful enzymes from the marine sediment samples.

IV. CONCLUSION

In this study, we isolated and screened the amylase and protease producing bacterial strains from marine sediment samples using starch agar and skim milk medium. Morphological and biochemical characterstics of the isolates were also carried out. This preliminary screening of amylase and protease producing bacterial isolates from marine sediments revealed the biotechnological potential of marine sediments. Isolation of bacterial strains from marine sediment samples would also provide extensive scope to asess their biotechnological potential.

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