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Isolation and Screening of Alkaline Protease Producing Bacteria from the Kavi-Kamboi, Jambusar, Gujarat

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ABSTRACT
A Protease is an enzyme that catalyses proteolysis, which is the breakdown of proteins in to smaller polypeptides or amino acids, allowing new protein products to develop. The most common application of alkaline protease is as a detergent component. They are also used in the leather industry, medical diagnostics, silver recovery from X- rays and so on. The samples were collected from Arabian seawater (Kavi-Kamboi village, Jambusar, Gujarat, India). The protease producing microorganisms were isolated using skim milk agar as substrate. It was revealed from the enzyme assay that the crude enzyme has 56 U/mL enzyme activity. The morphological studies showed that the isolate is Gram positive Bacillus. The biochemical studies unfolded various biochemical characters of the isolate. The bacterial growth optimization studies showed that bacteria possess optimum growth pH 10 and temperature 37°C. This study may provide an advantage of using the alkaline protease enzyme for commercial purpose in future.
Keywords: Protease, Skim milk agar, Casein, Bacterial screening.

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INTRODUCTION

Hydrolysates are enzymes that catalyze the destruction of peptide bonds in proteins [1]. This proteolytic enzyme is one of the most significant classes of enzymes used in industry, with use in a range of detergent-related activities, food, leather, and silk has expanded significantly in recent years [2]. Microbes may produce the protease in large amounts in a short period of time and are very affordable [3]. Bacteria, molds, and yeasts are among the 40 percent who produces today's enzymes. Using cultivating methods, in a short period of time, by aggregating, microbes may also be utilized to provide an adequate and consistent supply of the desired product, such as proteases. Recently, a lot of attention has been paid to the enzymes manufactured by microorganisms that are highly halophilic. They have great activity in high-salt environments, unlike other enzymes that are inhibited by high-salt environments, and might thus be employed in even more harsh industrial procedures [4]. Alkaliphiles are organisms that thrive at alkaline pH levels, with optimal growth developing potential at pH levels greater than pH 8, and many at pH rates more than 11[1]. Bacillus is a major source of industrial alkaline proteases, and it is likely the only genus being commercial for alkaline protease synthesis. Bacteria are extensively spread in soil and water, and some strains can tolerate harsh environmental conditions, including extreme alkalinity [5]. Proteases are divided into four categories: serine, cysteine, aspartic, and metalloproteases [6]. (A)Serine protease: serine protease is a protease subtype, serine alkaline a broad range of organisms, molds, yeasts, and fungus manufacture proteases. They hydrolyze peptide bonds with the carboxyl side of the breaking bond containing tyrosine, phenylalanine, or leucine. Alkaline proteases have an optimal pH of about 10 and9. Their average molecular weight range from 15 to 30 kDa.(B)Cysteine protease: every cysteine protease relies on catalytic pair of cysteine and histidine for its activity. Cysteine proteases activate only when reducing chemicals like hcn or cysteine are present. (C) Aspartic protease: aspartic acid proteases or acidic proteases are endopeptidases that catalyze their catalytic activity using aspartic acid residues. Most aspartic proteases have iso-electric points in the pH range of 3 to 4.5, with maximum low-pH activity (pH 3 to 4). Its molecular weights extend from 30 to 45 kilodalton. (D) Metalloprotease: the most common metalloproteases diversified of the protease catalytic types. They really are distinguished via means of

fact that they require a divalent metal ion to function. Collagenases by higher organisms, hemorrhagic toxins via snake venoms, and bacterial thermolysin, among others, are among them [7]. The alkaline protease-producing bacteria were identified in this research from kavi-kamboi, Jambusar, Gujarat.The isolate was morphologically and biochemically characterized. Also, the growth medium optimization was done to check the optimal pH and temperature of bacterium. The wild type isolate was having a good efficiency of producing the enzyme which may be used commercially in future for the production of protease.

MATERIAL AND METHODS

Collection of sample

The water samples was collected from Kavi-kamboi, near jambusar, Gujarat, India. The geographical coordinates are 22.1983° n and 72.6373° e.

Isolation and screening of protease producing bacteria

The bacterial culture was inoculated in a skim milk agar medium and then incubated at 37°C for 24 to 48 hours. The protease-producing bacteria were isolated on the basis of the formation of a hydrolytic zone around the colony on the skim milk agar medium [8].

Morphological characterization

For the study of morphological features of bacteria, Gram staining was performed using 24 hr old bacterial culture [9]

Biochemical characterization

To expose the biochemical characteristic features of the bacteria several biochemical tests were performed such as catalase test, oxidase test, methyl red test, Voges-Proskauer test, indole test, starch hydrolysis,Triple sugar iron test, Carbohydrate fermentation test as described in Bergey's manual of systematic bacteriology[10],[11].

Enzyme Assay

1 mL crude enzyme was combined with 1 mL of 100 mM Tris–HCl buffer (pH 9.0) containing 1% (w/v) casein and incubated at 37°C for 30 minutes. After injecting 5 mL of trichloroacetic acid (5 percent TCA) and sodium carbonate into the mixture, it was allowed to rest at room temperature for 15 minutes to terminate the activity. After that, add the folin reagent and wait 30 minutes. Separation at 5,000 rpm for 15 minutes separated the precipitate. Finally, at 660 nm, the effluent wereuv spectrophotometer analysed. The amount of enzyme necessary to liberate 1g of tyrosine inside one minute was defined as one grade of enzymatic activity.

The total protein concentration was identified using the Bradford's method.

Effect of pH on bacterial growth

The purpose of this experiment was to see how pH affects bacteria growth, the nutrient medium containing casein was mixed with buffer of various pH in the range of 5 to 11. To set the pH of medium the buffers such as sodium acetate, Tris HCl and Glycine -NaOH, Sodium carbonate were used. The pure culture of isolate was inoculated in the medium followed by the incubation at 37 for 24h. Later, the growth of bacteria was measured using UV-visible spec at 600nm.

Effect of Temperature on bacterial growth

The purpose of this experiment was to see how temperature affects bacteria growth, the nutrient medium containing casein and pH 10 was inoculated with the pure colony of bacterial isolate followed by the incubation at the temperature 25°C, 37°C and 50°C for 24h. Later, the growth of bacteria was measured using UV-visible spec at 600nm.

RESULTS AND DISCUSSION

Isolation and screening of protease producing bacteria

Protease-producing bacteria were isolated from saltwater sample on skim milk agar medium containing pH 10. The bacteria were screened based on their ability of production of hydrolytic zone on skim milk agar plate. It was observed that out of 6 colonies 2 produced a halo zone of 1.733 cm and 1.633cm respectively. The colony that produced the largest halo zone was selected for further studies (Figure 1).

Enzyme Activity

The quantitative assay was carried out to check the quantity of enzymes in the crude supernatant. The quantitative assay of enzyme activity revealed that the enzyme possesses56 U/mL enzyme activity. The total protein concentration in the sample was found to be 175.83 mg/mL.

Morphological characterization

To examine the morphological properties of a bacterial isolate, the Gram staining was performed and the bacteria showed a rod shape with, purple color when observed under the microscope which provides the information that the bacteria may belong to the *Bacillus* genus (Figure 2).

Figure 1. Bacterial isolation

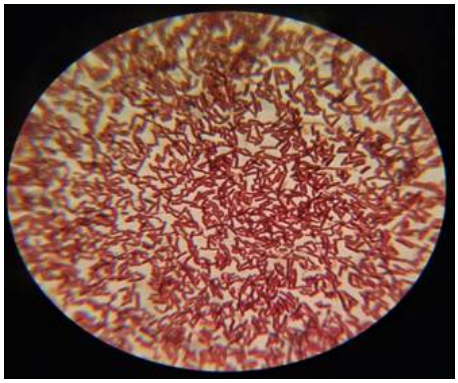

Figure 2. Gram staining

Test	Results
Morphological characteristic	
Colour	Creamy white
Shape	Rod
Surface	Smooth
Opacity	Opaque
Motility	Motile
Gram staining	Positive
Endospore	Positive
Biochemical characteristic	
Catalase test	Positive
Oxidase test	Negative
Methyl res test	Negative
Indole test	Negative
Voges-proskauer test	Positive
Starch hydrolysis test	Positive
Triple sugar ion test	Positive
Citrate utilization test	Negative
Sucrose	Positive
Lactose	Negative
Mannitol	Negative
Similarity of bacteria	*Bacillus sp.*

Table1. Morphological and Biochemical characterization of protease producing bacteria

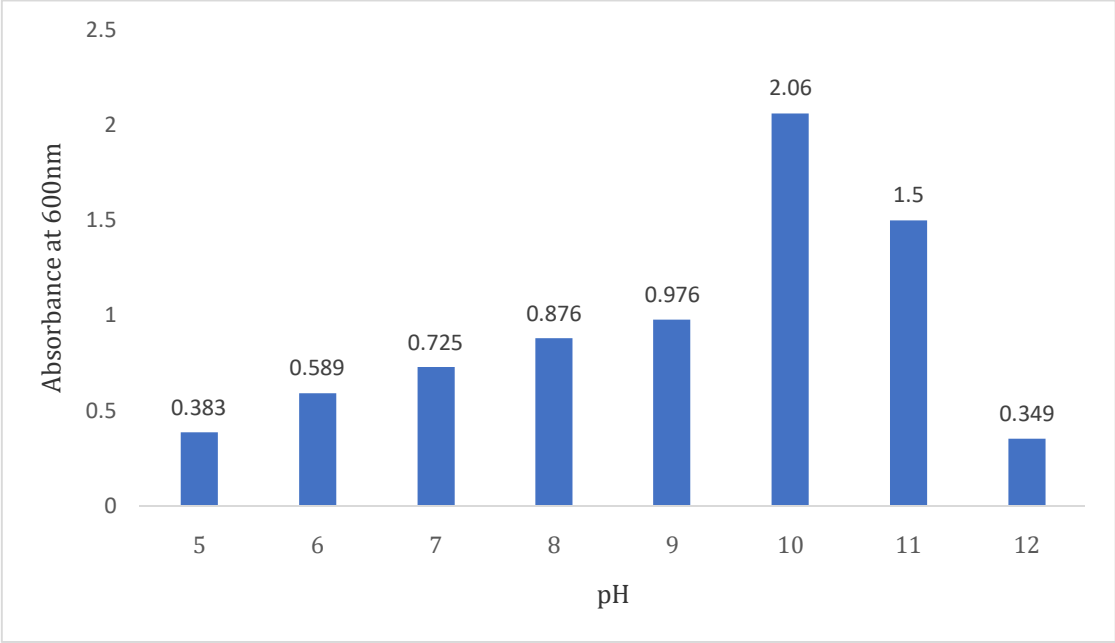

Figure 3.Effect of pH on the growth of bacteria

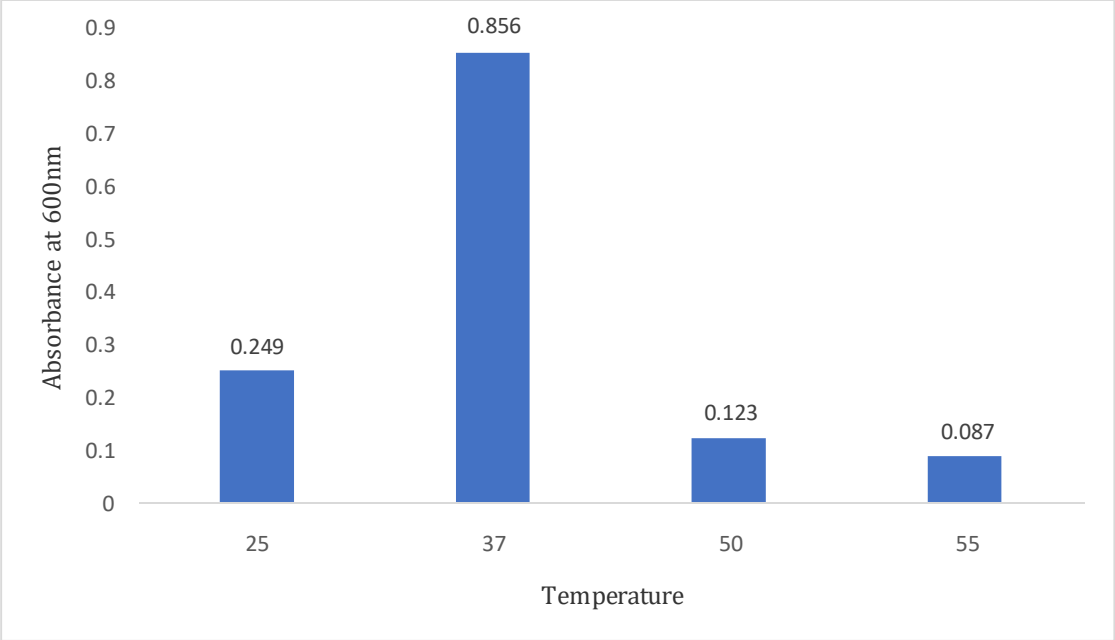

Figure 4. Effect of temperature on the growth of bacteria

Biochemical characterization

To expose the biochemical properties of bacteria several biochemical tests were performed such as catalase test, oxidase test, methyl red test, Voges-Proskauer test, indole test, starch hydrolysis, Triple sugar iron test, Carbohydrate fermentation test. The isolate showed a positive result for the Voges-Proskauer test and catalase test, also hydrolysis of starch was observed in the starch agar plate. The isolate fermented sucrose in a carbohydrate fermentation test(shown in Table 1).

Effect of pH on bacterial growth

The isolate was inoculated in a medium with a pH range of 5 to 12 to observe the effect of pH on bacterial growth. As shown in Figure 3, In the medium with a pH of 10, the isolate growth was maximum. The bacterial growth accelerated with increasing pH, reaching at pH 10 with the largest mass and later it starts declining. As a result, it can be concluded that the pH 10 was optimal for the growth of the isolate, but it can also thrive in the pH range of 6 to 11(Figure 3).

Effect of temperature on bacterial growth

The isolate was inoculated in a medium with a pH 10 at temperatures ranging from 25°C to 55°C to see how temperature affected bacterial growth. As shown in Figure 4, when the isolate was incubated at 37°C, it grew the fastest. The bacterial growth rate increased when the temperature was raised, and it reached its maximum at 37°C. Later, it began to decline. As a result, it can be concluded that the temperature of 37°C was optimal for the growth of the isolate, and it can also thrive in the temperature range of 25°C to 55°C(Figure 4).

CONCLUSION

Alkaliphilic amylase is very stable as compared to neutrophilic protease. The crude enzyme activity of the alkaliphilic protease generated by the bacteria isolated from Kavi Kamboi is 56 U/mL. The isolate's optimal pH and temperature are 10 and 37°C, respectively, according to the growth optimization data. As a result, the protease isolated from a sample of seawater may be employed in commercial manufacturing.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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