ISOLATION AND CHARACTERIZATION OF AMYLASE PRODUCING BACTERIA FROM SOIL SAMPLES

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1. Abstract:
Amylase is a digestive enzyme that is mostly released by the pancreas and salivary glands and is also present in very minute amounts in other tissues. The primary job of amylases is to hydrolyze the glycosidic linkages in molecules of starch, turning them to simple sugars. One of the most common enzymes utilised in industry is amylase. Due to the wide variety of biochemical processes found in microbes, they are the most favoured source of enzymes. The current study concentrated on isolating amylase producers from soil samples from sugarcane and areca plants. Out of the 10 isolates screened for amylolytic activity using the starch-agar plate method, 2 isolates provided a satisfactory clearance zone surrounding the colonies. Gram staining revealed that isolate 1 was gram negative, rod shaped. While isolate 2 was gram positive, rod shaped. Biochemical methods like indole, nitrate, methyl red and catalase were performed to characterize the isolates. Optimum amylase production was monitored by optimum temperature and optimum pH.

Keywords: Amylase, enzymes, catalase and Gram staining

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2. Introduction:

Enzymes are proteins made by living cells that function as catalysts for chemical reactions in biological systems. [1]. Enzymes are highly specialised molecules because each kind of enzyme has a unique binding site for the appropriate substrate. They are employed in a variety of fields, including those related to food production, fermentation, animal nutrition, detergents, cosmetics, brewing, textile to paper industries, pharmaceutical (medicine), and research and development. [2].

As one of the earliest enzymes to be formally researched, amylase was found in the early 1800s. Diastase was its original name before being changed to amylase in the early 20th century. [3]. Amylase is a digestive enzyme produced mostly by the pancreas and salivary glands, with minor amounts detected in other tissues[4].

Anselme Payen discovered the first amylase in 1833. Amylases have specialised substrates and are broadly dispersed in living systems. Amylase substrates are readily available from low-cost plant sources, making the enzyme's prospective applications more cost-effective. In comparison to plants and animals, microbes such as bacteria and fungi create the most amylase [3]. Fungi such as *Aspergillusniger*, *Aspergillusoryzea*, *Thermomyc-eslanuginosus* and *Penicilliumexpansum* are known to synthesis amylase. Bacillus spp. is a well-known bacterial species as an amylase manufacturer [6].

Microorganisms are utilised in industrial production because of their cost-effectiveness, uniformity, and reduced time and space requirements [7,8].Amylase, commonly known as 'glycoside hydrolases,' is an enzyme that breaks down carbohydrate molecules into smaller products[9]. Their main source of nutrition is starch, which is divided into smaller parts like as dextrin, maltose, maltotriose, and glucose [10].

The three types of amylase enzymes—alpha, beta, and gamma—each work on a different part of the carbohydrate molecule. Alpha-amylase is a substance found in humans, animals, plants, and microbes. Beta-amylase can be found in both microbes and plants. Gamma-amylase is present in both plants and animals [11].

Alpha-amylase ranks first among extracellular enzymes in terms of the commercial spectrum of uses, which has expanded to include clinical, pharmaceutical, and analytical chemistry [12].

Starch is a carbon source and the primary substrate of alpha-amylase, which is made up of two components: amylose (25–30%) and amylopectin (70–75%)[13,14]. Amylose is made up of glucose monomers connected by (1-4) glycosidic bands, and its molecular weight ranges from 1 105 to 1 106 Da. The other polymer is amylopectin, which is polymerized by (1-4) glycosidic bands and branched by (1-6) glycosidic bands, with a molecular weight of around 1107-1109 Da [15]. Fungi can be a good source for alpha-amylase production in the industry since their extracellular secretion of alpha-amylase is easily isolatable from the microbial growth medium [16].

Beta-amylase (EC 3.2.1.2) is an exo-enzyme with a high selectivity for cleaving the non-reducing end of starch's -1,4-glucosidic linkage, releasing maltose [17,18]. Beta-amylase belongs to the glycoside hydrolase family 14 [19] and is widely utilised in starch processing, especially in the production of maltose syrup and brewing [20]. Although microbial beta-amylase (20–40%) is structurally similar to plant beta-amylase, it has a number of benefits over the latter. First, there are no seasonal or climatic effects on the production process, simple downstream processes, and more consistent and reliable results. [21].

The exo-acting amylases known as glucoseamylases, also referred to as amyloglu-cosidases (1,4-D-glucan glucanohy- drolase; EC 3.2.1.3), produce glucose from the non-reducing end of starch and oligosaccharides. Glucoa-mylases that are offered for sale are used to convert malto-oligosaccharides into glucose. [22]. Many microorganisms have been discovered and selected as sources of amylase production due to their availability, high growth rates that result in short fermentation cycles, simplicity, ability to release proteins into an extracellular medium, maximum yield, and general handling safety. [23,24].

The use of amylase is allegedly rising annually. With almost 25% of the global enzyme market, the amylase category of enzymes is quite significant in the industrial sector. [25].Microbial amylases have been shown to be a viable alternative to chemical hydrolysis, and low enzyme yield has long been an issue in commercial amylase production [26].Bacillus is a common bacterial source for amylase production in industry.

Varied strains, on the other hand, have different optimal growth requirements and enzyme profiles. Bacillus strains such as *B. amyloliquefaciens*, *B. subtilis*, *B. licheniformis*, *B. stear othermophilus*,

B. megaterium, and *B. circulans* have reportedly been widely used in industry to produce alpha-amylase [27-31].

In addition to being used in the production of alcohol, detergents, bread, glucose and fructose syrup, fruit juices, and ethanol as a biofuel, amylase has a wide range of other uses in the food, textile, and paper industries. Bacterial alpha-amylases are also used in clinical, medicinal, and analytical chemistry [32].Currently, amylase manufacturing accounts for up to 30% of the global enzyme market and is steadily rising [33].

The most reliable test for identifying acute pancreatitis has long been amylase. A blood or urine test can be used to determine the levels of amylase. The pee test can be conducted using a clean catch or a 24-hour urine collection. Different laboratories have different serum amylase reference ranges [33].

Differentiating pancreatic amylase from other amylase isoforms is crucial in clinical practice. With normal lipase, an increased amylase level could indicate a disease outside the pancreas [34].

The production of processed foods, such as cakes, fruit juices, starch syrups, digestive aids, and beer, frequently uses amylases. (35). Alpha-amylases are frequently employed in the baking business. These enzymes can be added to bread dough to help the yeast ferment the flour's starch by dissolving it into smaller dextrins. Alpha-amylase is added to the dough to speed up fermentation and reduce dough viscosity, which enhances the volume and texture of the final product. [36,37].

In terms of volume and price, the detergent industry consumes enzymes the most. Enzymes are incorporated into detergent formulations to increase the detergent's ability to get rid of tough stains and make it more ecologically friendly.

Amylases are the second type of enzyme used in the formulation of enzymatic detergents, and 90% of all liquid detergents contain them. (36,38,39). These enzymes break down starchy food residues like potatoes, gravies, custard, chocolate, and other oligosaccharides into dextrins and other smaller oligosaccharides in laundry detergents and automatic dishwashing machines. (40,41).

In the textile business, amylases are used in the desizing procedure. Before making fabric, sizing agents like starch are applied to yarn to facilitate a quick and secure weaving process. Starch is a desirable size since it is accessible, affordable, and easily removed throughout the majority of the world. The starch in the woven fabric is then removed from it using a wet procedure in the textile finishing business.

The removal of starch, which serves as a strengthening ingredient to stop the warp thread from breaking during the weaving process, is the process of desizing. -Amylases eliminate size in a specific manner without harming the fibres. (42,43,36).

Alpha-amylases are employed in the pulp and paper industry to change the starch of coated paper, creating a low-viscosity, high molecular weight starch. (36, 37). The coating procedure makes the paper's surface sufficiently smooth and durable, which enhances the writing quality of the paper. In this application, the native starch has a viscosity that is too high for paper size, but by partially breaking down the polymer with alpha-amylases in batch or continuous operations, this can be changed.

In addition to being a great paper coating, starch works well as a finishing sizing agent to improve the quality and erasability of paper. The size has an impact on the stiffness and strength of the paper. (44, 36). Amizyme® (PMP Fermentation Products, Peoria, USA), Termamyl®, Fungamyl®, BAN® (Novozymes, Denmark), and -amylase G9995® (Enzyme Biosystems, USA) are examples of amylases derived from microorganisms used in the paper industry (45).

The starch liquefaction process, which turns starch into fructose and glucose syrups, uses alpha-amylases most frequently for starch hydrolysis. (46).

Liquefaction, which involves partial hydrolysis and the loss of viscosity, gelatinization, which entails the breakdown of starch granules and the creation of a viscous solution, and saccharification, which entails the production of glucose and maltose by further hydrolysis (36,47).

Bacillus amyloliquefaciens -amylase was initially used, but Bacillusstea- rothermophilus or Bacilluslich- eniformis alpha-amylase has since taken its place (37). Bacillus enzymes are particularly relevant for large-scale biotechnological activities because to their exceptional thermostability and the availability of efficient expression platforms (47).

5. MATERIALS AND METHODS:

5.2. Collection of soil samples:

A handful of areca and sugarcane soil were collected from B.C. Road, Bantwal in the regions of Dakshina Kannada district, in the sterilized two test tubes and transported to the laboratory.

5.3. Medium for isolation:

Medium used for isolation of bacterial species was Starch agar. The starch agar medium containing per liter (5g starch ; 5g peptone ; 5g NaCl ; 3g yeast extract ; 20g agar ; pH 7) .

5.4. Bacterial species isolation:

The serial dilution method was employed to isolate the bacteria. Samples were serially diluted up to 10-1 to 10-7 times and spread out on starch plates before being incubated at 37°C for 24 hours with 1g of soil added to the glass tube containing 9ml of sterilised distilled water.

5.5. Identification of Isolated Organisms:

According to the protocols outlined in Bergey's Manual of Systematic Bacteriology (Buchanan R. E. and Gibbons N. E., 1974), the isolated bacteria were identified using morphological and biochemical tests. The isolates were examined under a microscope to identify the bacteria that produce enzymes, and the bacterial colonies' dimensions, shapes, borders, and pigmentations were noted.

5.6. Microscopic observation:

Attempts have been made to identify the bacterial strains on the basis of Gram staining, Endospore staining.

5.6.1. Gram's staining procedure: A clean glass slide was produced with a bacterial stain. It was allowed to dry naturally before being quickly heated-fixed by passing the slide through a Bunsen burner's flame. It was initially stained for one minute with crystal violet, then cleaned.

Iodine from Gramme was added and removed. It was then treated with 95% ethyl alcohol to remove the colour. The smear was cleaned after being stained with safranin once again for 45 seconds. The smear was next examined with a 40X magnification microscope.

5.7. Biochemical characterization:

A variety of biochemical tests, including the Indole test, Methyl Red test, Citrate Utilisation test, Voges-Proskauer test, Nitrate Reduction test, and Catalase test, were used to characterise the biochemistry of bacterial isolates.

5.7.1. Indole test:

Take a test tube that has been sterilised and has 4 ml of tryptophan broth in it. Aseptically inoculate the tube by taking the growth after an 18 to 24-hour culture. the tube for 24-28 hours at 37 degrees Celsius. Kovac's reagent, 0.5 ml, should be added to the broth culture. Check to see if the ring is there or not.

5.7.2. Methyl Red Test:

The methyl red test determines a living thing's capacity for mixed-acid fermentation. Glucose, peptone, and a phosphate buffer are all components of MR-VP medium. The pH of the broth is lowered when mixed-acid fermentation organisms create an amount of acid that is greater than the broth's ability to act as a buffer.

Other forms of fermentation cannot defeat the broth's ability to act as a buffer. Inoculated isolates were cultured for 24 hours at 370C after being added to 10 ml of sterilised MR-VP broth. Each infected and control tube received five drops of the Methyl Red indicator. Bright red colour in the look denotes a positive test result.

5.7.3. Citrate Utilization Test:

The ability of intestinal bacteria to use citrate as their only carbon source is utilised to distinguish between them in the citrate utilisation test. Citrate must be used in conjunction with the "citrate permease" enzyme, which is created by living things and aids in the transfer of citrate into cells.

A specific composition of citrate medium was made in vials and sterilised. The vials were incubated at 370C for 48 hours following the inoculation of the media with cultures. It was seen that the colour changed from green to blue. Before deciding that the vials were negative findings, they were stored for up to 7 days.

5.7.4. Voges-Proskauer Test:

Each tube containing 2 ml of the liquid culture received 0.5 ml of -napthol solution in 95% alcohol and 0.5 ml of 16% KOH for the VP test. The development of red colour in the soup in both cases suggested that the reaction was positive..

5.7.5. Nitrate Reduction Test:

The nitrate reductase enzyme, which hydrolyzes nitrate to nitrite and can thereafter be degraded into a variety of nitrogen compounds depending on the enzyme system of the microbe, is used in the nitrate reduction test to distinguish between different bacteria.

Isolates were heavily inoculated and incubated in nitrate broth for 24 hours at 370°C. After incubation, Durham's tube contains nitrogen gas, which may be seen there. Six to eight drops of reagent A (sulfanilic acid) and six to eight drops of reagent B (-naphthylamine) were added.

For a minute, the colour shift was seen. Nitrite is transformed into nitrate sulfanilic acid in the presence of reagent A, and when reagent B is added, the result is red protonsil precipitates. In positive result the broth turns red and in negative results remains colorless.

5.7.6. Catalase Test:
The test finds out whether a microorganism has catalase, which is responsible for converting harmful hydrogen peroxide into oxygen and water. A drop of the 3% Hydrogen Peroxide reagent was applied after the bacterial colony had been placed to a glass slide. The good news is that the oxygen production causes bubbles to evolve quickly.

5.8. Screening for amylase enzyme activity (starch iodine test):
Using the dot method and sterile inoculating needles, bacterial colonies were subcultured in freshly prepared dishes containing starch agar. The plates were then placed in the incubator for 48 hours at 37°C. The plates were saturated with Gram's iodine following incubation. We noticed any creation of a clear zone around the colonies.

5.9. Classification of amylase enzyme:
Analysing the ideal temperature and pH for the enzyme's activity allowed scientists to distinguish the amylase enzyme.

5.9.1. Determining the Ideal Temperature:
A variety of screw cap test tubes were filled with 1 ml of 1% substrate solution (1% starch agar in phosphate buffer, pH 6.5), 1 ml of phosphate buffer (pH 6.5), and 1 ml of diluted (5 fold) crude enzyme. At temperatures ranging from 25°C to 85°C, the test tubes were incubated in a water bath for 30 minutes.

The reactions were then stopped with 1.5 ml of DNS solution. For 10 continuous minutes, the test tubes were left at room temperature. The test tubes were then heated to 100°C in a water bath for 10 minutes to boil them. Next, test tubes were placed in flowing water and warmed to room temperature. Each test tube's colour saturation at 540 nm was measured using a spectrophotometer. The data were contrasted with a blank solution at each temperature. When the most activity was observed, the temperature was given.

5.9.2. Determination of optimum pH:
By dissolving 1% soluble starch in a range of pH solutions from 3 to 10, several substrate solutions were created. One millilitre of each 1% substrate solution was added to separate screw-cap test tubes, along with one millilitre of each of the following buffers: 0.05 M glycine NaOH (pH 10), 0.05 M sodium phosphate buffer (pH 6 and 7), 0.05 M Tris-HCl (pH 8 and 9), and 0.05 M citrate buffer (pH 3 to 5). The buffers also included 1 ml of crude enzyme that had been diluted five times. The test tubes were incubated in a water bath for 30 minutes at 75°C (the ideal temperature). 1.5 ml of DNS solution was then used to cease the reactions. The test tubes were kept at room temperature for ten uninterrupted minutes. The test tubes were then placed in a water bath and boiled for 10 minutes at 100°C. The test tubes were then placed under running water to cool to room temperature. Using a spectrophotometer, the colour intensity for each test tube was measured at 540 nm. For each temperature, the values were compared to a blank solution. It was noted at what pH the most action was seen.

6. Results:
6.1. Isolation and selection of bacteria that produce amylase from soil:
In the study soil tasters of areca and sugarcane were collected from local areas of Dakshina Kannada district. After this the soil solution was diluted by serial dilution. After serial dilution0.1ml of sample was taken and spreaded on a Nutrient Agar Broth to observe the colonial morphology. Later, the starch agar plate that colonies in the starch agar plate iodine solution was put. Six isolates were reported to give a zone of clearing around their colonies after applying the iodine solution. In 6 isolates, 2 isolates were clearly visible around their zones. Signs of observations shows in the Table 2.

Later, these isolated colonies were separated and put in nutrient agar and their further regrown in nutrient broth and few are kept for further analysis.

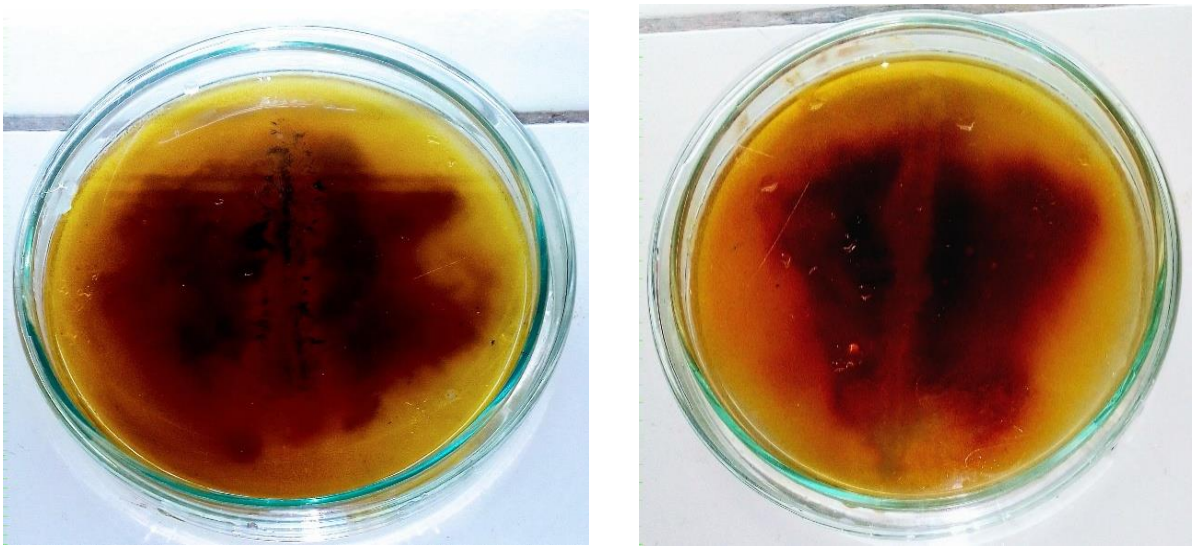

Figure 6.1: Zone of clearance produced by isolate 1 (left) and isolate 2 (right) on starch agar media

Table 2:- Bacterial isolates Morphological characteristics

S.No	Test	Isolate 1	Isolate 2
1	Culture medium	Starch medium	Starch medium
2	Growth	Good	Good
3	Form	Circular	Irregular
4	Elevation	Flat	Flat
5	Margin	Entire	Entire
6	Gram staining	Negative	Positive
7	Shape vegetative cell	Rod	Rod
8	Starch hydrolysis	Positive	Positive

6.2. Microscopic Observation:

The strains of bacteria were made in the nutrient broth and after this added to gram staining. The Isolate 1 gave gram negative since it was pink rod shaped bacteria, and isolate 2 gave gram positive shows purplish blue rod shaped organism. 2 isolates were later subjected to endospore staining, negative result shows in case of isolate 1.Another one isolate 2 strains gave a endospore positive result. As the green colour was observed and confirms the endospores presence.

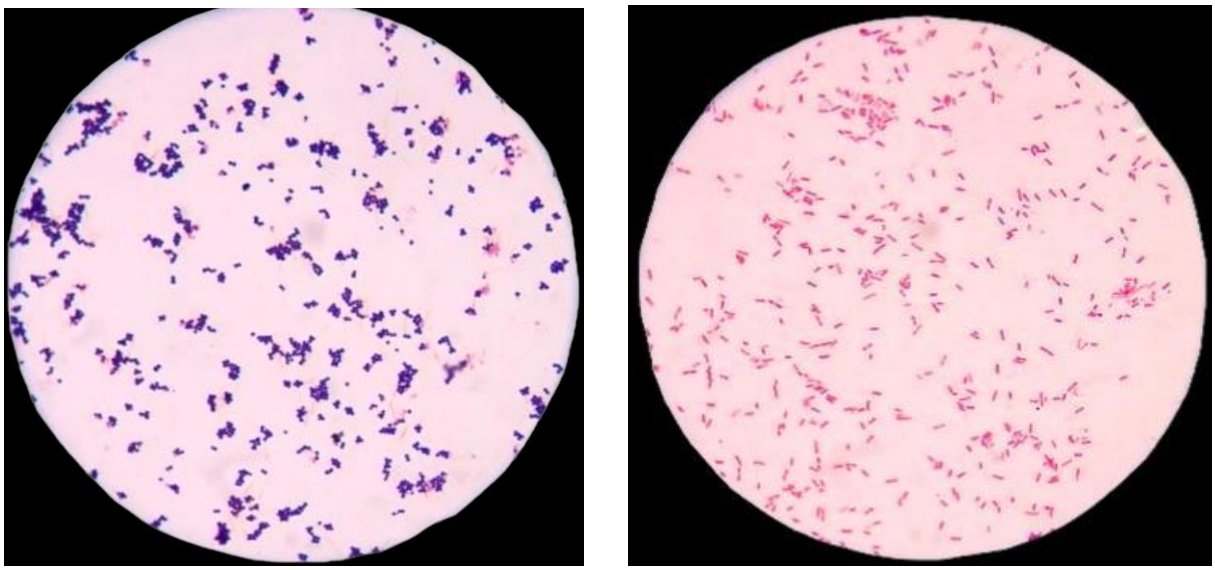

Figure 6.2: Appearance of isolate 1 and isolate 2 after gram staining under the microscope

6.3. Biochemical characteristics of bacterial isolates:

2 isolates were found to be Indole, Nitrate, positive capable of producing acid. The results were presented in Table 3.

Table 3:- Bacterial isolates

S.No	Name of the Test	Isolate 1	Isolate 2
1	Indole	+	+
2	Methyl Red	-	-
3	Voges-Proskauer	-	-
4	Citrate Utilization	-	-
5	Nitrate Reduction	+	+
6	Catalase	+	+

Biochemical characteristics

6.3. a) Indole Test:

From the Indole Test 2 isolates gave positive results. It is important for enterobacteria. Tryptophan with the release of indole with the help of enzyme tryptophanase. After the addition of Kovacs solution yellow to cherry red this shows the presence of indole.

6.3.b) Methyl Red Test:

On addition of Methyl Red to the organism shows yellow colour no red. This shows methyl red negative.

6.3.c) Voges-Proskauer Test: There is no red or pink colour found in 2 isolates, Voges-Proskauer was negative.

6.3.d) Citrate Utilization Test: After addition of bromthymol blue to the sample there is no colour changes from green to blue. This shows the negative result of citrate utilization test.

6.3.e) Nitrate Reduction Test: Addition of a dropper full of sulfanilic acid and alpha-napthylamine to the organism, red colour was observed this shows presence of nitrate reduction enzyme in 2 isolates.

6.3.f) Catalase Test: There is evolution of gas bubbles seen in the slide. Enzyme catalase was present.

a)Indole test **b)Nitrate reduction test** **c)Catalase test**

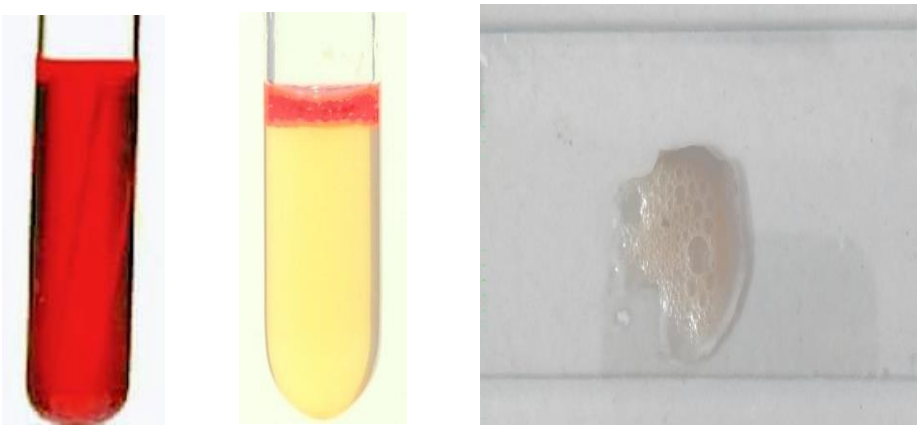

Figure 6.4: Results of biochemical tests

6.4. Characterization of amylase enzyme

6.4.1. Effect of temperature on enzyme activity isolate 1:

Up to 50 °C, the enzyme displayed a modest activity. The activity increased at 60°C, and it grew much more at 70°C (Figure 6.5).The enzyme's highest level of activity was reported at 70°C. This was thought to be the enzyme's optimum temperature. At 90°C, there was a noticeable drop in activity. .

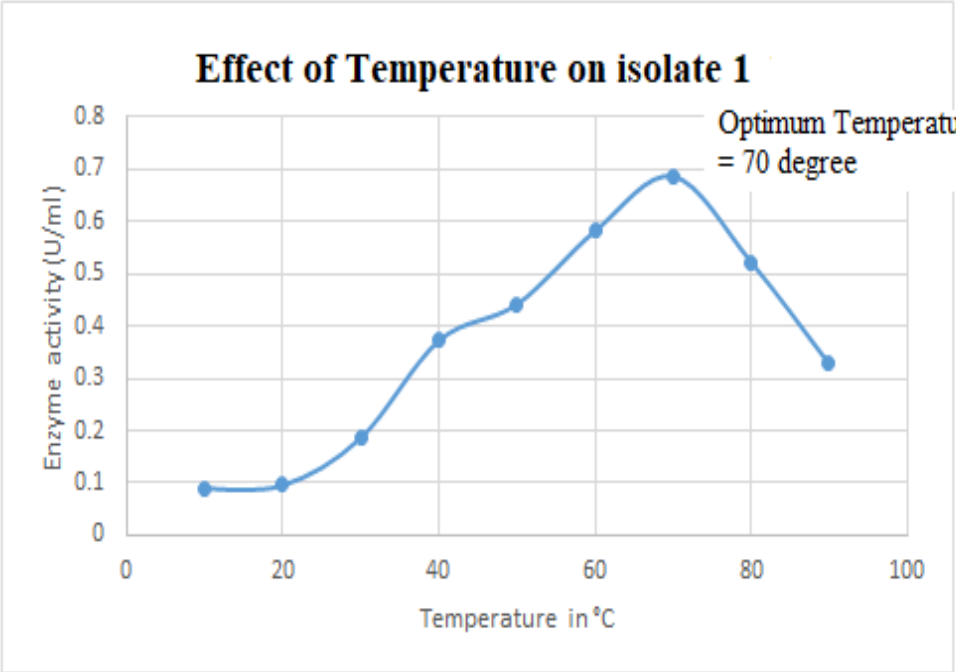

Figure6.5: isolate 1 activity of amylase effect of temperature

6.4.2. Effect of pH on the enzyme activity isolate 1:

At pH 1, the enzyme's activity was comparatively low. The activity of the enzyme was seen to rise along with an increase in pH. From pH 6 to pH 7, activity increases. From pH 8 to pH 10, however, the enzyme activity dropped. The ideal pH for this amylase enzyme is thought to be 6.8, or alkaline. (Figure6.6).

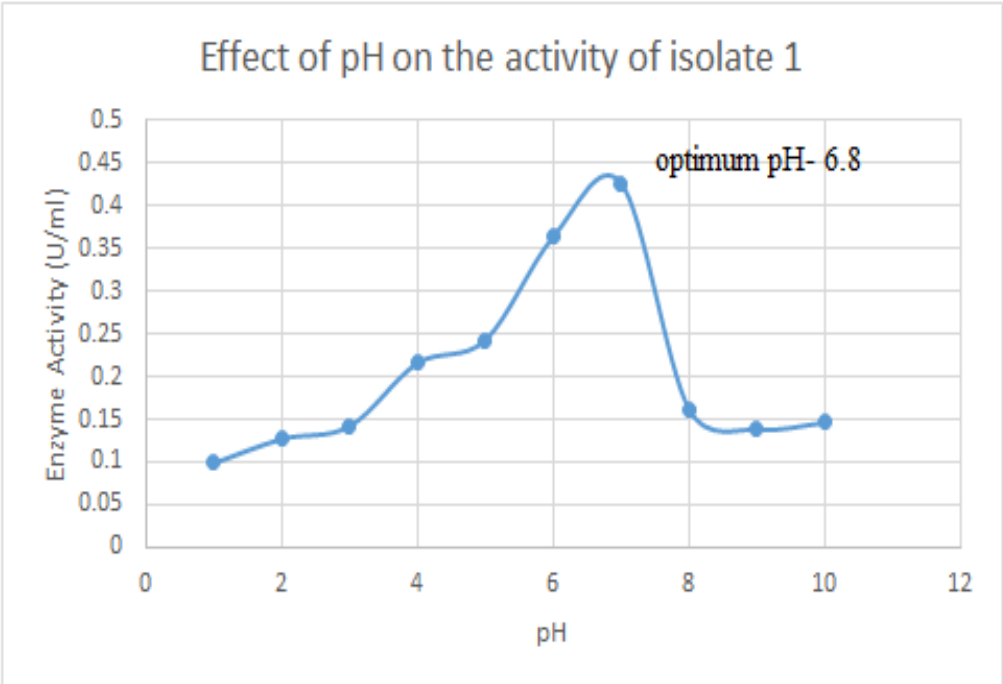

Figure 6.6. Effect of pH on the activity of amylase of isolate 1

6.4.3.Effect of temperature on enzyme activity isolate 2:

From 15°C to 25°C, the enzyme's activity was very low. The activity increased at 60°C, and at 68°C, it grew even more (Figure). The enzyme's highest level of activity was noted at 68°C. This was thought to be the enzyme's optimum temperature. At 90°C, the activity was shown to diminish. (Figure 6.7).

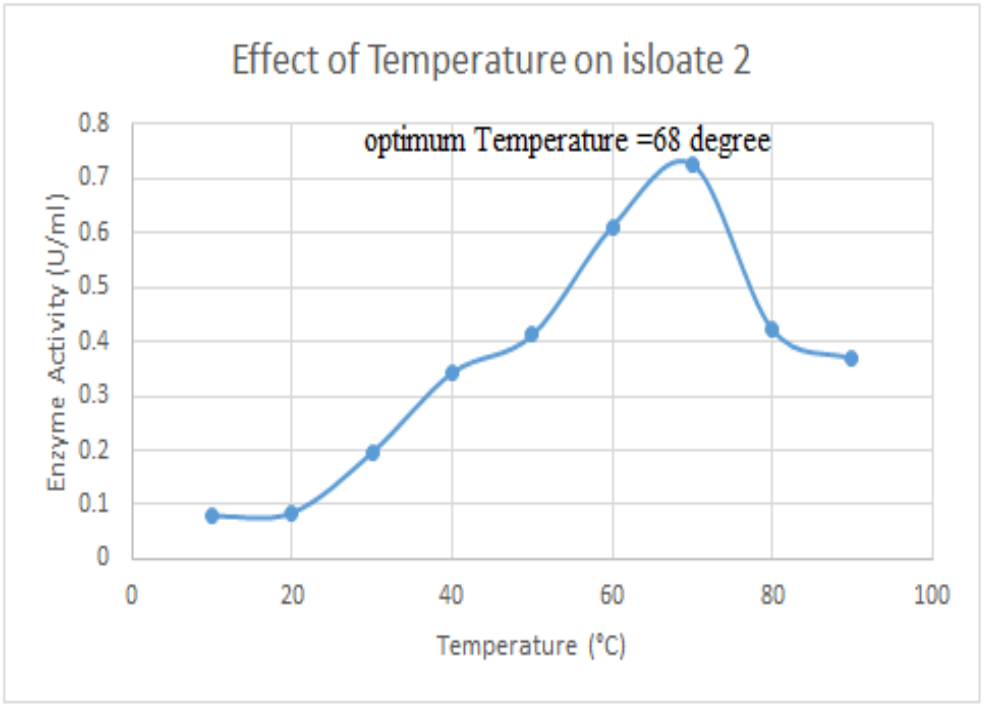

Figure 6.7: Effect of temperature on the activity of amylase of isolate 1

6.4.4. Effect of pH on the enzyme activity isolate 2:

At pH 1, the enzyme's activity was comparatively low. The activity of the enzyme was seen to rise along with an increase in pH. From pH 6 to pH 7, activity increases. From pH 8 to pH 10, however, the enzyme activity dropped. The ideal pH for this amylase enzyme is thought to be 6.6. (Figure 6.8).

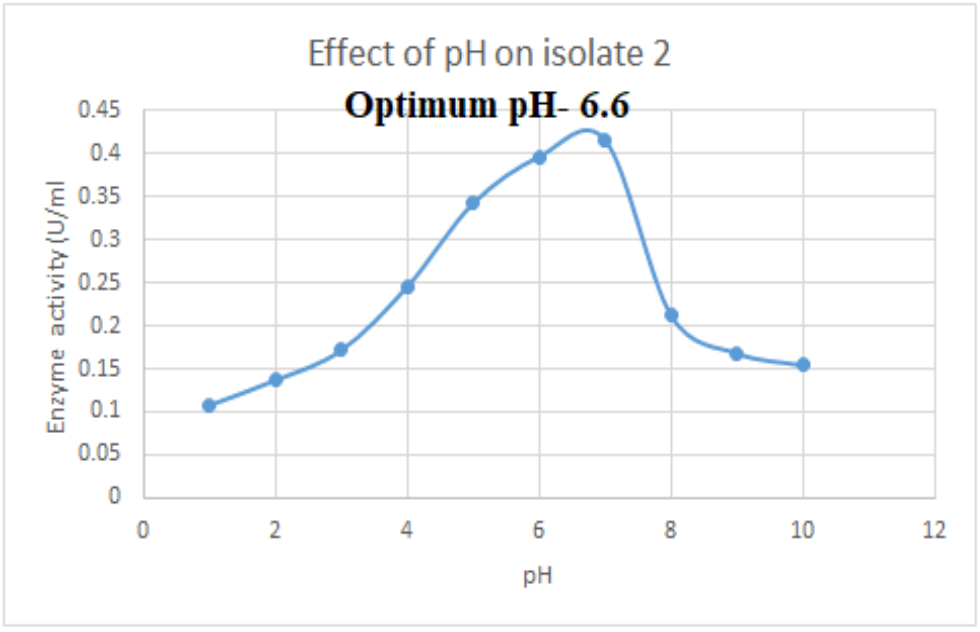

Figure 6.8: Effect of pH on the activity of amylase of isolate 2

7. Discussion:

Enzymes are not only necessary for carrying out biochemical reactions within an organism, but also for the synthesis of a wide variety of products due to their great specificity and catalytic properties. One of those enzymes is amylase. Amylase is commonly utilised in the food, textile, detergent, and paper industries on a global scale. Additionally, the chemical and pharmaceutical companies regularly use amylase to produce their goods. (El-Fallal et al., 2012).

It is essential to isolate a local strain that produces a lot of amylase because industrial amylase is often

derived from bacteria and fungi. Although amylase-producing microbes can be isolated from a variety of environments, soil is generally considered to be the best source of these organisms. Because soil has a wide variety of bacteria, it was chosen as the source of bacterial isolation and is occasionally thought of as the repository of amylolytic microorganisms. [36].

Two separate soil samples from the root-adjacent soil of sugarcane and coffee were taken for this study. The major goal was to isolate an amylase-producing bacterial strain from soil and characterize the amylase enzyme generated by figuring out the ideal temperature and pH for the enzyme to function at.

Four out of every ten isolates of the bacterial strains were found to generate amylase during the initial screening. To find out, the isolates were grown on starch agar medium, then Gram's iodine was added to look for any clear zone generation surrounding the bacterial colonies. The absence of starch, which was digested by the amylase enzyme secreted by the bacteria, was what caused the clear zones to form.

Gramm staining, a technique used for microscopic inspection, revealed that isolate 1 displays gramme negative, pink rod-shaped bacteria while isolate 2 displays gramme positive, purple rod-shaped bacteria. The biochemical features of the bacterial isolate were verified using a number of biochemical assays, and it was found that in addition to producing amylase, this bacterial isolate is also capable of producing the enzymes catalase and nitrate reductase.

Characterization of the amylase enzyme produced by this strain of Bacillus cereus served as the foundation for the study's second section. Several factors affect how quickly amylase breaks down starch (Sivaramakrishnan et al., 2006). When an enzyme is characterised, the ideal fermentation conditions for that enzyme are found. Additionally, it is possible to verify the inhibitory Concentrations of salts and metal ions for that specific enzyme.
Since the characteristics of amylase must match the application, it is essential to determine the ideal circumstances, which can be achieved through characterisation (Sivaramakrishnan et al., 2006). Ones of the most significant ones are pH and ideal temperature. Hence, the enzyme was characterized by carrying out enzyme assay at different temperatures and pH in order to detect the optimum conditions. There are various ways to characterize an enzyme.

In this study, the DNS method was used which determines the amount of reducing sugar produced at different temperatures and pH by the enzyme. Isolate 1 was detected that the activity of amylase was low at temperatures from 30°C to 50°C. However, a drastic elevation of activity was observed from 50°C which maximized at 60°C.

At a higher temperature of 70°C, the activity started to recede. So, this particular amylase showed highest activity at 70°C. Thus it can be concluded that the amylase is most active a moderately high temperature as compared to amylases worked with on different studies. While isolate 2 shows optimum temperature at 68°C.
When the enzyme was characterized to identify optimum pH, isolate 1 was observed that the amylase showed the highest activity at pH 6.8. From pH 3 to pH 5 the activity was moderate and gradually increasing but at pH 6 it elevated drastically up to pH 7. However, the enzyme started showing lower activity as the pH moved to the alkaline range from 8 to 10. From this result, it can be concluded that the enzyme works best at a neutral pH. Isolate 2 was observed that the amylase showed the highest activity at pH 6.6.

8. Conclusion :

The present study focused with the isolation of amylase producers from areca and sugarcane soil samples. The starch-agar plate method was used to screen the amylolytic activities of 10 isolates, out of 10, 2 isolates gave a good clearance zone around the colonies. Gram staining revealed that isolate 1 was gram negative, rod shaped.

While isolate 2 was gram positive, rod shaped. Biochemical methods like indole, nitrate, methyl red and catalase were performed to characterize the isolates. Optimum amylase production was monitored by optimum temperature and optimum pH. Amylase activity of 0.186 U/mL for isolate 1 and 0.127 U/mL for isolate 2 with an optimum temperature of 70°C and 68°C. Optimum pH for the enzyme activity of 0.427 for isolate 1 and 0.404 for isolate 2 with an optimum pH of 6.8 and 6.6.

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