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ISOLATION OF AMYLASE PRODUCING BACTERIA FROM SOIL AND ITS PLANT GROWTH PROMOTION ACTIVITY

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ABSTRACT

Amylase is an enzyme that hydrolyses starch into its monomeric compounds, the littlest being glucose. There are great advances in the use of amylase in industrial sectors additionally. Soil may be a primary source of those bacteria which might be isolated and commercially grown in large numbers to supply an unlimited amount of amylase. Different species of *Bacillus* were stated to be producing α-amylase by fermentation. Bacillus sp. KR-8104 produces α-amylase, which is Ca independent and active at low pH, used for large-scale α-amylase development. Some *Bacillus* strains produce thermostable α-amylases while others develop acid-resistant α-amylases. An option for commercial production of thermostable α-amylases in the thermophilic bacterium *Bacillus stearothermophilus*. *Bacillus* sp. produces alkaline and thermotolerant amylases. such as *Bacillus licheniformis* and *Bacillus halodurans*. A total of 190 bacterial colonies were taken out of which 27 showed clear halos on starch agar plates. The 27 colonies were again inoculated into wheat bran and checked for enzyme production. The crude enzyme extract for all 27 isolates was assayed at 50 and 70°C, pH 6.5.

Keywords: Amylase Production, Microorganisms, Soil, Starch, Growth promotion Activity.

1. INTRODUCTION

Microorganisms are the foremost important enzyme production sources. Choosing the right species plays a key role in generating high yields of suitable enzymes for industrial use. Bacteria, fungi, actinomycetes, algae, and protozoa could even be classified as soil microorganisms. Each of those groups possesses characteristics that characterize them and their soil work. The foremost common microorganisms in soil are bacteria and Archaea. Enzymes are the biological catalysts of a macromolecular nature. At the start of the cycle, the molecules are called substrates, and also the enzyme transforms these into various molecules called products (Bertrand *et al.*, 2004). The definition of a microorganism has allowed a real understanding of the microbial environment united which will be analyzed using similar methods and techniques, although it reflects very differing types of reproductive units and cell organizations. Biologists began to grasp the commonality of all species within the late 20th century following the work of Carl Woese and other molecular evolutionists, all consisting of cells with a stimulating resemblance to every other and having a typical evolutionary heritage, and consequently with major features of a widely shared genetic ordination and biology. Microbiology and biology as a whole were united during this context, as they'd never been before (Bruin Enberg *et al.*,1996).

Amylase is an enzyme that hydrolyses starch into its monomeric compounds, the tiniest being glucose. The glycosidic bonds that hold the monomers together are dampened by the enzyme. This will be an awfully common and essential reaction within various living organisms to come back up with or store energy. Hence, amylase could also be a prevalent enzyme produced biologically by various varieties of living beings. This includes plants, animals, humans, and other microorganisms. There are great advances in the use of amylase in industrial sectors additionally. A huge portion of the enzyme market share is owned by amylase. A large range of industries such as foods, garments, textiles, and beverage industries and medicinal and clinical chemistry use amylase to manufacture their products. This needs a continuous production of amylase enzyme. Extraction of this huge quantity of amylase directly from nature isn't feasible and hence various methods are being constantly established to develop the production of business or commercial amylase.

Among the assorted kinds of amylase, microbial amylase meets demands. quite commercial large quite microorganisms are identified and chosen due to the source of amylase production due to the supply and simple the ways during which they yield amylase. Fungal amylases are used worldwide along with different strains of bacteria. Each strain of bacteria requires specific growth conditions and nutrients to provide amylase. Soil could even be a primary source of these bacteria which can be isolated and commercially grown in large numbers to supply an infinite amount of amylase. to supply this, industries use fermentation shake flasks to grow bacteria. Additionally, the amylases that are extracted require optimum conditions to mean the foremost effective activity. This includes parameters like temperature and pH. For this reason, it's important to hunt out the optimum conditions for amylase activity through research before it'll be used for industrial purposes.

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2. MATERIALS AND METHODS

2.1 Apparatus and glassware

All fresh glassware like conical flasks, glass pipettes, beakers, and glass rods were washed once with H2O and thrice with H2O. They were then air-dried before use. Petri dishes were first sterilized at 160°C during a sterilizer and then used. All previously used glassware was autoclaved first at 121 °C at 15 psi for a quarter-hour in an autoclave machine so used. Micropipette tips, Eppendorf's, and falcon tubes were autoclaved first so used. every kind of procedure was distributed by the scholar under the supervisionof the lab officers and teaching assistants.

2.2 Solutions and reagents

Required solutions and reagents were freshly ready before use. All chemicals required were nonheritable from the laboratory's shelves. None of the solutions or chemical agents were more refined since they were reagent grade. Nutrient agar medium was used for short preservation of microorganism cultures and social group functions. Luria Bertani (LB) broth was used for the expansion of microorganism culture before polymer extraction. Starch agar medium was accustomed screen amylase-producing bacterium. A selective media was used throughout the fermentation of bacterium for enzyme production during a shake flask.

3. ISOLATION AND SELECTION OF AMYLASE PRODUCING BACTERIA FROM SOIL

A handful of soil containing decomposed manure was collected from a farm, in an exceedingly bag and transported to the laboratory. From the soil sample, 1 g of sample underwent serial dilution and, the spread plate technique was wont to transfer the bacteria to agar media. The plate showing distinct colonies of bacteria was chosen for further work.

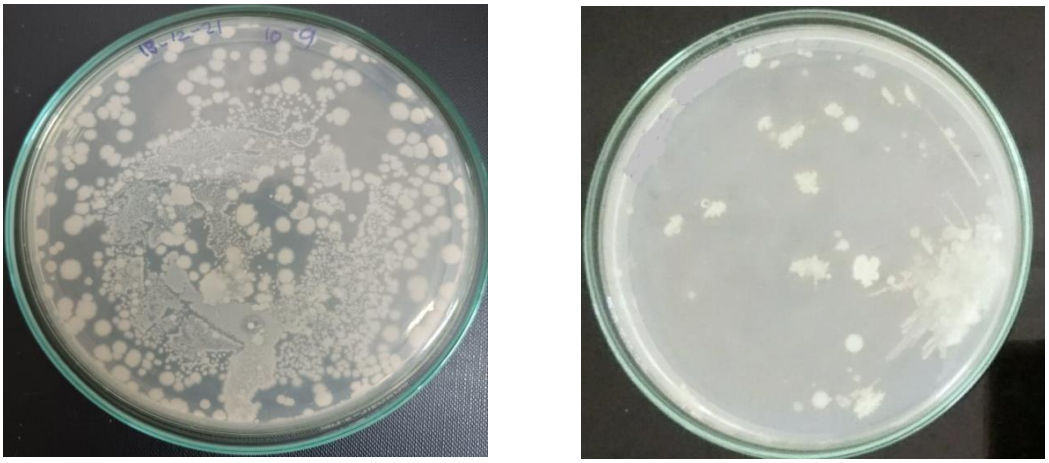

Figure 1: Isolation of amylase enzyme-producing bacteria from soil

3.1 Gram staining

A bacterial smear was prepared on a clean glass slide. it had been allowed to air dry and heat-fixed by passing the slide through the flame of a Bunsen burner very swiftly. At first, it was stained withantibacterial for 1 minute and washed. Gram's iodine was added and washed off. After that, it was decolorized with 95% ethyl alcohol. The smear was stained again with safranin for 45 seconds and washed. The smear was then observed under the microscope with 1000X magnification. The selected bacteria were observed to be Gram-positive rods arranged and enchained when visualized under the microscope after Gram staining.

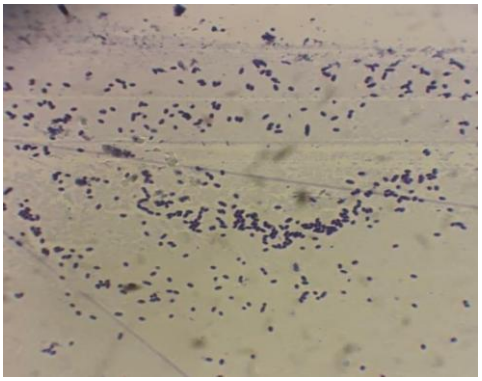

Figure 2: Gram Staining

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4. BIOCHEMICAL TEST

It was observed that a positive result was obtained for the catalase check, indole production, cellulose degradation, gelatin, and casein chemical reaction, and reduction of nitrate. On the opposite hand, the result was negative for the oxidase test, each MR and VP test, utilization of citrate, motility, enzyme production, and MSA plate check. Also, the anaerobic condition and 45°C favored the expansion of the microorganism whereas the alternative was discovered for 65°C and seven% NaCl. Colony morphology, size, color, shape, gum production, and growth pattern were recorded when twenty-four h of growth on LB agar plates at twenty-eight ± 2◦C as delineated by Hoben Cell size and motility were discovered by light microscopy. Acid/alkali production was tested on LB agar plates containing 0.025% (w/v) bromothymol as a pH indicator. Amino-peptidase and hemoprotein enzyme tests were performed by victimization commercially accessible strips (Merck, Darmstadt, Germany). Catalase enzyme production was checked by putting a drop of H_2O_2 onto the bacterial colony on a glass slide.

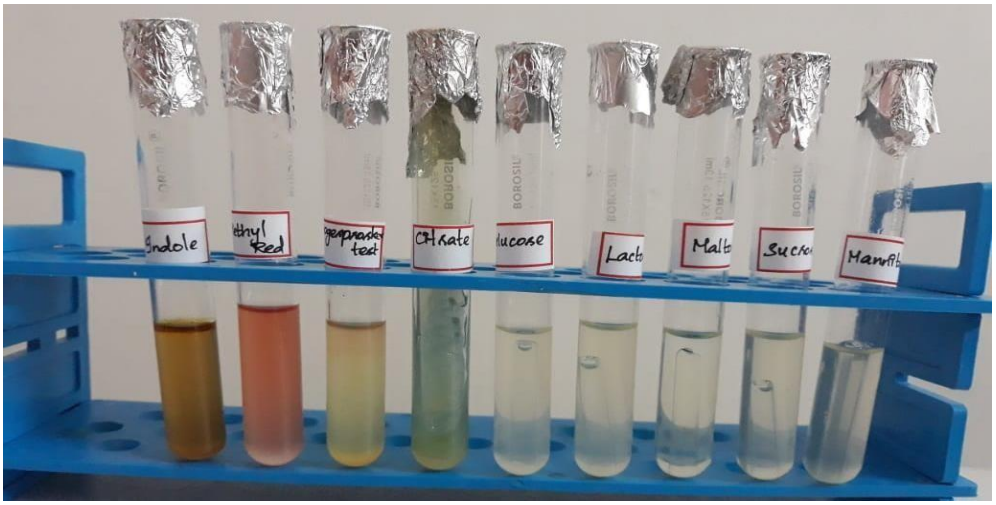

Figure 3: Biochemical Test

Table 1: Biochemical Test

TEST	RESULTS
INDOLE	+
METHYL-RED	-
VOGES PROSKAUER	-
CITRATE	-
LACTOSE	+
GLUCOSE	+
SUCROSE	+
MALTOSE	+
MANNITOL	+
CATALASE	+
STARCH HYDROLYSIS	+

5. RESULTS AND DISCUSSION

5.1 Effect of different Temperature on the Selected isolate *Bacillus* Sp. and Maximum Enzyme Activities

The test tubes containing broth were inoculated with the isolate *Bacillus* sp. *and* were incubated at different temperature zones from 30 ◦C to 70 ◦C and the enzyme activity was assayed. The enzyme activity was maximum at the temperature of 30to 50 ◦C for all the enzymes. The peak activities were 137.20, 137.22, 137.16, 90.87, and 87.68U/L for the Amylase respectively. Results are shown in Table 2 & Figure 4.

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Table 2: Optimum Temperature for *Bacillus* Sp.

Optimum Temperature for *Bacillus* Sp.		
S. No	**Temperature (◦C)**	**Enzyme activity(U/L)**
01	30	137.20
02	40	137.22
03	50	137.16
04	60	90.87
05	70	87.68

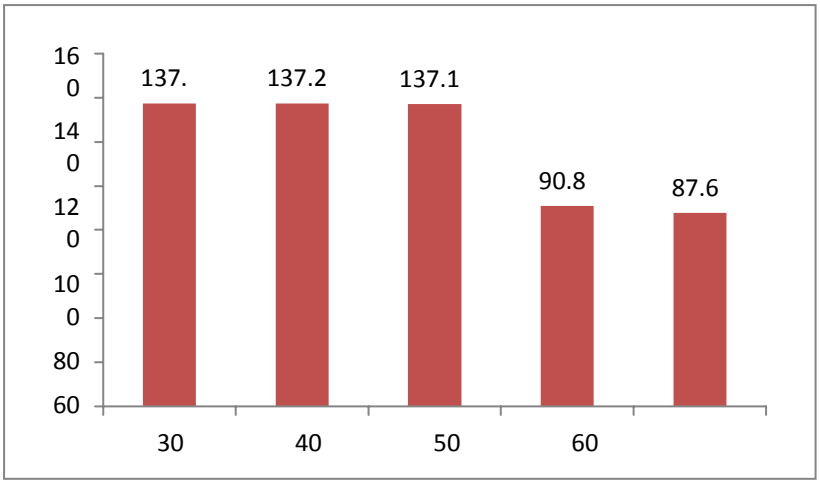

Figure 4: Optimum Temperature for *Bacillus* Sp.

5.2 Effect of different pH on the Selected isolate *Bacillus* Sp. and Maximum Enzyme Activities

The organism was grown over a wide range of pH values from 6.5 to 8.5. The enzyme activity at these pH ranges is given in Table 3. The maximum activity of 137.03 U/L and 140.20 U/L was observed at the pH of 6.5 and 7.5 for an enzyme.

Table 3: Optimum pH for *Bacillus* Sp.

Optimum pH for *Bacillus* Sp.		
S.No	**pH**	**Enzyme activity (U/L)**
01	6.5	137.03
02	7.0	133.20
03	7.5	140.20
04	8.0	125.18
05	8.5	136.20

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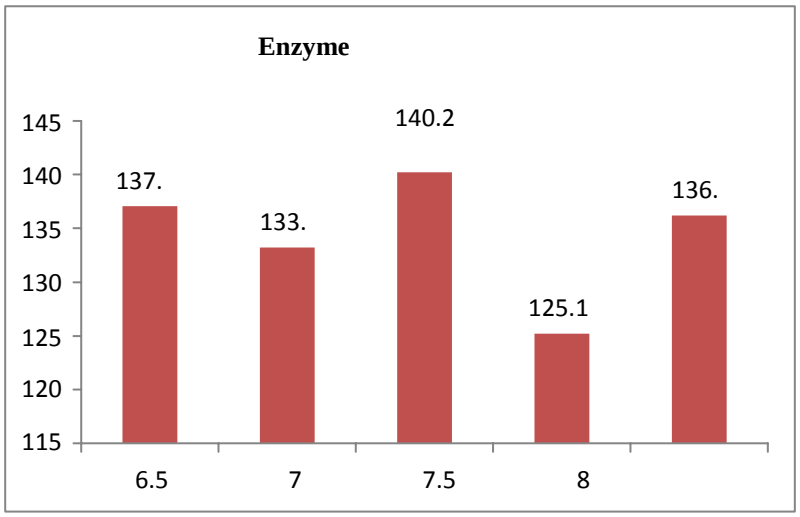

Figure 5: Different pH on the Selected isolate *Bacillus* Sp.

6. CONCLUSION

The use of amylase enzyme is widespread globally in industrial sectors for the manufacture of a range of products. Within the food industry, they were used in the assembly of glucose syrups, maltose syrups, and high fructose corn syrups. However, the scarcity of economicamylase production within the country may be a major problem for such industries. to beat this, the soil samples were collected and isolated the amylase-producing bacteria using spread plate techniques under microscopic examinations which induce the expansion promotion activity for the assembly of varied industrial uses.

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