Comparative analysis of crude and partial purified amylases from soil isolates

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Abstract

Amylases are the enzymes which have various applications in food, detergent, pharmaceutical, textile industries as well as other industries. This research is focused on Screening of amylase-producing microorganisms from various soil samples and extraction and partial purification of amylase. Amylase activity was measured using the dinitro salicylic acid (DNSA) method, which detects reducing sugars. The isolate with the highest activity was selected for further study. Partial purification of amylase was carried out through ammonium sulfate precipitation followed by dialysis.

This study underscores soil's potential as a source of amylase-producing microbes. Basic screening and activity assays are vital for identifying strains suitable for large-scale biotechnological use. Comparative analysis of amylases from soil isolates reveals that in crude 200 activity was there which rise up to 300

Results indicate soil contains diverse microbes capable of producing industrially relevant enzymes, with some isolates showing high amylolytic potential.

Introduction

Microbes play a crucial role in their surroundings by carrying out various biochemical activities. Soil acts as a habitat for a wide range of microbes like bacteria, fungi, algae, viruses, and protozoa. Investigating microbial enzymes such as amylases is key to grasping biochemical processes and leveraging their biotechnological capabilities. Amylases break down starch into useful products and are utilized in industries like food, textiles, and paper. The preference for microbial amylases stems from their stability and varied properties [1].

Focusing on the importance of amylases in industrial settings and considering they are produced by microbes like Bacillus species, this research aims to isolate and identify the isolates from Christ College, Rajkot's soil samples that produce amylase. Research similar to this has shown the potential of microbes in industrial uses [1][2]. By exploring the microbial diversity in Christ College's soil, there's a possibility of discovering new amylase-producing bacteria with distinct properties. This could enhance the understanding of biochemical activities and biotechnological potential of soil microbes. Since soil microbial communities contribute significantly to nutrient cycles, decomposition of organic matter, and maintaining soil health, examining amylase-producing bacteria provides insights into ecological dynamics [5]. Application of thermophilic microorganisms to produce amylolytic enzymes for industrial use is a general practice because they provide broader temperature range and higher thermostability compared to enzymes from mesophilic microorganisms.

Material & Method

- **Isolation of amylase producing microorganism from soil and screening of organism based on its amylase activity**

  Soil suspension was prepared after sample collection and heated to kill vegetative forms present in soil. After cooling sample was streaked on Starch agar plate and kept in incubation for 48hrs at 37^{O}C. Zone of utilization was obtained on starch agar by application of Iodine. (1)

- **Morphological characterization of microorganisms and colony character.**

  Colony on starch agar plates, giving high zone of utilization was transferred on other N agar plate to study further morphological examinations and to characterize the organism on the basis of cell arrangement as well as cell size and shape was performed by Gram staining

- **Maintenance of Isolates**

  The pure cultures were maintained at refrigeration temperature by use of Nutrient broth

- **Amylase activity assay by DNSA method**

Activated cultures were grown for 48 hrs. at 37^{O}C and supernatant was collected. Broth was used to study Amylase activity. Amylase activity was carried out by DNSA Method. Reacting reducing sugars with 3,5-dinitrosalicylic acid (DNSA), which is heated in a boiling water bath to produce a colored product, is the DNSA procedure, a colorimetric assay used to measure reducing sugars. At a wavelength of 540 nm, the intensity of the reddish-brown color that develops is directly proportional to the amount of reducing sugars present in the sample. A solution of potassium sodium tartrate or sodium sulfate is frequently added after heating to produce a stable color.

Take 0.5 ml test solution add 0.5 ml of starch solution and incubate it for 20 mins then later add 1 ml of DNSA in the incubated solution then keep it for water bath for 10 min then after completing the water bath add 8 ml of distilled water in it and shake vigorously and to check the optical density of the sample

- **Partial purification of Amylase**

The first steps in partial purification of the enzyme from the fermented broth was filtering the broth to get rid of bigger particles and then centrifuging it for 20 minutes at 3000 rpm and 4°C. The crude enzyme was present in the cell-free broth that was produced by this process, which successfully eliminated cellular components. The following purification procedures were then carried out using this unrefined amylase preparation. Precipitation of Ammonium Sulfate for the Purification of Enzymes Ammonium sulfate was used to precipitate proteins from the broth that was devoid of cells. To reach a 50% (w/v) saturation level, a predetermined quantity of ammonium sulphate was added to the supernatant. Centrifugation at 3000 rpm for 10 minutes at 4°C was used to gather the precipitated proteins following sufficient mixing. After that, the pellet with the precipitated enzyme was reconstituted in a small amount of 2-4 mL of phosphate buffer that had a pH of 6 ± 0.2 and a concentration

of 0.1 M. Dialysis to Remove Salt from an enzyme solution the reconstituted enzyme preparation was put in a dialysis bag with a molecular weight cut off of 12 kDa in order to eliminate salts from the enzyme solution that was obtained after precipitation. The dialysis bag was immersed in 0.1 M phosphate buffer at pH 6 after being firmly tied at both ends. Dialysis was performed at 4 degrees Celsius for the entire night while the buffer was constantly stirred. Activity of Partial purified enzyme was carried out by DNSA method.

Results and Discussion

Isolation of amylase producing microorganism from soil and screening of organism based on its amylase activity

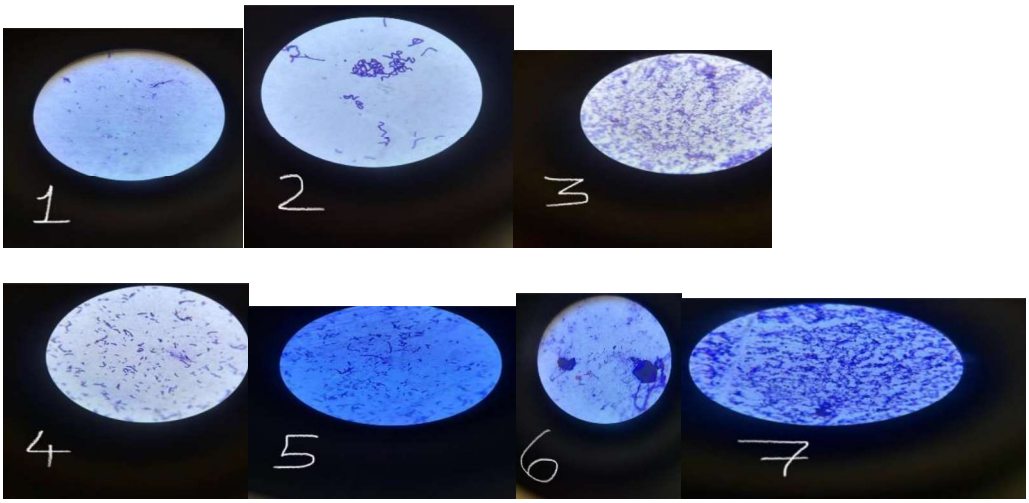
Soil isolates obtained from different soil samples of Christ College, Rajkot, were screened for amylase using starch agar. Morphological and colony characters of isolates were studied on N agar medium are as follows:

Soil as a Reservoir for Amylase-Producing Microbes. This study highlights soil as a valuable reservoir for amylase-producing microbes. Basic screening and activity assays were crucial in identifying potent strains suitable for large-scale biotechnological applications.

Diverse Microbial Populations in Soil: Results showed that soil harbors diverse microbial populations capable of producing industrially relevant enzymes like amylase. Specific isolates exhibited high amylolytic potential

Morphological and Colony characterization of amylase producers

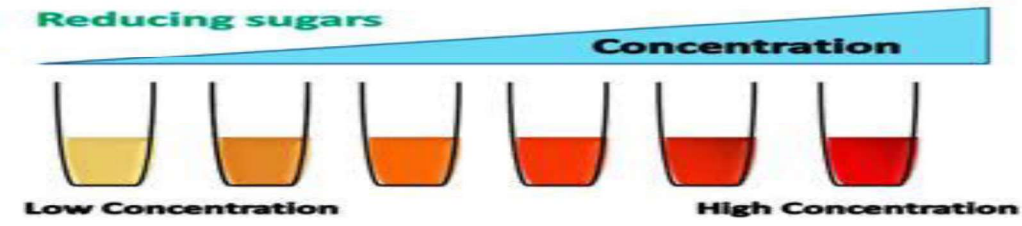
Colony characteristic	1	2	3	4	5	6	7
Size	Large	Medium	Medium	Large	Large	Large	Large
Shape	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular	Irregular
Margin	Entire	Entire	Entire	Entire	Entire	Entire	Entire
Texture	Rough	smooth	rough	smooth	Rough	smooth	Rough
Elevation	Flat	Elevated	Elevated	Raised	Flat	Flat	Flat
Pigmentation	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
Opacity	Translucent	Opaque	Opaque	Opaque	Translucent	Opaque	Opaque



Characters / Isolates No.	Name	Shape	Arrangement	Color	Gram's Reaction
1	1	Small Rod	Single	Violet	Gram Positive
2	2	Long rod	Single and chain	Violet	Gram Positive
3	3	Small rod	Single	Violet	Gram Positive
4	4	Long rod and short rods	Single	Violet	Gram Positive
5	5	Medium rods	Single and in bunch-chains	Violet	Gram Positive
6	6	Long rod	Single	Violet	Gram Positive
7	7	Small rod	Single	Violet	Gram Positive

Amylase activity of Crude and Partial purified enzyme:

The dinitro salicylic acid (DNSA) method was used to measure amylase activity in crude enzyme extracts from culture supernatants while those isolates which were giving highest activity was selected for partial purification. Partial purified enzyme also studied for confirming its effectiveness in extracellular amylase production.



Crude enzyme activity shows potential of isolates for production of amylases. Selected potent isolates were further proceeded for activity after Partial purification. Comparison of Crude and partial purified enzyme activity shows that isolates may possess potential to produce amylase and can be used for industrial purposes.

Summary:

Amylase producing soil isolates can be obtained. Isolates can be studied for their potential of amylase activity. Partial purified enzyme shows enhanced activity of enzyme. Further Optimization of Enzyme Yield and Factors like pH, temperature, and incubation time can be evaluated to maximize enzyme yield. Microbial amylase is preferred due to their stability and varied properties.

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