Online at: https://researchtrendsjournal.com ISSN No: 2584-282X

INTERNATIONAL JOURNAL OF TRENDS IN EMERGING RESEARCH AND DEVELOPMENT

Volume 2; Issue 1; 2024; Page No. 50-53

Received: 13-12-2023
Accepted: 27-02-2024

Amylase enzyme isolation, identification, and characterization of different microorganisms isolated from soil

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Abstract

One of the most often employed enzymes in industries over the years is Amylase. Its primary job is to hydrolyze starch molecules into oligosaccharides and glucose units. Of the various varieties of amylases, α-amylase is highly sought-after because of its extensive application in the food, textile, baking, and detergent sectors. Microorganisms, plants, and animals can all provide it. The purpose of this investigation was to separate, identify, and describe the amylase enzyme that soil microbes create. Soil samples were gathered from several locations. Using starch agar plates and a starch hydrolysis test, bacteria that produced amylase were screened for. After being discovered, the organisms generating the largest zone of clearance were employed in the manufacture and characterisation of amylase. While the isolated fungus was identified as Aspergillus Niger based on colonial features and LPCB staining, the organisms were identified as Bacillus subtilis based on staining, cultural characteristics, colonial characteristics, and biochemical testing. The found organisms were further employed in submerged fermentation to produce amylase. The DNS technique was used to measure the amylase activity. Ammonium sulphate was used to partially purify the generated enzyme after centrifugation. The highest amylase production was seen for Bacillus amylase at 40 ºC and pH 6.5, and for Aspergillus Niger at 30 ºC and pH 7.5. In addition, the enzyme was characterized in preparation for its industrial use. For Bacillus subtilis, crude amylase exhibited maximum activity at pH 7 and 45 ºC, whereas for Aspergillus Niger, it was at pH 6.5 and 30 ºC. The detergent business indicated considerable possibilities for crude amylase's application.

Keywords: Aspergillus Niger, amylase, soil, detergent, organisms

1. Introduction

The use of living things-whether in their native or altered forms-for industrial or commercial purposes is known as biotechnology? Bio is the scientific study of life, encompassing all living things. The use of science in the creation of industrial and commercial goods is known as technology. Biotechnology should be understood as a collection of enabling technologies that involve the practical application of organisms or their cellular components to the manufacturing and service industries related to environmental management. It is not a product or range of products in and of itself. Of all the applied sciences, biotechnology is the one that is expanding the fastest at the moment. As opposed to being a single scientific field, biotechnology is also known as the clever science of biology, which connects the physical and biological sciences with technological advancements. Microbiology, biochemistry, molecular biology, cell biology, immunology, protein engineering, enzymology, classified breeding techniques, and the entire spectrum of bioprocess technologies are actually combined in this field. In the past, biotechnology was more of an art than a science. This was demonstrated by the production of cheeses, wines, and beers, for example, where the manufacturing processes were well-developed and reproducible but the molecular mechanisms were unclear (Linden A *et al*., 2000) [9]. These processes have been improved and better understood thanks to significant advancements in microbiology and biochemistry. Today's biotechnological procedures produce a vast array of novel products, such as monoclonal antibodies, enzymes, antibiotics, and vaccines. The production of these products has been enhanced by a plethora of novel molecular discoveries, enabling previously unheard-of modifications to biological systems.

1.1 Industrial importance of microbial enzymes

Many industries, including detergent, food processing, brewing, and pharmaceuticals, use microbial enzymes extensively. As point out, their use has been documented in oriental countries since antiquity, even if their functional utility is unknown. Additionally, they are employed in scientific, analytical, and diagnostic capacities (Asgher M, *et al*., 2007) [14]. Enzymes including pectinases, glucoamylases, proteases, and glucose isomerase have become commonplace in modern times and are widely used as commodities (Kunanmeni A, *et al*., 2005) [4]. A portion of the microbial enzymes utilized in industry. The majority of these are hydrolases, as may be observed. The majority of industrially significant enzymes are extracellular, meaning that cells secrete them into the surrounding media. These enzymes must be retrieved by being taken out of cells and other solid materials.

1.2 Fermentation studies for improved product production

The word "fermentation," which means "to boil," comes from the Latin word "fervere," which describes how yeast appears on fruit or malted grain extracts. The boiling sensation results from the anaerobic breakdown of the extract's carbohydrates, which produces CO_2 bubbles.

The anaerobic reactions that provided microorganisms with energy for growth in the absence of oxygen were referred to as "fermentation" by Pasteur. These days, the definition of fermentation is far wider. It is applicable to both aerobic and anaerobic metabolic processes in which an organic substance in the substrate causes particular chemical changes in the microorganisms.

Various microbes are used in fermentation processes to create a wide range of chemicals, including alcohols, organic acids, amino acids, vitamins, antibiotics, enzymes, single-cell proteins, hormones, and more. Using the proper kind of organism that can generate the required product in big quantities and at low cost is crucial to the success of fermentation.

1.3 Solid state fermentation

Solid-state fermentation (SSF) is defined as fermentation involving solids in the absence or almost absence of free water. However, adequate moisture is necessary for the substrate, which the microbes utilize for development and metabolism. SSF is recognized as the original fermentation process and has the capacity to produce enzymes. Thus, it should come as no surprise that the foundation of all ancient fermentation processes was SSF.

When treating agricultural and industrial leftovers, SSF may be the optimum option. Since solid-state procedures are more environmentally friendly, need less energy, and generate less wastewater, they are a good solution for the solid waste disposal issue. A novel process development for the value addition of these inexpensive wastes is provided by the SSF's continued use of agroindustrial residues. Currently, microbial products like feed, fuel, food, industrial chemicals, and pharmaceuticals are produced on a commercial scale using SSF techniques. Numerous benefits have resulted from its use in bioprocesses as bioleaching, biobeneficiation, bioremediation, and biopulping, among others.

The right substrate must be chosen for the SSF; it must be insoluble and serve as a source of nutrients as well as a physical support. A solid substrate is required, and these can be found naturally in agricultural products, agricultural industry waste, or inert supports. When choosing a substrate, there are two main factors to take into account: the first is the particular substrate, which calls for appropriate value addition or disposal. The second objective can have to do with creating a particular product from an appropriate substrate.

The ideal substrates for SSF procedures are typically thought to be agricultural industrial leftovers. Canned bagasse, wheat bran, maize bran, gram bran, rice bran, rice husk, soy hull, grape vine, trimmings, sawdust, banana waste, tea waste, palm oil waste, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meat, coconut & mustard oil cake, wheat & corn flours, steamed rice, and starch, among other substrates, have all been used in the SSF process.

But wheat bran is the most important and widely utilized material in a variety of processes. The choice of substrates for enzymes is influenced by a number of variables, chief among them being substrate cost. The microbes growing in the substrate might receive all the nutrients they require from it, but some of the nutrients might be present at less than ideal amounts or sometimes not at all. In some situations, exogenous nutrition supply can overcome this.

The substrate's particle size is crucial because, generally speaking, smaller substrate particles offer a bigger surface area for microbial attack. The moisture content is another crucial element. It has been suggested that the water activity (aw) of the medium is a vital parameter for the mass transfer of water and solutes between the microbial cells. Water has a significant impact on the physicochemical properties of the solids, which in turn affects the overall process productivity. This parameter can be exploited to alter the metabolic synthesis or excretion of microbial products. The accessible surface area, medium pH, and incubation temperature are other factors that affect the product production under SSF.

2. Materials and Methods

Green gram, rice bran, and wheat bran are among the various agro-industrial waste materials that were gathered from the neighborhood market. They were then processed using the USA standard sieve set, Nos. 7, 10, 14, 18, and 50, to produce mean particle sizes of 2.0–1.4, 1.4–1.0, and 1.0–0.3 mm, which were then stored until needed again. Sigma (USA) provided the gel filtration matrix with a medium viscosity of 500 m Pas, sodium alginate, and Sephadex G-100. Qualigens, an Indian company, supplied all other analytically grade compounds that were used. Samples were gathered from a variety of locations, in order to isolate the amylase enzyme from Aspergillus species. The samples were then transported to the laboratory and kept at 4 ºC until needed.

3. Results and Discussion

This section describes how to isolate bacteria from various environments, check for amylase activity, identify the isolate using ribotyping and Bergey's manual of bacteriology, and optimize medium for amylase synthesis in

both submerged and solid state fermentation. Furthermore, the amylase's characterisation and purification processes as well as possible uses are covered.

3.1 Isolation and screening for amylase producing organisms

The amylase-producing bacterial strains were isolated using soil samples gathered from several alien environments. Before being used for isolation tests, one gram of soil was suspended in sterile distilled water and well stirred for an hour at room temperature. To solidify the agar solution, sterile 1% potato dextrose starch agar plates were made and incubated for 15 minutes at 4 ºC. After bringing the plates to room temperature and maintaining sterility, 0.1 cc of soil solution was sprinkled across them. These plates were kept in an incubator at 30 degrees Celsius. The plates were examined for microbial colonies with a distinct zone of hydrolysis after being incubated for 24 hours. Agar slants containing starch were used to cultivate over thirty distinct microbial strains that were chosen from several plates. Using 1% potato dextrose starch agar plates, each isolate's production pattern was further validated. 17 strains were further tested based on the zone of clearance, and one colony that displayed an extremely clean zone was chosen for additional research and given the designation MK 07.

Table 1: Effect of incubation period on amylase activity

Incubation period (Hrs.)	Amylase activity (U/ml)
12	7
24	21
36	29
48	41
60	49
72	55
84	38
96	32
120	25

Table 2: Amylase activity at different temperatures

Amylase Activity at Different Temperatures	
Temperature (°C)	**Amylase activity (U/ml)**
20	26
25	42
30	59
35	46
40	31

Table 3: Amylase activity at different pH

pH	Amylase activity (U/ml)
3.5	11
4	30
4.5	43
5	63
5.5	51
6	27
6.5	8

Table 4: Varying percentages of inoculum levels

Inoculum Levels (%)	Amylase activity (U/ml)
3	57
5	61
7	54
10	48
15	41

Table 5: Effect of different carbon substrates

Carbon substrate	Amylase activity (U/ml)
Sucrose	69
Glucose	63
Starch	58
Maltose	42
Lactose	13

The current study, "Isolation, identification, characterization, and fermentative production of alpha amylase by Aspergillus sp." found that

- A soil sample collected from the dump yards of a local starch processing industry contained an efficient Aspergillus sp. amylase-producing microbial species.
- Using Bergey's manual of bacteriology and ribotyping, the strain was identified.
- Using traditional and statistical software, the Aspergillus sp. MK 07 strain was described for its production of amylase.
- With the fermentation settings optimized, the enzyme output increased from 55 U/ml to 85 U/ml in SmF and from 106 U/g to 164 U/g in SSF.
- Studies on solid state fermentation have demonstrated that the type of agricultural material used, along with other fermentation parameters such as medium pH, incubation temperature, inoculum level, moisture content, particle size, and carbon and nitrogen supplementation, all have an impact on the generation of enzymes.
- With optimized solid state conditions, the overall output of enzymes was enhanced from 106 U/g to 164 U/g using wheat bran as the substrate material.
- The Fermentor research yielded the greatest amylase activity of 1734 U/ml at 250 RPM. However, after optimizing all process variables, the activity might reach up to 2112 U/ml.

4. Conclusion

Because biotechnology has so many advantages over convectional chemical methods, it is growing quickly. The fundamental components of biotechnology operations are industrial enzymes. Three application segments make up the industrial enzyme market: technical enzymes account for 60% of the market. Enzymes found in food (32%) and animal feed (8%). Of all industrial enzymes, amylase is essential for a wide range of applications, including home processing, leather processing, environmental pollution reduction, the development of diagnostic kits, health care

products, value-added product manufacturing, and clinical applications. With a growth rate of 6.5%, the global amylase market is predicted to reach over 25 billion US dollars by 2010–11. Currently, various forms of amylases account for 60% of the global enzyme market. Most enzymes used in industry today are derived from microorganisms. These enzymes are manufactured on an industrial scale through the fermentation of bio-based materials.

Thirty distinct microbial strains that produce amylase were identified from effluent soil samples derived from a nearby starch plant. One of the strains exhibiting increased activity was chosen from among them and given the name MK 07. This strain was evaluated using a number of methods listed in Bergey's Manual of Bacteriology. The strain was identified as Aspergillus niger after it thrived best at pH 5.0 and in the temperature range of 20 to 40 degrees Celsius. This strain was therefore given the designation Aspergillus niger MK 07.

PDA was used to study the effects of various fermentation parameters, such as medium pH, incubation temperature, inoculum concentration, incubation time, and RPM. Plackett-Burman design was used to assess the effects of various nitrogen and carbon sources, including soybean meal, yeast extract, corn steep liquor, beef extract, potassium nitrate, ammonium sulphate, and ammonium nitrate, as well as glucose, starch, arabinose, ribose, xylose, sucrose, fructose, and mannose. The synthesis of the enzyme was enhanced to 164 U/g in the medium containing 3% sucrose, at optimal conditions of 30 °C and pH 5.0.

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