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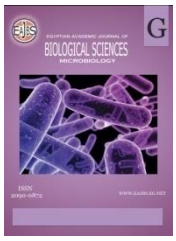

Screening and Optimization of the Production of Alpha-Amylase Enzyme from Streptomycetes

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ABSTRACT

Amylases are enzymes that help starch break down into sugars. These Enzymes make up about 25% of the market for enzymes and have numerous industrial applications. Amylases are virtually entirely gone supplanted chemical methods in the starch hydrolysis business, microbial amylases being the most abundant and widest range of applications in the industries related to food, fermentation, textiles, and paper. Many different types of plants have developed this enzyme, including microorganisms like bacteria, fungi, algae, and actinomycetes. 37 isolates of the actinomycetes from various Egyptian governorates' soil were used in this investigation. Only 12 Streptomyces isolates were found to have the best capacity for the production of extracellular alpha-amylase, as shown by the existence of a clear zone surrounding their colonial growth. Starch and yeast extract were efficient suppliers of carbon and nitrogen, respectively. Alpha amylase requires a temperature of 30 degrees, a pH of 7, and an incubation period of seven days.

INTRODUCTION

The internal 1,4-glycosidic acid's hydrolysis connections substances of low molecular weight, such as glucose and maltose from starch units is catalysed by Alpha-amylases (E.C.3.2.1.1). (Gupta *et al.*, 2003; Kandra, 2003; Rajagopalan & Krishnan, 2008). Amylases, a type of Industrial enzyme, which makes up around 25% of the market for enzymes worldwide, are among the most significant enzymes and have significant biotech implications. (Rajagopalan & Krishnan, 2008; Reddy *et al.*, 2003). They come from a variety of sources, including plants, animals, and microbes. Many microbial amylases are now commercially accessible, and they have nearly entirely supplanted chemical starch hydrolysis in the starch processing business. Microorganisms' amylases have a wide range of industrial uses because They are stronger than those produced using Alpha-amylases from plants and animals. (Tanyildizi *et al.*, 2005).

The ability to produce amylases in large quantities at low cost Considering how simple it is to engineer bacteria to create enzymes with certain qualities are the two main benefits of employing microbes for this purpose. Several fungi, yeasts, and bacteria have been used to create Alpha-amylase. However, applications for enzymes from bacterial and fungal sources have predominated in the industrial sectors. (Gupta *et al.,* 2003). Alpha-Amylases may be a variety of industrial procedures, such as those used in the food, paper, detergent, fermentation, textile, and pharmaceutical sectors. Amylases produced by fungi and bacteria may have applications in the fine-chemical and pharmaceutical sectors. However, because of advancements in biotechnology, the use of amylases has increased in a variety of sectors, including clinical, pharmaceutical, and brewing as well as analytical chemistry, distilling, food, textile, and food sectors. (Gupta *et al.,* 2003; Kandra, 2003; Pandey *et al.,* 2000). The present review highlights the microorganisms that create Alpha-amylases, one of the most well-known and significant types of industrial amylases. Alpha-Amylase is used in a variety of processes, including the liquefaction of sugar and the gelatinization of starch. (Pranay *et al.,* 2019; Lin *et al.,* 1998; Ullah *et al.,* 2021), in the detergent and textile industries (Pranay *et al.,* 2019; Gupta *et al.,* 2003; Ullah *et al.,* 2021), for reducing paper size (Porfirif *et al.,* 2016; Porfirif *et al.,* 2020), medicines, the manufacture of biofuels, and analytical chemistry (Gupta *et al.,* 2003; Abdullah *et al.,* 2014; Toda *et al.,* 1982; Machius *et al.,* 1995). Many scientists have previously investigated the synthesis of extracellular Alpha-amylase from various actinobacterial isolates. But up until this point, there has been no report on the statistical optimization of the production of Alpha-amylase from the actinobacterium Streptomyces fragilis DA7-7 found in desert soil. Therefore, the objectives of the current study are to: (1) isolate and screen actinobacterial producers of -amylase from desert soil; (2) characterize the potential isolate DA7-7; (3) pre-optimize and statistically optimize Alpha-amylase production; and (4) extract, purify, and characterize Alpha-amylase produced by S. fragilis DA7-7. To withstand harsh climatic conditions, wild-type Alpha-amylases need to undergo several important changes. The application of some of the most promising methods to modify the structural domains of wild-type amylases so that they may be used under harsh settings is currently the main focus, rather than only the manufacture of amylase. Therefore, the aim of this publication is to present the state-of-the-art and emerging trends in microbially generated Alpha-amylases. It will concentrate on the several synthetic and agricultural waste substrates, the downstream methods, particularly the most recent and appropriate immobilization techniques, and genetic engineering techniques tailored for the generation and stabilization of Alpha-amylase. Offering solutions to satisfy the industry demand, will also solve the flaws in each stage of Alpha-amylase production and stability.

MATERIALS AND METHODS

Isolation of Streptomycetes:

Streptomycetes isolates remained separate from the soil at different governorates in Egypt from Giza (G), Dakahlia (D), and Gharbia (GH). Using serial dilution method on marine media containing: starch 10 g/L, K_2HPO_4 1.0 g/L, $MgSO_4.7H_2O$ 0.5 g/L, NaCl 0.5 g/L, KNO_3 2.0 g/L, $CaCO_3$ 2.0 g/L, $FeSO_4.7H_2O$ 0.01 g/L, agar 20.0 g/L and an adjusted pH. Streptomycetes were isolated using agar slants that had the same constitution as the main plating medium. and then purification to obtain single colonies.

The Rapid Assay Plate Method:

To examine the production of the amylase enzyme by using nutritive starch agar, streptomycetes isolates were streaked on the plates in a single line and incubated at 28 ºC for 4 days. A positive test result meant that the medium around the colonies had formed a zone of clearance, which was then measured in millimetres (mm) after being flooded Mix

100 mL of distilled water with iodine solution (5 g of iodine and 10 g of potassium iodide).

Enzyme Production and Amylase Assay:

Twelve isolates of streptomycetes are capable of maximizing alpha-amylase production. Further testing for enzyme production was done on the isolates' maximal zones of clearance. Individually injected into 500 mL 100 mL of ISP-2 yeast extract-malt extract broth is the name for the International Streptomyces Project-2 broth. was placed in Erlenmeyer flasks. the prospective isolates were cultured at 28 ºC for 4 days with a rotary shaker running at 120 rpm. The supernatant was recovered after centrifuging the fermented broth at 5000 rpm for 20 min at 4 ºC. Bernfeld (1955) described a spectrophotometric method for measuring the amylase test. A test tube containing 1 mL of the culture filtrate was then filled with 1 mL of 1% soluble starch in 0.1 M sodium phosphate buffer (pH 7). For 10 minutes, the tubes were covered and heated to 35 ºC. After stopping the reaction with 2 mL of DNS reagent in each tube, the incubation period in a boiling water bath was extended by 10 minutes. The final volume was brought down to 10 mL by adding distilled water after chilling at room temperature, and the absorbance was measured at 540 nm using a spectrophotometer.

Determination of Extracellular Protein:

According to Bradford (1976), the Bradford reagent was used to measure the protein concentration of the produced crude.

Medium Optimization for the Production of Alpha-Amylase:

Optimisation of media components needed for the streptomyces strain to produce the most methininase was assessed at broth media. (C). The effects of various incubation times (4, 5, 6, 7, 8 and 9 days), various pH levels (3, 4, 5, 6, 7, 8 and 9 adjusted with 1 N HCl or 1 N NaOH), and various incubation times were then tested on the medium componenta range of additional carbon sources (glucose, xylose, arabinose, manitole, galactose, fructose, succrose, lactose, maltose, and starch at 1% w/v) at a range of temperatures (20, 25, 30, 35, and 40 C). and other nitrogen sources (ammonium oxalate, ammonium chloride, ammonium molybidate, and ammonium sulphate), as well as yeast extract, malt extract, peptone, beef extract, and sodium nitrate.

RESULTS

1. Isolation of Streptomycetes:

Isolates of streptomycetes were chosen. depending on the unique morphology of the colony, which is usually rounded, convexed in shape, having a growth that is firmly rooted in the medium. Spore masses usually cover the colonies' surface, they are dry and powdery. Randomly 37 Streptomycetes isolates have been found in a variety of habitats, including soil. The highest number of streptomycetes isolates was recovered from Dakahlia (15 isolates) followed by Giza (12 isolates) and then Gharbia (10 isolates).

2. Rapid Assay for The Production of Alpha-Amylase:

Rapid plate assay test methods are used for screening the ability of thirty-seven *streptomyces* isolates for the production of alpha-amylase. Table (1) showed that only Nine isolates of Streptomyces were distinguished by the pink tint around their imperial expansion as proof and by having the highest capacity for the production of extracellular alpha-amylase.

Table 1. Screening for production of alpha-amylase from streptomycetes isolated from soil by qualitative rapid plate assay test.

Sample	Results	Sample	Results
D 1	-	**G 5**	-
D 2	+	**G 6**	+
D 3	-	**G 7**	+
D 4	-	**G 8**	-
D 5	+	**G 9**	-
D 6	-	**G 10**	-
D 7	+	**G11**	-
D 8	-	**G12**	-
D 9	-	**GH 1**	-
D 10	+	**GH 2**	+
D 11	-	**GH 3**	-
D 12	-	**GH 4**	-
D 13	-	**GH 5**	+
D 14	-	**GH 6**	+
D 15	-	**GH 7**	+
G 1	-	**GH 8**	-
G 2	+	**GH 9**	-
G 3	+	**GH 10**	-
G 4	-		

3. Quantitative Screening for Production of Alpha-Amylase:

Twelve streptomyces isolates (D2, D5, D7, D10, G2, G3, G6, G7, GH2, GH5, GH6 and GH7) were quantitively screened for alpha-amylase production and distinguished by the presence of a distinct zone surrounding their colonial expansion as proof of extracellular alpha-amylase production. (Table 2). Every isolate's protein estimate, enzyme production, and specific activity were assessed, and isolate (D10) revealed the highest specific activity.

Table 2. Quantitative screening of *Streptomyces* isolates for alpha-amylase formation.

Samples	Total activity (U)	Total protein (mg)	Specific activity (U/mg)
D2	410.55	5.7	72.02
D5	589.03	5.3	111.13
D7	600.53	4.9	122.55
D10	825.33	4.7	175.60
G2	798.67	5.1	156.60
G3	683.90	5.8	117.91
G6	710.63	6.2	114.61
G7	723.89	5.0	144.77
GH2	529.71	4.3	123.18
GH5	598.09	5.6	106.80
GH6	476.38	5.3	89.88
GH7	511.82	6.2	82.55

4. The Outcome of Features Affecting Alpha-amylase:

The Outcome of Time: alpha-amylase production by Streptomycete (D10) was affected by an incubation time test. Results in (Fig. 1) reveal that alpha-amylase synthesis gradually increases until 7 days, at which point it reaches its maximum (826.87 U), and subsequently, enzyme activity declines.

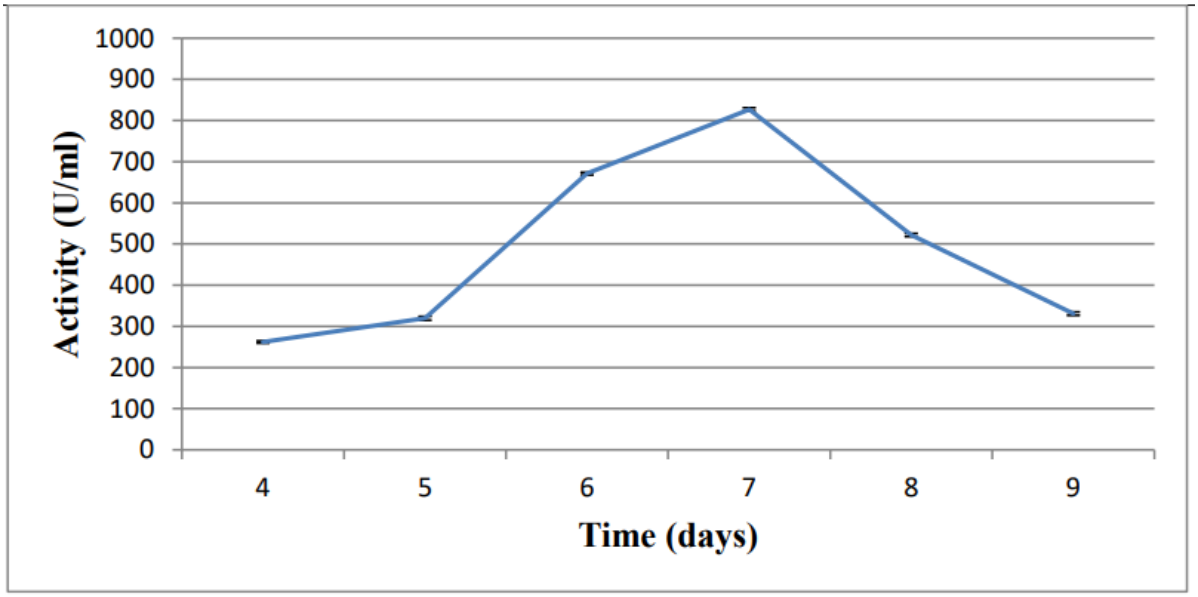

Fig. 1. The outcome of incubation time on alpha-amylase production

The Outcome of pH: Streptomycete (D10) synthesis of alpha-amylase is influenced by pH. The pH of the synthesis of enzymes is influenced by the fermentation media, as shown in (Fig. 2). Thus, at pH 7.0, the maximum enzyme yield was observed to be (829.21 U). Reduced enzyme synthesis was brought on by either an increase or a drop in the medium's pH.

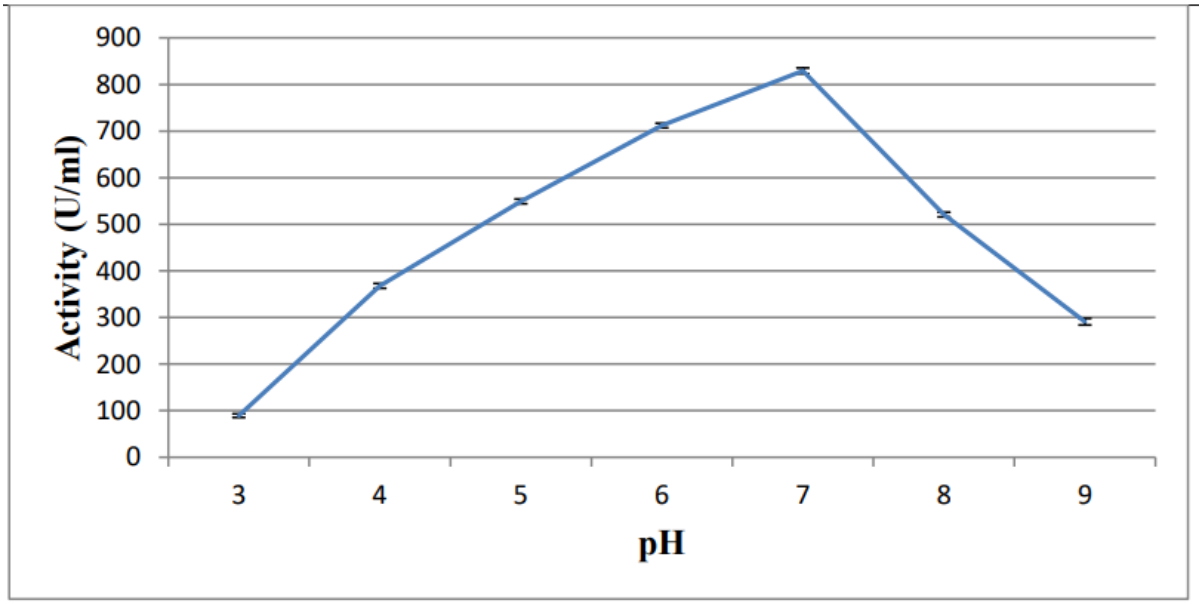

Fig. 2. The outcome of incubation pH on alpha- amylase production**.**

The Outcome of Temperature: The results validated a substantial correlation between alpha-amylase synthesis and incubation temperature up to 30 °C (Fig. 3), where an ASNase output of 856.92 U was recorded. Compared to the optimal temperature value, there was a significant drop in enzyme synthesis at 35 and 40 °C, respectively.

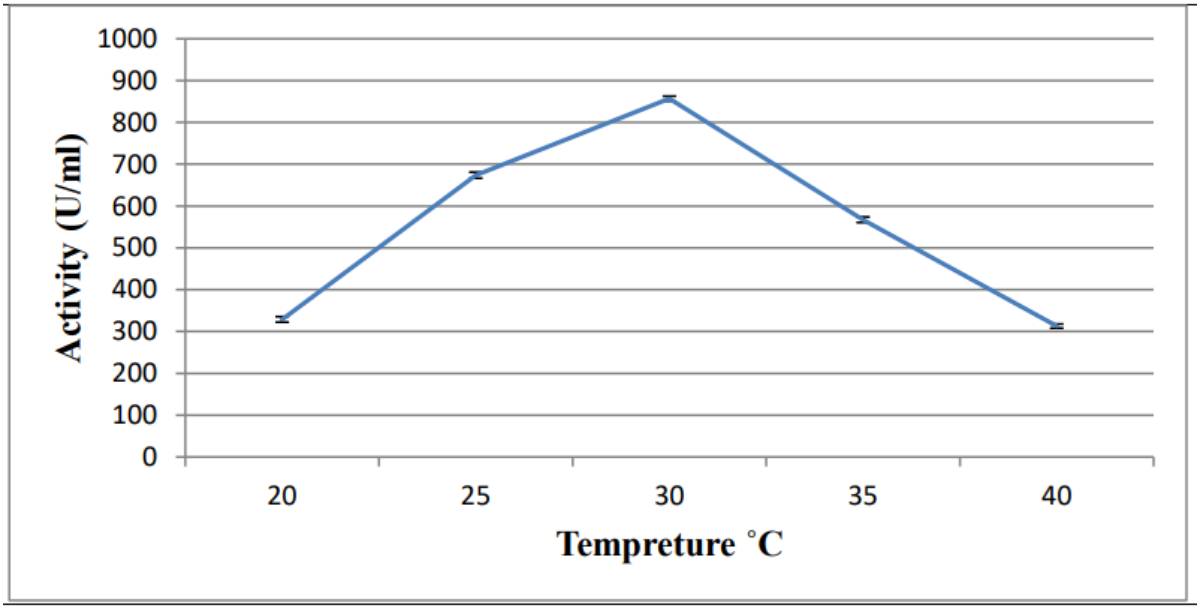

Fig. 3. The outcome of incubation temperature on alpha-amylase production.

The Outcome of Different Carbon Sources: In our data, the ability of the Streptomycete (D10) isolate to utilize several carbon sources to produce alpha-amylase was examined. The carbon source employed significantly affected the synthesis of alpha-amylase, with starch being the favored carbon source and yielding enzymes with an activity of 879.99 U (Fig. 4). It is interesting to note that the other carbon sources examined couldn't sustain bacterial growth and enzyme synthesis.

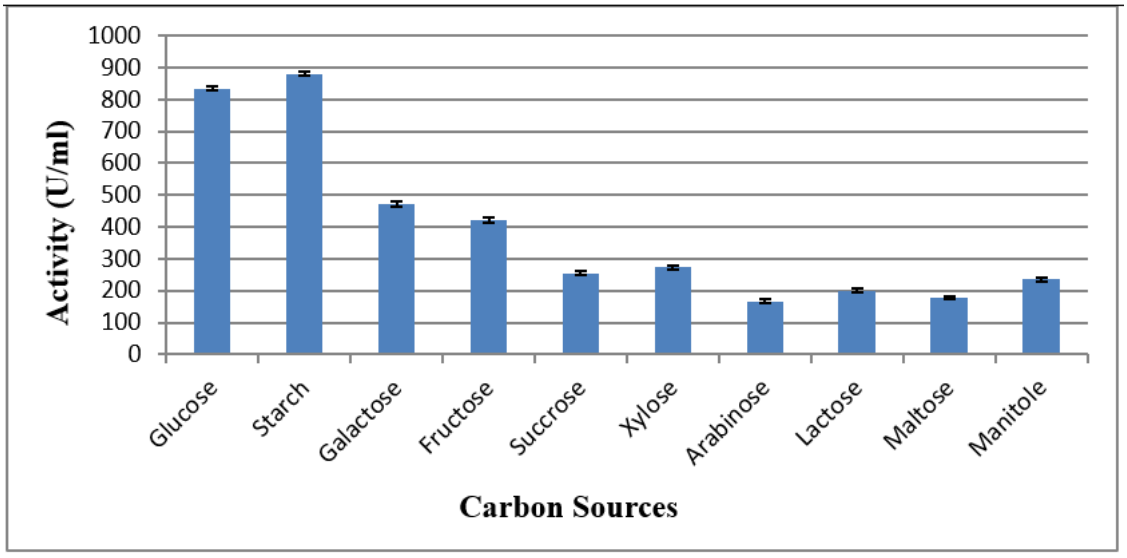

Fig. 4. Effect of different carbon sources on alpha-amylase production**.**

Effect of Different Nitrogen Sources: One of the nitrogen sources studied for its possible impact on alpha-amylase synthesis was yeast extract, along with peptone, beef extract, ammonium sulfate, and ammonium chloride. The results displayed that yeast extract was the greatest source of nitrogen for alpha-amylase production878.45 U For Streptomycete (D10) (Fig. 5).

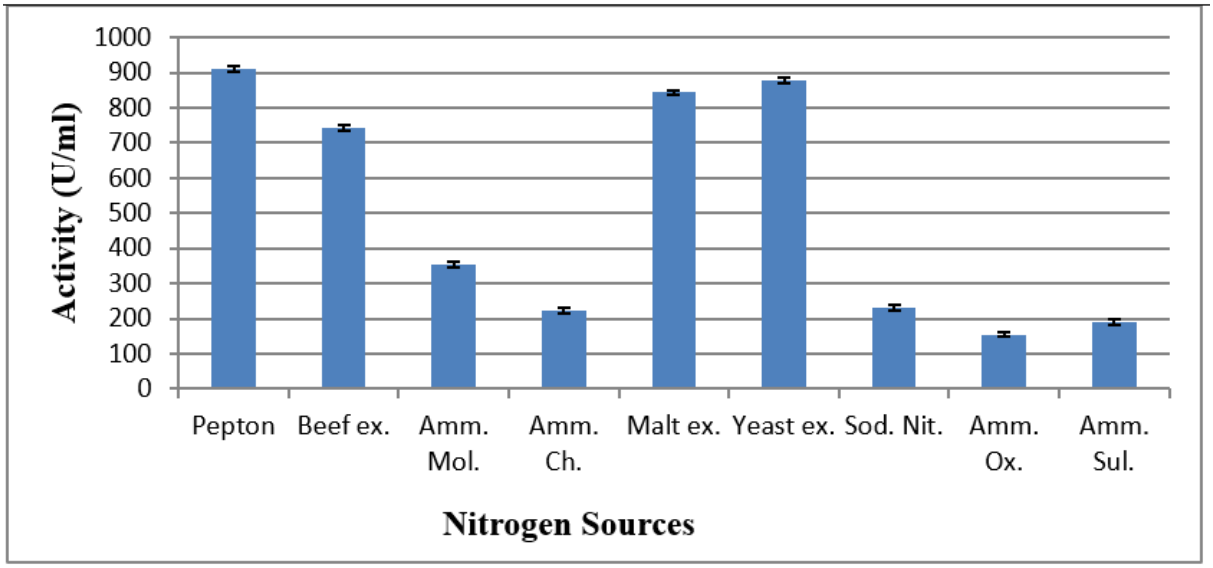

Fig. 5. Effect of different nitrogen sources on alpha-amylase production**.**

DISCUSSION

Among the most significant commercial enzymes, amylase is used globally in a variety of industrial fields. Actinomycetes' diversity is significant in many branches of research and medicine. They are a plentiful source of bioactive and varied chemicals. (Bernan *et al.*, 1997). The goal of this investigation was to identify a strong alpha-amylase production from a distinct soil source. The extraordinary source of extracellular and intercellular enzymes produced by streptomyces is essential for usage in industry and commerce. Thirty-seven isolates of actinomycetes from different Egyptian governorates could produce alpha-amylase by serial dilution method. Several studies have shown that Egypt's soil is abundant in microbial species that could be helpful in the hunt for novel bioactive substances. (Debbab *et al.*, 2010 & El-Gendy *et al.*, 2000).

The plate assay method was selected as a suitable method for qualitative and Hydrolysis zones on starch agar plates discovered during the initial screening of investigated strains for Alpha-amylase production. The clear zone showed that starch had been hydrolyzed by Streptomyces. Our analysis revealed that twelve isolates from 37 actinomycetes isolates produce alpha-amylase to varied degrees by producing clear zones.

There is more research being done that uses starch agar plates to isolate and screen amylase. Bacillus subtilis B19 was used to detect amylolytic activity. (Dash *et al.*, 2015). Aspergillus versicolor and Penicillium sp. confirmed the existence of a generation of amylase on the agar plate. (Gopinath, *et al.*, 2017). Numerous strains, including S. gulbargensis, Streptomyces strain A3, S. avermitilis, and S. rochei BTSS 1001, have produced amylase. (Syed *et al.*, 2009 & Hwang *et al.*, 2013). This investigation was done at the same time as earlier amylase on starch agar analyses that came to similar conclusions.

The Amylase activity and protein content were checked for 12 streptomycetes isolates and the specific activity was identified, the maximum specific activity was shown by isolate (D10). among the key industrial enzymes is amylase. Several tonnes of amylase are utilised annually in Iran's numerous industrial uses. (Moghbeli *et al.*, 2009). Streptomyces gulbargensis DAS 131, a newly discovered alkali-thermotolerant strain, produced a high amount of extracellular amylase. (Syed *et al.*, 2009).

The optimal conditions for Streptomycete (D10) alpha-amylase

production found in this study were consistent with those found that alpha-amylase production is significantly inclined by the conformation media for fermentation and the cultural circumstances, with time, temperature, pH, and different sources of carbon and nitrogen. The maximum synthesis of the alpha-amylase under investigation in this work was seen at 72 hours, during the stationary phase of bacterial development, demonstrating a relationship between the creation of enzymes and bacterial development. The strain used for alpha-amylase production will determine the best pH, with variations perhaps caused by the fermentation process or the unique genetics of the microbial species.

On the contrary, the makeup of the medium affects the growth of actinomycetes and the synthesis of enzymes. In order to change the productivity of enzymes, it is critically necessary to maximise bacterial production and utilisation on an industrial scale using physical variables and medium components. (Al-Dhabi, *et al.,* 2020). Actinomycetes' proliferation and synthesis of extracellular hydrolytic enzymes are influenced by physical conditions and the makeup of the culture medium. To boost the output of bacterial enzymes, it is therefore vitally necessary to optimise physical parameters and medium components. (Al-Dhabi *et al.,* 2020). We looked into the ideal situations for strain NAA-28 to produce amylase. According to the findings, the strain produced the most amylase when peptone and starch were added to the growth medium as Additional sources of both carbon and nitrogen.

Conclusion

Streptomyces sp. was isolated from soil environments obtained from different governorates in Egypt for maximum amylase production with optimum pH 7, and 30 degrees Fahrenheit and a 7-day incubation period. The best sources of carbon and nitrogen were starch and yeast extract, respectively.

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