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# Screening and optimization of the production of xylanase enzyme from streptomycetes

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**Abstract**

Xylan is recognized as the primary constituent of plant hemicelluloses and holds potential as a suitable initial material for synthesizing a range of artificial compounds and mixtures. Following cellulose, xylan ranks as the second most abundant polysaccharide found in the natural environment. The extraction of xylooligosaccharides from heteroxylans is achieved through both chemical and enzymatic approaches, with the latter method offering several advantages such as precision, substitution pattern and linkage type specificity, reaction manipulation, and elevated reaction rates. Xylanase is an innate enzyme present in organisms and fungi, falling under the category of pentosanases—an enzyme group used for breaking down plant cell wall matrices through xylan hydrolysis into xylose. Consequently, this inherent characteristic of the xylanase enzyme has bestowed it with commercial significance. Xylanases, classified as glucosidases (O-glycoside hydrolases, EC 3.2.1.x), facilitate the endo hydrolysis of 1,4-β-D-glycosidic bonds within xylan molecules. Among the most noteworthy xylanolytic microorganisms are Aspergilli, Trichoderma, streptomycetes, Bacilli, and others. In this investigation, twenty-one streptomycete isolates were obtained from various locations across Egypt and subjected to quantitative screening for xylanase production. The most potent isolate, Streptomyces (C5), was isolated from the Cairo region. Xylan and sodium nitrate were identified as effective carbon and nitrogen sources, respectively. The optimal pH for xylanase activity was determined to be 7, with an ideal temperature of 30°C, and an incubation period of seven days.

**Keywords:**

Actinomycetes, xylanase, isolation, optimization, screening.

environments (e.g., the backwoods) where material of plant gather and weakens. It has been determined that xylanolytic microorganisms are capable of delivering extremophilic xylanases. They have been secluded from various sources like soda deserts, soda lakes, warm springs marine solfateric fields, antarctic conditions **(16)**.

The primary benefit of employing enzymes in the industrial context stems from their effectiveness, precision, and environmentally conscious characteristics. Microbial enzymes are favored over their plant and animal counterparts due to their cost-effectiveness, substantial production output, reliability, adaptability for product customization, continuous availability unaffected by seasonal fluctuations, rapid growth of microorganisms in economical media, stability, and enhanced catalytic activity **(9)**.

Xylanase is a normally occurring enzyme that is tracked down in organisms and growths. Xylanase enzymes have a place with pentosanases, a group of enzymes that is used for hydrolysis of the plant cell wall matrix by hydrolysis of xylan into xylose. Consequently, this characteristic of the xylanase enzyme renders it commercially significant. Recently, genetically modified organisms (fungi and bacteria) have been used to produce xylanases. Xylanases have several applications across multiple industries. These include beverage industry, paper and textile industry, pharmaceutical industry, biofuel production and bakery industry **(16)**.

The present work was carried out for the collection of different local soil samples, isolation of different types of streptomycetes isolates, screening, and optimization for enzyme production from the isolated streptomycetes.

## 2.Materials and Methods

1. **Sampling:** In 2020, samples of agricultural soils from three distinct governorates, namely Giza, Cairo, Gharbia, and Dakahlia, were collected in polyethylene bags and then kept at 4°C in sterile containers.

## 1.Introduction

Xylan stands as a noteworthy polysaccharide within the natural realm and plant cellular structure. It emerges as the predominant polysaccharide, comprising roughly one-third of the sustainable carbon sources present on Earth. This polysaccharide constitutes a major portion of hemicellulose, which is an intricate amalgamation of carbohydrate hydrolases encompassing xylan, xyloglucan, galactoglucomannan, arabinogalactan, and glucomannan. Sizeable quantities of xylans are prominently located in angiosperm hardwood, where they comprise 15-30% of the cell wall content, and in gymnosperm softwood, constituting 7-10%, relatively lower than in annual plants measuring less than 30% **(17)**. The configuration of xylan varies across plant species, with the homopolymeric backbone chain of 1,4-linked beta-D xylopyranosyl units capable of being adorned with a range of alternate side chain groups. Due to its diversity and intricacy, the complete breakdown of xylan varies in structure among different plant species, and the homopolymeric backbone of 1,4-linked beta-D xylopyranosyl units can be augmented with various substituent side chains. Owing to its heterogeneous and complex nature, the thorough degradation of xylan necessitates a diverse array of enzymes that function synergistically, including beta-D xylosidases (which cleave the xylan backbone), endo-1,4-beta-D-xylanases (which break the xylan backbone), beta-D xylosidases (which release xylose monomers from the non-reducing end of xylooligosaccharides and xylobiose), alpha-D-glucuronidases, alpha-L-arabinofuranosidases, and so forth, to effectively remove the distinct substituent side chains. A Total xylanolytic compound framework including this action is all very much presented in the microorganisms like fungi **(19)**. The absolute most significant xylanolytic organisms like Aspergilli, Trichoderma, Ruminococci, Pheanerochaetas, Chyridiomycetes, Streptomycetes, Clostridia, Bacilli, Fibrobacters etc. These microorganisms' ecological niches are diverse and widespread, typically including

5. **Determination of Extracellular Protein:** The protein content of the crude preparation was determined using the Bradford reagent **(Bradford, 1976).**
6. **Medium Optimization for production of Xylanase:**

Optimization of media components needed for the Streptomyces isolates has high Xylanase activity was assessed. The effects of various incubation periods (4, 5, 6, 7, 8, and 9 days), various pH levels (3, 4, 5, 6, 7, 8, and 9 adjusted with 1 N NaOH or1 N HCl), and various incubation temperatures (20, 25, 30, 35, and 40 C) were then tested on the medium component and a variety of additional sources of carbon (glucose, lactose, fructose, arabinose, xylose, mannitol, galactose, sucrose, starch and maltose at 1% w/v) and other nitrogen sources (ammonium chloride, ammonium oxalate, ammonium sulfate and ammonium molybdate), as well as malt extract, beef extract, peptone, yeast extract, and sodium nitrate.

**3.Results**

**1. Isolation of streptomycetes**

Streptomycetes isolates were selected depending on the unique morphology of the colony, which is usually rounded, convexed in shape, with deeply rooting growth into the medium. Spore masses usually cover the colony's surface, they are dry and powdery. Randomly 21 streptomycetes isolates were isolated from different environments. The highest number of streptomycetes isolates was recovered from Cairo followed by Dakahlia then followed by Gharbia and Giza.

(Table 1) showed the distribution of the streptomycetes isolates.

2. **Isolation of Xylanases produced Streptomycetes:** The fermentation medium composition included (in grams per liter): 10 g of birchwood xylan, 4.5 g of KNO3, 0.075 g of K2HPO4, 1.5 g of KH2PO4, and a trace element solution of 2.7 ml/l, containing (in grams per liter): 0.16 g of MnSO4·H2O, 0.14 g of ZnSO4·7H2O, 0.5 g of FeSO4·7H2O, and 0.2 g of CoCl2·2H2O in distilled water. The pH of the medium was adjusted to 7.2 after sterilization using sterile 1 N NaOH. An inoculum of 2.5 ml was introduced into 250-ml Erlenmeyer flasks containing 50 ml of sterile culture medium. The flasks were placed on an orbital shaker and incubated at 28°C for 120 hours. After incubation, the extract was subjected to centrifugation at 10,000 g and 4°C for 10 minutes, and the clear supernatant was evaluated for its xylanase activity.
4. **Xylanase assay:** Using the xylan compound, the activity of the xylanase enzyme was measured **(Bailey, Biely, & Poutanen, 1992)**. After introducing 0.2 ml of culture supernatant into a 2 ml xylan solution (1.5% concentration; pH 5.0; using 200 mM acetate buffer), the mixture was incubated at 50°C. After 20 minutes, the reaction was halted by adding 2 ml of 3,5-dinitro salicylic acid reagent. The quantity of reducing sugars produced in the reaction was estimated by measuring the absorbance at 535 nm **(Miller, 1959)**. Under assay conditions, 1 UA of xylanase activity corresponds to the quantity of enzyme required for releasing 1 mol of xylose per minute.

**Table 1:** Total number of streptomycetes isolates.

| Locality | No. of isolates | Incidence percent (%) |
|---|---|---|
| Cairo (C) | 8 | 38.1 |
| Dakahlia (D) | 6 | 28.6 |
| Gharbia (GH) | 4 | 19 |
| Giza (G) | 3 | 14.3 |
| Total | **21** | **100** |

**2. Quantitative screening for the production of Xylanase:**

Twenty-one Streptomyces isolates were screened quantitatively for the production of Xylanase (Table 2). For every isolate, enzyme formation, protein estimation and specific activity were measured, and isolate C5 demonstrated the highest specific activity. Table 2. Quantitative screening of Streptomyces isolates for Xylanase formation.

| Samples | Total activity (U) | Total protein(mg) | Specific activity(U/mg) |
|---|---|---|---|
| C1 | 14.5 | 5.2 | 2.79 |
| C2 | 19.4 | 5.4 | 3.59 |
| C3 | 13.7 | 4.6 | 2.978 |
| C4 | 18.5 | 4.1 | 4.51 |
| C5 | 27.4 | 4.4 | 6.23 |
| C6 | 21.3 | 5.4 | 3.94 |
| C7 | 22.5 | 6.0 | 3.75 |
| C8 | 17.3 | 5.2 | 3.33 |
| D1 | 15.3 | 4.9 | 3.12 |
| D2 | 12.8 | 5.2 | 2.46 |
| D3 | 19.0 | 5.6 | 3.39 |
| D4 | 15.9 | 6.3 | 2.52 |
| D5 | 8.4 | 4.5 | 1.87 |
| D6 | 16.6 | 5.3 | 3.13 |
| GH1 | 19.0 | 4.7 | 4.04 |
| GH2 | 21.8 | 4.9 | 4.45 |
| GH3 | 19.7 | 5.1 | 3.86 |
| GH4 | 25.4 | 6.4 | 3.97 |
| G1 | 11.1 | 6.3 | 1.76 |
| G2 | 13.5 | 5.4 | 2.5 |
| G3 | 13.7 | 5.7 | 2.4 |

**3. The Outcome of Features Affecting Xylanase:**

**The Outcome of Time**: Xylanase production by Streptomycete (C5) was affected by an incubation time test. Results in (Fig. 1) reveal that Xylanase synthesis gradually increases until 7 days, at which point it reaches its maximum (27.8 U), and subsequently, enzyme activity declines.

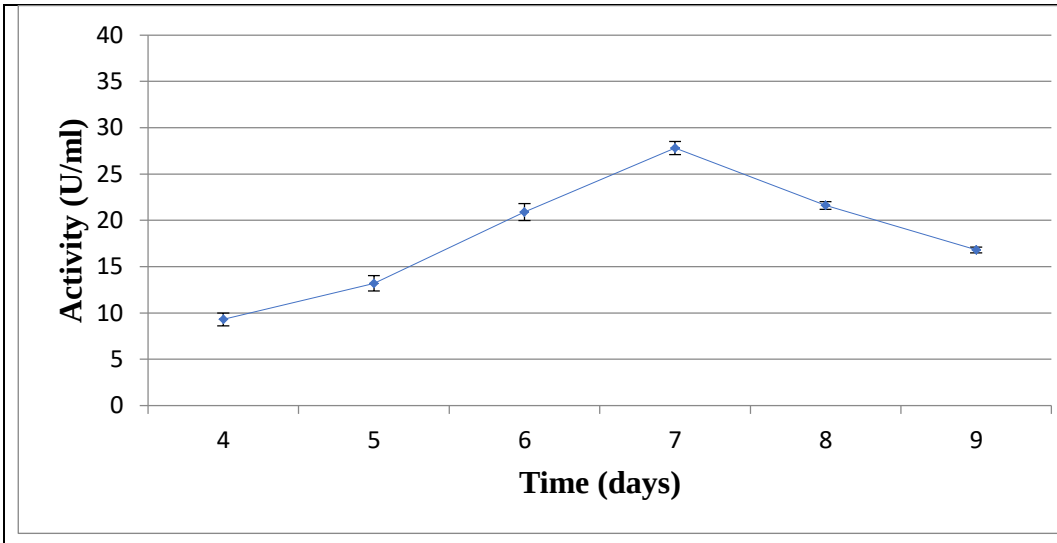


**Fig. 1.** The outcome of incubation time on Xylanase production

**The Outcome of pH:** Streptomycete (C5) synthesis of Xylanase 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 (28.3 U). Reduced enzyme synthesis was brought on by either an increase or a drop in the medium's PH.

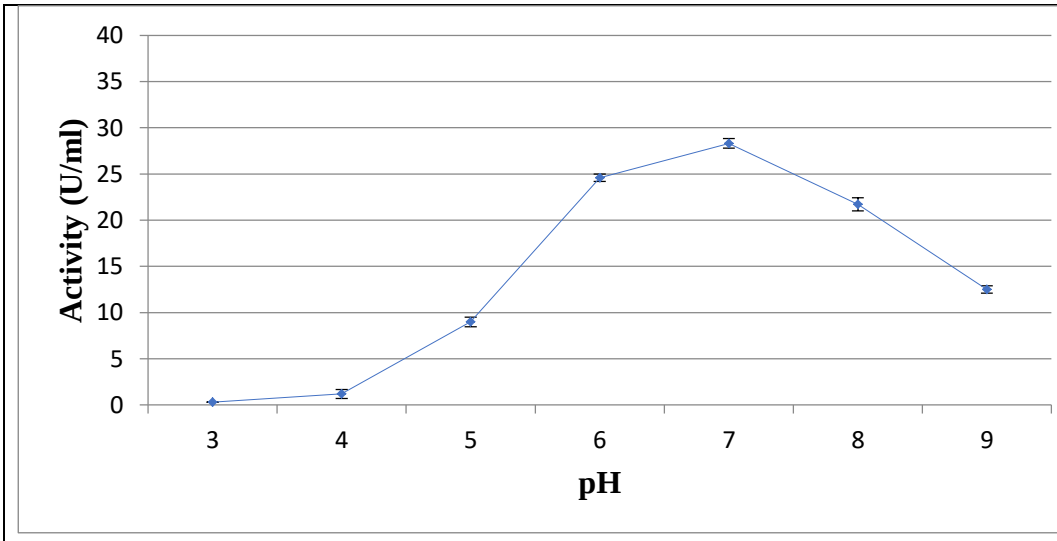


**Fig. 2.** The outcome of incubation pH on Xylanase production

**The Outcome of Temperature**: The results validated a substantial correlation between Xylanase synthesis and incubation temperature up to 30 °C **(Fig. 3)**, where a Xylanase output of 28.7 U was recorded. Compared to the optimal temperature value, there was a significant drop in enzyme synthesis at 35 and 40 °C, respectively.

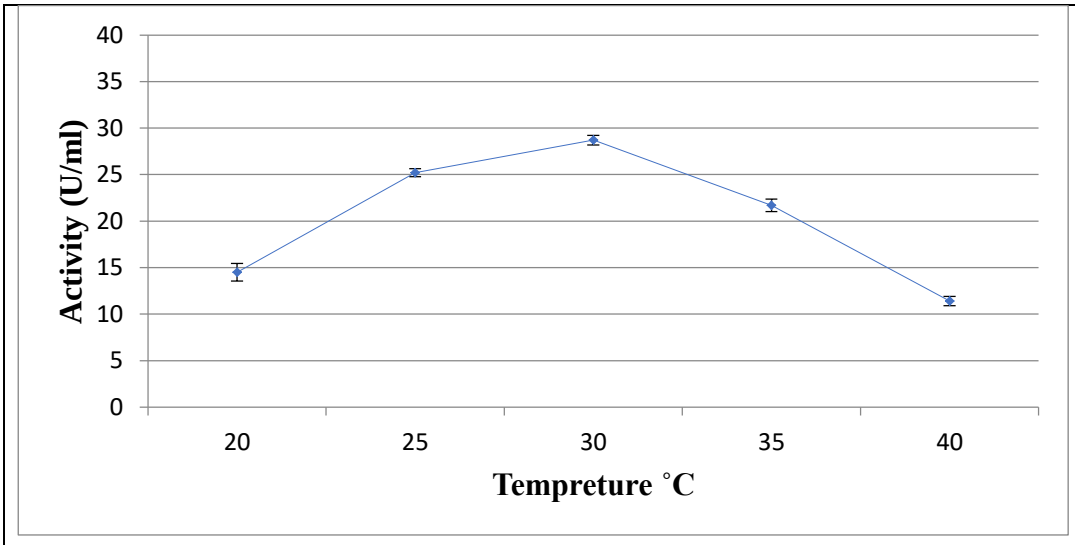


**Fig. 3.** The outcome of incubation temperature on Xylanase production

**The Outcome of Different Carbon Sources**: In our data, the ability of the Streptomycete (C5) isolate to utilize several carbon sources to produce Xylanase was examined. The carbon source employed significantly affected the synthesis of Xylanase, with Xylan being the favored carbon source and yielding enzymes with an activity of 30.1 U **(Fig. 4)**. It is interesting to note that the other carbon sources examined couldn't sustain bacterial growth and enzyme synthesis.

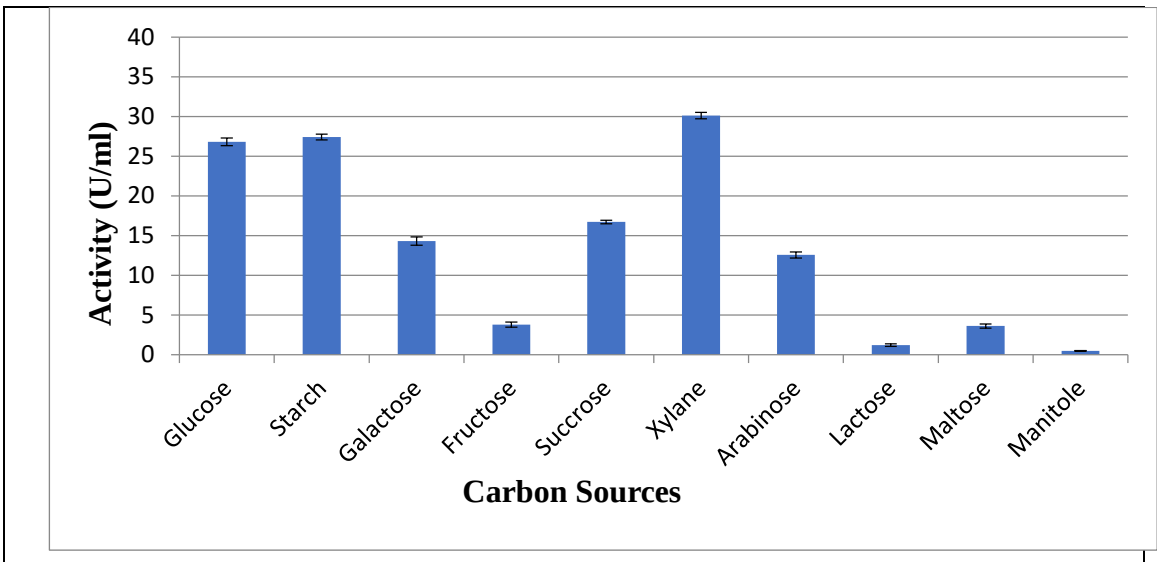


**Fig. 4.** Different carbon sources effect on Xylanase production

**Effect of Different Nitrogen Sources:** One of the nitrogen sources studied for its possible impact on Xylanase synthesis was sodium nitrate, along with peptone, beef extract, malt extract, and yeast extract. The results displayed that yeast extract was the greatest source of nitrogen for Xylanase production 30.4 U for Streptomycete (C5) **(Fig. 5)**.

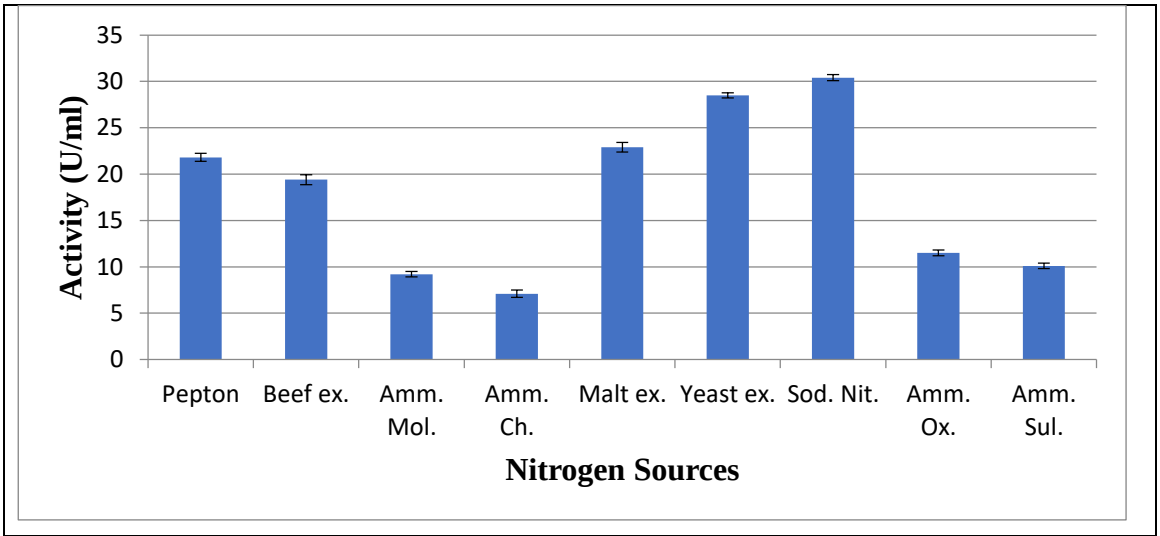


**Fig. 5.** Effect of different nitrogen sources on Xylanase production

Previous investigations have explored the optimal growth conditions to enhance xylanase synthesis across diverse fungal and bacterial strains. For instance, various fungal genera like Trichoderma, Aspergillus, and Penicillium have undergone such studies **(1)**. Likewise, bacterial genera including Cellulomonas, Bacillus, Thermonospora, Clostridium, and Arthrobacter have also been investigated for the same purpose **(14)**. Interestingly, despite the extensive metabolite production observed in actinomycetes, this group of microorganisms has not been fully harnessed for the production of hydrolytic enzymes (Trincone, 2011). Streptomyces species, which fall within the actinomycete category, exhibit significant potential in generating a plethora of highly active secondary metabolites and extracellular enzymes **(10)**.

Streptomyces (C5) underwent a process of optimizing the growth medium to enhance the production of the xylanase enzyme. After a seven-day incubation period, it was determined that the optimal conditions for increased xylanase production were at pH 7 and a temperature of 30°C. Furthermore, xylene was identified as the ideal carbon source, while sodium nitrate served as the optimal nitrogen source. This outcome aligns with the observations made by **(2)**, who documented xylanase from S. albus ATCC 3005 displaying optimal activity at pH 6.5. Strain Ib 24D exhibited a wide pH range for xylanase activity, consistent with the findings of **(5)**, who reported that

## 4.Discussion

Xylanases are widely distributed in nature, present in both eukaryotic and prokaryotic organisms. A diverse array of microorganisms, including fungi and bacteria from various environments, have been identified as sources of xylanases. Prokaryotic sources of xylanase production encompass bacteria like Streptomyces, Actinomadura, and Nonomuraea, as well as diverse species of actinomycetes **(23)**. Research has revealed that specific Streptomyces strains have the capacity to synthesize thermostable xylanases that remain active within the temperature range of 60 to 70°C **(12)**. The fact that xylanases function effectively at elevated pH levels and temperatures holds significant advantages for numerous biotechnological applications. For instance, the breakdown of xylan into xylooligosaccharides (XOS) from corncobs is facilitated, and the employment of heat-stable alkaline xylanases enables the direct extraction of xylan from raw corncobs using a mild salt-based technique. This approach eliminates the need for temperature and pH adjustments, leading to time and cost savings **(6)**.

Therefore, this study involved the isolation of twenty-one streptomycete strains from various locations within Egypt. These isolates were subjected to quantitative assessment to evaluate their capacity for xylanase enzyme production. The most robust strain turned out to be Streptomyces (C5), obtained from the government district of Cairo.

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xylanase produced by S. chromogens strain 5028 (S1) demonstrated significant activity within the pH range of 4.5 to 8.5. Similarly, **(15)**, in their study of xylanase produced by S. halstedii JM8, yielded comparable outcomes. The utilization of xylan as the carbon source led to notably high xylanase activity, signifying that the synthesis of xylanase remained unimpeded by xylose **(18).**

In light of increasing demand for xylanases and the resulting need for greater productivity, it is imperative to identify novel enzymes and enzyme-producing microbial strains **4)**. Thus, the selection of microorganisms and the process techniques for a successful commercial production of xylanase enzymes are of the highest importance **(4)**. Several metabolites and biomolecules, such as hydrolytic enzymes, have been attributed to the genus Streptomyces throughout the years **(10)**. After all, the hemicellulolytic capability of Streptomyces albidoflavus has received minimal attention. The production of microbial hydrolytic enzymes is a process governed by multiple variables, including the structure and culture conditions of microbial cells. In order to increase enzyme titer and productivity, it is crucial to measure the effect of these variables (**8)**.

## 5.Conclusion

Streptomycetes were isolated from soil environments obtained from different governorates in Egypt for maximum xylanase production with optimum pH 7, and 30 º C and a 7-day incubation period. The best sources of carbon and nitrogen were xylan and sodium nitrate, respectively.

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