Journal of Basic and Environmental Sciences
ISSN

Online:2356-6388

Print:2536-9202

Research Paper Open Access

Microbial Interdisciplinary Experiment: Unravelling the outcome of L-glutaminase Produced by Streptomycetes isolates

Mohamed O. Abdelmonem [1], Mohamed E. El awady[2], Mohamed I. El-khonezy[3] Mervat G. Hassan[1], Mona A. Kishk [1]

[1] Botany and Microbiology Department, Faculty of Science, Benha University, Benha, Egypt.

[2] Microbial Biotechnology Department, Biotechnology Research Institute, National Research Centre, El-Buhouth St. 33, Dokki, Cairo, Egypt. https://orcid.org/0000-0001-5155-3949.

[3] Molecular Biology Department, National Research Centre, 33-El Bohouth St., Dokki, Giza, P.O.12622, Egypt.

Abstract:

It is well known that Streptomyces produces beneficial pharmaceuticals, particularly antibiotics and anticancer agents. This investigation uncovered 34 randomly selected streptomycetes isolates in sediment, marine water, soil, and other habitats. Using a qualitative rapid plate assay to test for GluNase synthesis, 9 of them produced a significant amount of L-glutamine pink colour.The maximum levels of GluNase synthesis were obtained at a pH of 7.5, an incubation temperature of 40 °C, and the utilization of glucose and peptone as the carbon and nitrogen sources, respectively.

Keywords: *Streptomycetes* , GluNase, isolation, screening, optimiza

Introduction:

A potential cancer-fighting enzyme called L-glutaminase breaks down the amide bond in L-glutamine to produce ammonium ions and glutamate. It functions as an antioxidant, a flavour enhancer, as well as a biosensor for measuring glutamine levels.

A well-known example of a microbial source that can can create a wide different chemical structures is actinomycetes, which include some extremely costly pharmaceuticals, agrochemicals, and industrial goods such as enzymes (Balagurunathan et al., 2010). Due to their production of extracellular enzymes, actinomycetes are regarded as preferred enzyme sources (Prakash, 2013). Actinomycetes produce a variety of enzymes, including L-glutamine amid hydrolase, also known as L-glutaminase (GluNase), which has been used as a medication(Awad et al., 2019) .

GluNase is a potential antineoplasm enzyme that hydrolyzes the amide bond of L-glutamine to provide glutamate and ammonium ions while the detection of its anti-neoplasm properties is ongoing(More et al., 2018) . Both prokaryotes and eukaryotes utilise gluNase, which plays an important role in nitrogen metabolism (Amobonye et al., 2022) . Recently, GluNase has attracted a lot of attention due to its extensive use in pharmaceuticals and its antagonistic nature towards leukaemia (Brumanoet al., 2019) .

Whereas some types of cancerous cells have a high glutamine consumption rate as a defining feature. In light of this personality, novel therapies to starve tumour cells of L-glutamine have been developed (DeBerardinis and Cheng, 2010). Preventing glutamine from being taken up by the tumour cells is one potential method for slowing tumour development; GluNase is effective at doing this. Tumour cells are selectively starved in contrast to ordinary cells because they lack fully functional machinery for the biosynthesis of glutamine (Van Geldermalsen et al., 2016).

The diverse class of bacteria known as actinomycetes creates filaments in the soil that resemble threads. They are widely dispersed in the natural environment and involved in a variety of biological and metabolic activities, including as the production of extracellular enzymes. Actinomycetes are considered to be the most significant category of microbes in the field of biotechnology because they create bioactive secondary metabolites that have uses in agriculture, industry, and medicine. Streptomyces are well known for producing industrial products like enzymes for money-making discovery platforms as well as valuable pharmaceuticals, particularly antibiotics and anticancer mediators(Awad et al., 2019). For microbial GluNase

producers like *Streptomyces rimosus*, *Streptomyces avermitilis*, and *Streptomyces labedae*, a wealth of information has been provided (SILVA, 2017).

Materials and Methods

Sample Collection:

In this study, two different types of samples were used. The first type is made up of soil samples that were taken from agricultural soils in Giza, Dakahlia, and Gharbia governorates in 2020 and placed in polyethylene bags. In sterile containers kept at 4°C, a second type of sample was taken from water (sea water) in two different governorates: South Sinai (sharm El sheekh) and Alexandria.

Isolation of Streptomycetes isolates:

A sediment and water sample weighing ten grammes or ten millilitres was transferred into 90 millilitres of sterilised saline (0.85% w/v aqueous NaCl solution). Suspension plates were used to plate streptomycetes using the serial dilution technique of (Hayakawa and Nonomura, 1987). To remove moisture film from the surface of agar, The day before plating, petri dishes were prepared and overnight incubated at 37 °C (Shearer, 1987). Each plate received a 0.1 ml inoculum of the appropriate dilution, which was spread with a sterile glass rod. Streptomycetes were isolated using starch nitrate agar (Waksman, 1961). For the isolation of the marine water sample (50%) sea water containing (g/l) of starch nitrate agar medium was used. Agar 20.0, H2O 1000.0 ml, pH 7.0–7.4; Starch 10.0; KNO3 2.0; K2HPO4(anhydrous) 1.0; MgSO4.7H2O 0.5; NaCl 0.5; CaCO3 2.0; FeSO4.7H2O 0.01.

Screening for L-glutaminase producer isolates:

Before pouring the plates, 34 samples of Streptomyces were examined to see if they could break down L-glutamine as the sole nitrogen source in an altered medium with starch agar that had 0.07% (W/V) of phenol red added to it. This medium's pH was raised to 7.0. According to (William and Hariharan, 2013). it was possible to identify L- glutaminase producing isolates by observing the pink colonies or the region surrounding the growth, which was caused by L-glutaminase's reaction with L-glutamine to produce ammonia.

L- glutaminase assay

According to (Thompson and Morrison, 1951). the Nesslerization method was used to determine the L- glutaminase activity. The standard is composed of 0.1 millilitres of pyridoxal phosphate, 1 millilitres of undiluted enzyme, and

1 ml of 1% L- glutaminase in (0.2 M) buffer composed of phosphate with (7.0) of pH. It is incubated for one hour at 30°C. Then, by adding 0.5 ml of 20% trichloroacetic acid, the activity stopped.

Hence, to remove the precipitated protein, centrifuged for five minutes at 5,000 rpm. 0.1 ml of the above mixture was added to0.2 ml of Nessler reagent and3.7 ml of distilled water. In order to measure the developed coloured compound, a UV/VIS-2401 PC visible spectrophotometer (Shimadzu, Kyoto, Japan) was used. The amount of L- glutaminase that, under ideal assay conditions, releases ammonia at a rate of 1 mol/min was designated as one unit. The units of enzyme activity per milligramme of protein were used to express the specific activity of L-glutaminase.

Medium optimization for production of L- glutaminase

It was determined that broth media provided the best conditions for streptomyces strain to produce the most glutaminase. 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 NaOH or 1 N HCl), and various incubation times were then studied on the medium component. . different temperature (20, 25, 30, 35 and 40 ˚C), different glutaminase concentration (0, 0.4, 0.8, 1.2 and 1.6%), Various additional carbon sources (glucose, arabinose, xylose, mannitol, , lactose, fructose, galactose, sucrose, maltose, and starch at 1% w/v) and various nitrogen sources, including yeast extract, peptone, beef extract, malt extract, sodium nitrate, ammonium oxalate, ammonium molybidate, ammonium sulphate, and ammonium chloride.

Results

Isolation and screening of *Streptomycetes* strains

The streptomycetes isolates were chosen based on the colony's distinctive morphology, which frequently has a spherical and curved shape and has firmly rooted the medium's development. Dry, powdery spore masses frequently cover the colonies' surface. In addition to other environments, silt, seawater, and soil all contained 34 random isolates of the streptomycetes genus.

The distribution of the streptomycetes isolates is shown in Tables 1-6, with Dakahlia having the highest concentration, followed by Gharbia, Giza, and South Sinai (Sharm Elshiekh), and Alexandria having the lowest concentration. Streptomycetes isolates were most frequently found in Dakahlia, followed by Gharbia and Giza, and fewer times in Alexandria and South Sinai

(Sharm El-Sheikh). When the ability of 34 Streptomyces isolates to release GluNase was tested using a rapid plate assay technique, the presence of a pink hue around the colonial growth of only nine Streptomyces isolates, which also have the greatest capacity for the production of extracellular GluNase, allowed for their identification. **(Fig. 1)**.

Table 1: Total number of *streptomycetes* isolates.

Source	Locality	No. of isolates	Incidence percent (%)
Soil	Dakahlia	11	**32.35**
	Gharbia	9	**26.47**
	Giza	8	**23.52**
Marine	Alexandria	4	**11.76**
	South Sinai (Sharm Elshiekh)	2	**5.88**
	Total	**34**	**100**

Table2 Screening for production of ASNase from *streptomycetes* isolated from Dakahlia by qualitative rapid plate assay test.

Sample	Results
DK 1	**-**
DK 2	**-**
DK 3	**-**
DK 4	**-**
DK 5	**-**
DK 6	**-**
DK 7	**-**
DK 8	**-**
DK 9	**-**

DK 10	**+**
DK 11	**-**

Table 3. Screening for production of GluNase from *Streptomyces's* isolated from Gharbia by qualitative rapid plate assay test.

Sample	Results
GH 1	**-**
GH 2	**-**
GH 3	**-**
GH 4	**-**
GH 5	**-**
GH 6	**-**
GH 7	**-**
GH 8	**+**
GH 9	**-**

Table 4. Screening for production of GluNase from *Streptomyces's* isolated from Giza by qualitative rapid plate assay test.

Sample	Results
GZ 1	**-**
GZ 2	**-**
GZ 3	**+**
GZ 4	**-**
GZ 5	**+**
GZ 6	**-**
GZ 7	**-**
GZ 8	**-**

Table 5. Screening for production of GluNase from *Streptomyces's* isolated from Alexandria by qualitative rapid plate assay test.

Sample	Results
Alx 1	**-**
Alx 2	**-**
Alx 3	**-**

Alx 4	**+**

Table 6. Screening for production of GluNase from *Streptomyces's* isolated from Sharm Elshiekh by qualitative rapid plate assay test.

Sample	Results
SH 1	**+**
SH 2	**-**

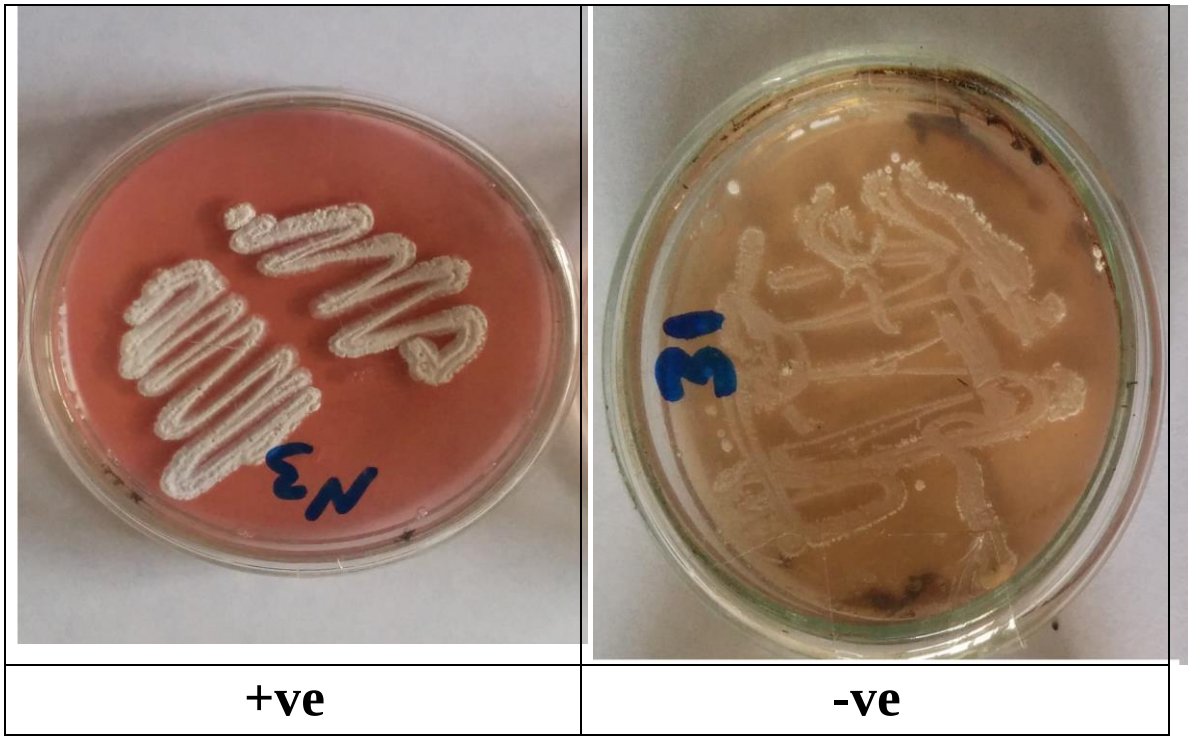

Fig. 1: GluNase production screening using rapid plate assays.

Factors Affecting the Production of GluNase

Effect of time: L- glutaminase synthesis by Streptomyces sp. strain 5M was affected by an incubation time test. The data in **(Fig. 2)** demonstrate that L-glutamine synthesis steadily increases until day 7, at which point it reaches its maximum level (5.26 U/ml), and thereafter enzyme activity declines.

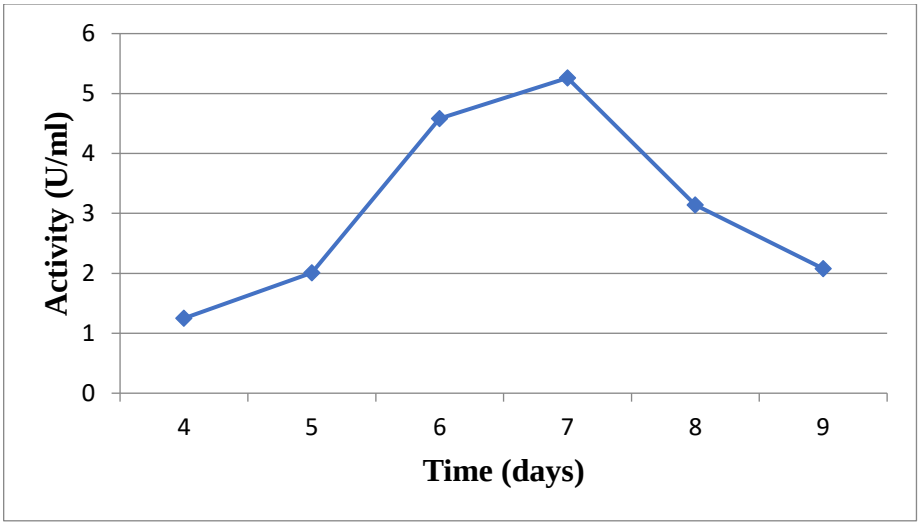

Fig. 2. Effect of incubation time on GluNase production

Effect of pH: *Streptomyces sp.* strain 5M's synthesis of L- glutaminase is influenced by pH. The pH of the fermentation medium is stated as having an impact on the synthesis of enzymes in **(Fig. 3)**. A maximum enzyme yield of 5.57 U/ml was therefore observed at pH 7.0. Enzyme production was decreased by either an increase or a drop in the medium's pH.

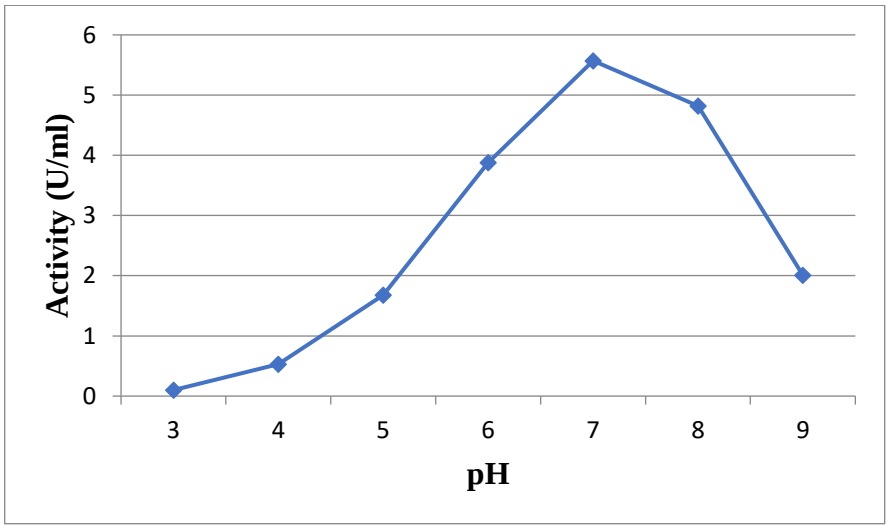

Fig. 3. Effect of pH on GluNase production

Effect of temperature: Any microbial strain can grow at a certain rate depending on the fermentation medium's incubation temperature. That at 30°C, the highest enzyme production (5.85 U/ml) was observed. Reduced GluNase synthesis results from any variation in temperature **(Fig. 4)**.

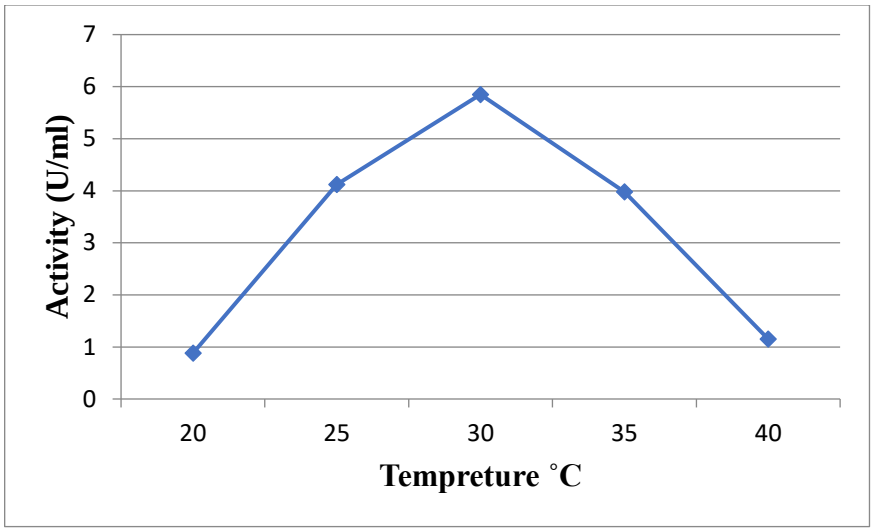

Fig. 4. The impact of Temperature on GluNase production

Effect of glutamine concentration: The synthesis of GluNase is induced by the amino acid glutamine. As a result, the medium for producing enzymes was mixed with varying quantities of glutamine. Based on the findings in **(Fig. 5)**, the enzyme synthesis was at its highest level (6.07 U/ml) at a 0.4% concentration. However, the synthesis of enzymes was decreased when glutamine concentration rose.

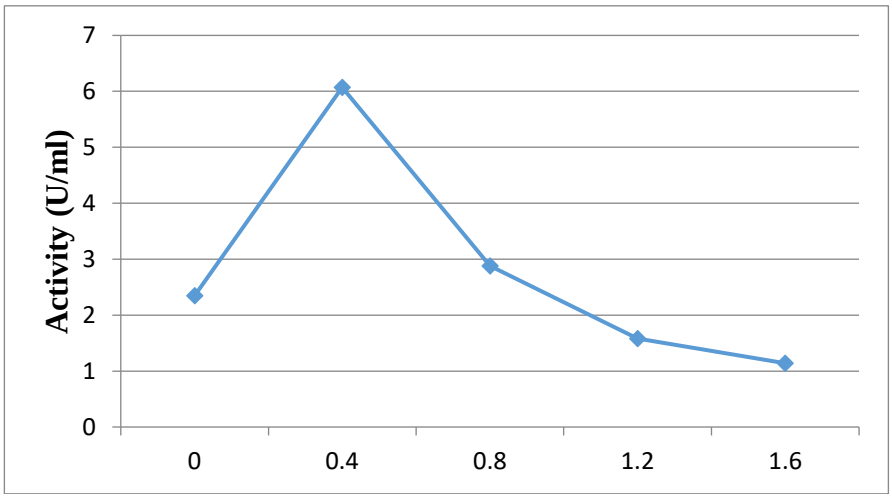

Fig. 5. Effect of glutamine concentration on GluNase production

Effect of different carbon sources: When various sources of carbon were integrated to the medium at a 1% level, the synthesis of enzymes increased. The generation of enzymes from all other carbon sources was much higher than that from glucose (6.21 U/ml), which supported the highest enzyme yield. Arabinose had the lowest efficiency (0.95 U/ml) as a carbon source **(Fig. 6)**.

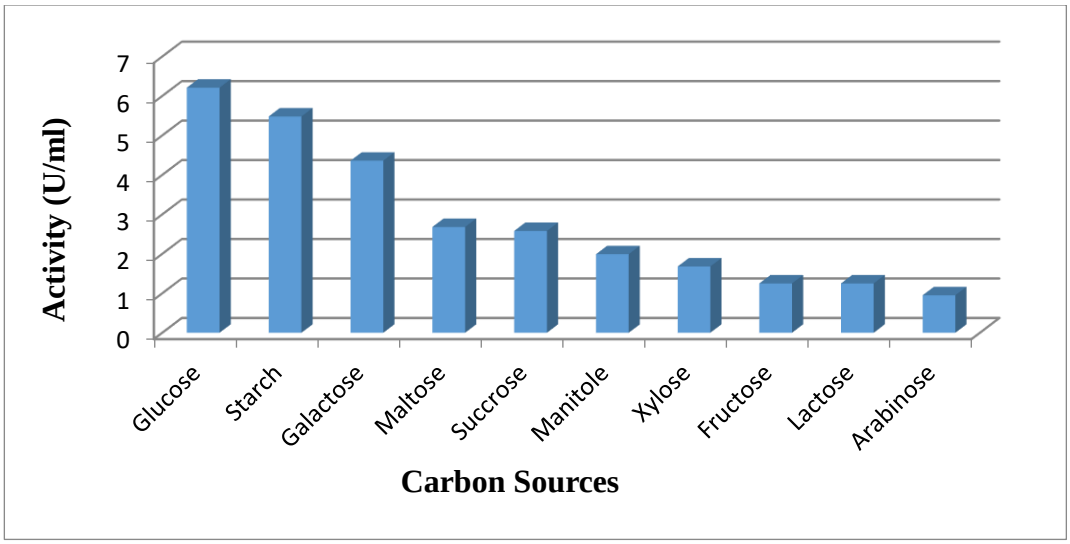

Fig. 6. The impact of different carbon sources on GluNase production

Effect of different nitrogen sources: A substantial increase in the synthesis of enzymes was seen when extra organic and inorganic nitrogen sources were added to the medium. The highest enzyme production (6.33 U/ml) was supported by peptone out of all the other nitrogen sources examined. All other nitrogen sources produced varying amounts of enzymes as well. The least efficient nitrogen source overall was sodium nitrate (1.25 U/ml) according to **(Fig. 7)**.

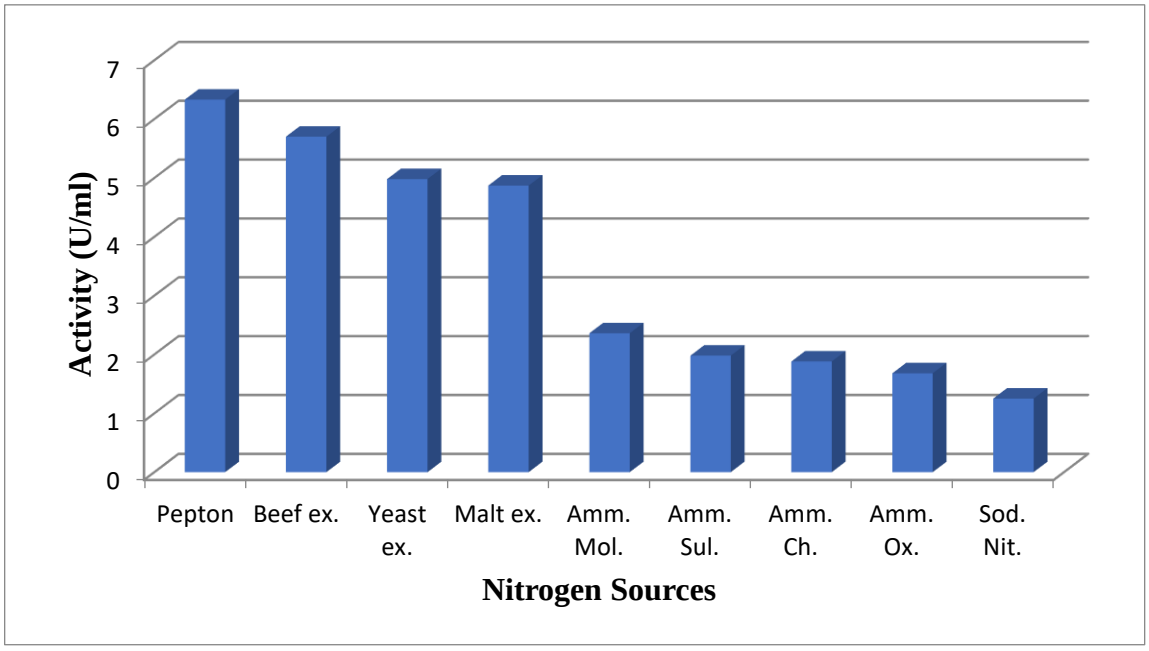

Fig. 7. Effect of different nitrogen sources on GluNase production

Discussion

Gram-positive filamentous bacteria called actinomycetes produce a variety of industrial enzymes. Because of its capacity to produce a variety of enzymes, Streptomyces sp. is one of the most significant and industrially useful organisms among actinomycetes. The ability of actinomycetes to manufacture several enzymes, including protease, amylase, lipase, pectinase, cellulase, xylanase, glutaminase, and asparaginase, is well documented (Balagurunathan et al., 2010).

Different extracellular enzymes are secreted by streptomyces species, and these enzymes are used extensively in both industry and agriculture. Large-scale improvements in enzyme manufacturing have been made thanks to the development of molecular techniques, which have replaced the conventional microbiological method. The development of efficient and stable enzymes to enhance metabolic processes has benefited from the application of functional genomics, proteomics, and other genetic approaches such as metabolic engineering and combinatorial biosynthesis(Awad et al., 2019) . The streptomycetes isolates were chosen based on the colony's distinctive morphology, which is frequently spherical and shaped convexly with extensively rooted the medium's development. Dry, powdery spore masses frequently cover the colonies' surface. In addition to other environments, silt, seawater, and soil all contained 34 random isolates of the streptomycetes genus. (Table S1, 2, 3,4,5,6 Supplementary Data) displays the distribution of the streptomycetes isolates, with the most isolating strains coming from Dakahlia, the next is Gharbia and Giza, with the lowest percentage coming from South Sinai (Sharm Elshiekh)and Alexandria.

The greatest number of streptomycetes isolates were discovered in Dakahlia, and to assess their purity, gluNase was purified from Streptomyces sp. strain 5M. Crude GluNase fractionally precipitates after salting (30-80% ammonium sulphate). These conclusions concurred with those looked at by (Reda, 2014). The GluNase activity of Bacillus sp. was also purified 49-fold after gel filtration, according to (Kumar et al., 2012). with a 25% retrieval and a specific activity of 584.2 U/mg protein. Contrarily, Penicillium brevicompactum NRC829's intracellular GluNase was isolated to homogeneity (162.75 fold) and had a distinct molecular mass of 71 kDa, according to (Elshafei et al., 2014). This indicates that the method used in the current study was effective at producing and purifying GluNase. The initial optimisation resulted in an increase

in enzyme productivity while taking into account the potential impacts of the factors mentioned previously, such as temperature, pH, time, incubation time, nitrogen sources, and carbon sources.

Over the pH range of 5.0 to 9, the L-glutaminase maintained its activity, with pH 8.0 being the optimal value. pH values that are both low and high resulted in a significant decline in activity. L-glutaminase's pH stability followed a similar pattern, remaining constant across a wide pH range (5.0-11.0), with pH 7.0–9.0 being its most stable range. Practically, assuming the enzyme had a basic identity, an increased rate of enzyme inactivation was observed at acidic pH values when compared to the alkaline side.

The adverse impact enzyme activity at both high and low pH levels, implying the impact on the ionisation state of the enzyme, altering the enzyme's surface charge, dissociating the subunits/coenzyme, and consequently disrupting its bond with substrate. The alkaline pH 7-8 optimum pH of S. canarius L-glutaminase activity was consistently observed, and the enzyme's stability at a neutral pH was a preferred indicator of how the enzyme would behave in vivo. At an alkaline pH as opposed to an acidic one, Streptomyces gulbargensis' L-asparaginase was also more stable. (Amena et al., 2010; Kumar et al., 2012).

L-glutamine is used as the reaction's substrate, and the reaction mixture is incubated at different temperatures (25–50 °C) in a 0.1 M potassium phosphate buffer (pH 8.0), it was possible to determine the ideal temperature for the L-glutaminase activity. At 40 °C, the greatest amount (53.3 U/mg protein) of specific enzyme activity was attained. The enzyme activity decreased at both this temperature and above it. Under 60 °C, the enzyme was thermally stable for catalysis; at 70 °C, it started to lose activity slightly; at 80 °C, it was completely inactive.

At, respectively, 50, 60, 70, and 80 °C, the enzyme half-lives (T1/2) were 45.7, 38.8, 29.3, and 17.3 min. Additionally, considering the enzyme's Tm to be 57.5 °C, presuming that heating it for 60 minutes would retain about 50% of its initial activity. Theoretically, at 50, 60, 70, and 80°C, respectively, thermal inactivation rates (Kr) are 0.0184, 0.0227, 0.0544, and 0.0449 S-1, indicating the

dissociation of coenzyme or denaturation of subunits by heating per unit time.These findings agreed with those made public by (Amena et al., 2010; Kumar et al., 2012).

Additionally, (Singh and Banik, 2013) reported the ideal pH and temperature for the purified L-glutaminase enzyme made by Bacillus cereus MTCC 1305 were 7.5 and 35 °C, respectively.

Even after 30 minutes of treatment at 50 and 55 °C, respectively, the enzyme maintained stable up to 50 and 20%. Additionally, (Elshafei et al., 2014) reported that the thermo-stability of this enzyme was demonstrated by the purified L-glutaminase from Penicillium brevicompactum, which displayed its greatest activity against L-glutamine after 30 minutes of incubation at pH 8.5 and 50 °C. The sixth day generated the highest yield (6.04 U/ml) after screening glutaminase productivity over a wide time span of 1- 9 days. The isolate began to exhibit activity on day 1 (1.04 U/ml), then activity gradually increased up until day 6, and production ability remained at 3.03 U/ml on day 9 (fig 4). According to the isolate used, the optimal incubation times were 4.5 days (Keerthi, 1999) and 7 days in another study (El-Sayed, 2009). where the optimal production of glutaminase time was shorter (4.5) days.

The highest productive organic nitrogen source for producing L-glutaminase by S. avermitilis was found to be peptone, followed by malt extract and yeast extract, with peptone reaching a maximum productivity of 9.52 U/ml. With a productivity of 10.30 U/ml, sodium nitrate is regarded as the best inorganic nitrogen source for the production of enzymes.The synthesis of S. avermitilis's enzymes was inhibited by urea. As reported by (Sivakumar et al., 2006) S. rimosus was found to benefit from malt extract in order to produce L-glutaminase.

The production of biomass and glutaminase was stimulated by glucose, one of the different carbon sources. The marine organisms' preference for slowly metabolizable sugars like glucose is consistent with earlier reports. Inhibitors of glutaminase activity and biomass include lactose, citric acid, sucrose, mannitol, and maltose. Both biomass and activity (65.74 U/l) were produced after the free sugars were removed from the medium. According to (Iyer

and Singhal, 2009) this demonstrated the organism's capacity to use other media components as a source of energy.

Conclusion:

Using a semi-qualitative method, various Actinomycetes isolated from soil were examined for their ability to produce L-glutaminase in the current study. Using morphological and biochemical characteristics, Streptomyces sp. was further identified as a potential L-glutamine producing actinomycete. Additionally, the impact of variables on Streptomyces sp.'s production of enzymes was investigated.

References:

[1] Albina, J. E., Henry, W. I. L. L. I. A. M., King, P. A., Shearer, J. E. F. F. R. Y., Mastrofrancesco, B. A. L. D. U. I. N. O., Goldstein, L. E. O. N., & Caldwell, M. D. (1987). Glutamine metabolism in rat skeletal muscle wounded with lambda-carrageenan. American Journal of Physiology-Endocrinology And Metabolism, 252(1), E49-E56.

[2] Amena S, Vishalakshi N, Prabhakar M et al. (2010) Production, purification and characterization of L-aparaginase from Streptomyces gulbargensis Braz J Microbiol 41:1517-8382.

[3] Amobonye, A., Singh, S., Mukherjee, K., Jobichen, C., Qureshi, I. A., & Pillai, S. (2022). Structural and functional insights into fungal glutaminase using a computational approach. Process Biochemistry, 117, 76-89.

[4] Awad, H. M., El-Deen, A. M. N., Mostafa, E. S. E., & Hassabo, A. A. (2019). Biochemical studies and biological activities on L-glutaminase from rhizosphere soil Streptomyces rochei SAH2_CWMSG. Egyptian Pharmaceutical Journal, 18(1), 27.

[5] Balagurunathan, R., Radhakrishnan, M., & Somasundaram, S. T. (2010). L-Glutaminase producing actinomycetes from marine sediments–selective isolation, semi quantitative assay and characterization of potential strain. Aust J Basic Appl Sci, 4(5), 698-705.

[6] Brumano, L. P., da Silva, F. V. S., Costa-Silva, T. A., Apolinário, A. C., Santos, J. H. P. M., Kleingesinds, E. K., ... & Junior, A. P. (2019). Development of L-asparaginase biobetters: current research status and review of the desirable quality profiles. Frontiers in bioengineering and biotechnology, 6, 212.

[7] DeBerardinis, R. J., & Cheng, T. (2010). Q's next: the diverse functions of glutamine in metabolism, cell biology and cancer. Oncogene, 29(3), 313-324.

[8] El-Sayed ASA. L- glutaminase production by Trichoderma koningii under solid state fermentation. Indian J. Microbiol. 2009; 49:243-250.

[9] Elshafei, A. M., Hassan, M. M., Ali, N. H., Abouzeid, M. A. E., Mahmoud, D. A., & Elghonemy, D. H. (2014). Purification, kinetic properties and antitumor activity of L-glutaminase from Penicillium brevicompactum NRC 829. British Microbiology Research Journal, 4(1), 97-115.

[10] Hayakawa, M., & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. Journal of Fermentation Technology, 65(5), 501-509.

[11] Iyer PV and Singhal RS. Screening and selection of marine isolate for L-glutaminase production and media optimization using response surface methodology. Applied Biochemistry and Biotechnology, 2009; 159: 233–250.

[12] Keerthi TR. (1999). L- glutaminase production by marine Pseudomonas sp. PhD Thesis, Cochin University of Science and Technology, Cochin. India.

[13] Kumar, L., Singh, B., Adhikari, D. K., Mukherjee, J., & Ghosh, D. (2012). A temperature and salt-tolerant L-glutaminase from gangotri region of uttarakhand himalaya: enzyme purification and characterization. Applied biochemistry and biotechnology, 166, 1723-1735.

[14] More, S. S., Swamy, R., Mohan, N., Navyashree, M., Janardhan, B., & Niyonzima, F. N. (2018). Purification and characterization of anti-cancer L-glutaminase of Bacillus cereus strain LC13. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, 88, 695-705.

[15] Prakash, D., Nawani, N., Prakash, M., Bodas, M., Mandal, A., Khetmalas, M., & Kapadnis, B. (2013). Actinomycetes: a repertory of green catalysts with a potential revenue resource. BioMed research international, 2013.

[16] Reda, F. M. (2014). Purification and characterization of Streptomyces canarius L-glutaminase and its anticancer activity. Egypt J Bot, 54(1), 137-157.

[17] Shearer, M. C. 1987 Methods for the isolation of non-streptomycete actinomycetes J. Indus. Microbiol. Suppl. 2 91–97

[18] SILVA, W. D. O. (2017). Otimização e caracterização parcial de L-asparaginase e L-glutaminase de actnobactéria da rizosfera de Poincianela pyramidalis (Master's thesis, Universidade Federal de Pernambuco).

[19] Singh P and Banik RM. Biochemical Characterization and antitumor study of L-glutaminase from Bacillus cereus MTCC 1305. Applied Biochemistry and Biotechnology, 2013; 171: 522–531.

[20] Sivakumar K, Sahu MK, Manivel PR, Kannan L (2006). Optimum conditions for L-Glutaminase production by actinomycete strain isolated from estuarine fish Chanos chanos (Forskal 1975). Ind. J. Exp. Biol.,44: 256-258.

[21] Thompson JF, Morrison GR. Determination of organic nitrogen: control of variables in the use of Nessler's reagent. Anal Chem.,1951, 23:1153–1157.
[22] Van Geldermalsen, M., Wang, Q., Nagarajah, R., Marshall, A. D., Thoeng, A., Gao, D., ... & Holst, J. (2016). ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. Oncogene, 35(24), 3201-3208.
[23] Waksman, S. A. (1961). The Actinomycetes. Vol. II. Classification, identification and descriptions of genera and species. The Actinomycetes. Vol. II. Classification, identification and descriptions of genera and species.
[24] Willis, W. L., Hariharan, S., David, J. J., & Strauch, A. R. (2013). Transglutaminase-2 mediates calcium-regulated crosslinking of the Y-Box 1 (YB-1) translation-regulatory protein in TGFβ1-activated myofibroblasts. Journal of Cellular Biochemistry, 114(12), 2753-2769.