Indian Journal of Biotechnology and Pharmaceutical Research
Journal homepage : www.ijbpr.in
ISSN: 2347-3266
2023, 11 (3): 1 – 07

Research Article

Studies on the production and optimization of L-Glutaminase by *Actinomycetes* isolated from soil sample using mixed substrate of jowar powder and coconut Kernel powder under solid-state fermentation

Vidya Douluri[1]* and K. Jaya Raju[1]

1 Center for Biotechnology, Department of Chemical Engineering, AU College of Engineering, Andhra University, Visakhapatnam

ABSTRACT

Actinomycetes are gram-positive microorganisms that are unicellular and minute in size. These organisms are capable of producing a wide array of optional metabolites, which may include enzymes. L-glutaminase, which has both anticancer and antiretroviral characteristics, has been shown to be effective against HIV. Glutaminase has the ability to reduce the quantities of glutamine found in blood and tissues. L-glutaminases have been put to use in the business that deals with the preparation of food over a very extended period of time. In the present study *actinomycetes* produced glutaminase enzyme under solid-state fermentation and the purpose of this experiment was to locate and isolate an actinomycete strain from the soil sample. On a medium consisting of starch casein and nutrient agar and on media consisting of little glutamine, it was tested for L-Glutaminase activity. The optimum conditions for the maximum production of L-Glutaminase were found to be at a temperature of 28oC with a pH of 7.5 when incubated for 5 days with 3 days old culture and 4 milliliters of the inoculum volume and 60% of the moisture content using mixed substrate concentration (Jowar powder and coconut kernel powder of each 2.5 grams) of 5 grams and the addition of Maltose as a good carbon source. The effect of nitrogen source and the effect of metal ion Barium chloride were studied. The maximum activity of L-glutaminase 327.56U/gds was observed with the presence of Barium chloride in the production media

KEY WORDS: *Actinomycetes*, L-Glutaminase, Solid state fermentation, Jowar powder Coconut kernel powder, Anti-retroviral drugs.

Introduction

Both the food and the pharmaceutical sectors have shown a significant amount of interest in L-glutaminase in recent years. The enzyme L-glutaminase is used in the food business to increase the glutamic acid content of products. Glutaminolysis, the process by which L-glutamine is broken down into L-glutamic acid and ammonia, is how it functions. In addition, it is used in the therapy of cancer using enzymes, most notably for acute lymphocytic leukemia. L-glutaminase is also used in biosensors in order to determine the amount of glutamine that is present in mammalian and hybridoma cells.

Received: 24.07.2023, Revised: 09.08.2023, Accepted: 17.08.2023
***Address for Correspondence** Center for Biotechnology, AU College of Engineering, Andhra University, Visakhapatnam, India
E-mail: dvidva2k@amail.com

L-glutaminase is employed as an oncolytic enzyme and an antiretroviral medication due to its capacity to degrade small molecules like glutamine. Furthermore, glutaminase is used in the food industry as a flavoring agent

Materials and Methods

Sample Collection:

Garden plots close to the boy's hostel at the Andhra University College of Engineering were chosen for the collection of soil samples. Each sample was made from a depth of ten to fifteen inches in the soil, and these were allowed to air dry for one week before being crushed and sieved. After being screened, the soils were put to use in an experiment to isolate *actinomycetes*.

Isolation of *Actinomycetes*:
A gram of soil was suspended in 10 milliliters of physiological water, and the mixture was then agitated vigorously while being heated to 28oC for 200 cycles per second. After letting the mixtures settle for a while, 0.1 milliliters of each dilution from 10-3 to 10-5 was removed and placed uniformly over the outer layer of *actinomycetes* starch casein agar. The vertex was then perturbed in a more expedient manner by utilizing sterile physiological water to pre-arrange consecutive dilutions up to a concentration of 10-5. Rifampicin at a concentration of 2.5 mg/ml and amphotericin B at a concentration of 75 mg/ml was added to the media in order to prevent bacterial and parasitic contamination, respectively. After forty-eight, seventy-two, and ninety-six hours of continuous streaking, purified bacterial provinces that resembled A*ctinomycetes* were identified on plates of starch casein agar. Plates were bred and monitored at temperatures of 28 and 37oC. Separated samples were poured over a SCA medium while being carried along by a laminar wind stream. After the pour plating was finished, the Petri dishes were placed in an orbital shaker and incubated at a temperature of 28oC for 14 days. After a period of 14 days, colonies of A*ctinomycetes* were painstakingly removed from several plates in order to prevent them from being contaminated by bacteria or parasites. On a plate, colonies of a*ctinomycetes* are clearly distinguishable from those of other microorganisms as shown in Figure 1. They have a sparkling surface, are noticeably more diminutive, and because of the effects of weathering, they have the appearance of a funnel. The A*ctinomycetes* colonies were maintained on starch casein agar plates in addition to being relocated and incubated at a temperature of 28oC for an extended length of time. The naked eye gave the impression that these colonies were distinct from one another in a number of ways, including the color of the elevated mycelium, the diversity of the turnaround, the dissolvable color, and the state surface. We were able to get knowledge on A*ctinomycetes* segregates from the given sample.

Maintenance of pure cultures:
The a*ctinomycetes* that had been isolated were kept alive in test tubes that were filled with starch casein agar media. They were sub-cultured once every four weeks and kept in the refrigerator at a temperature of 4oC. The results are shown in Figure 1.

Mixed substrate:
Substrates are essential to the growth of a*ctinomycetes* and the production of glutaminase enzyme. These substrates may come from a variety of household and agricultural waste products. The production process involves the use of substrates such as tea powder, wheat grain, jowar powder, coconut kernel powder, and groundnut oil cake.

Both jowar powder and coconut bit offer a significant return when used in the production of L-glutaminase enzyme. These two are combined and employed as blended substrates in the production medium.

Jowar Powder:
The jowar plant is an important crop that may be used for food, animal feed, and fodder in dry and semi-arid tropical locations across the globe. Jowar is an excellent source of several essential nutrients, including but not limited to thiamin, niacin, magnesium, copper, manganese, iron, and potassium. This millet is of the highest quality. Sorghum bicolor, more often known as jowar, is the scientific name for this grain. The most jowar is produced in Maharashtra, which is India's most populous state.

Jowar is used to lower the amount of sugar in the blood, put an end to cravings, promote weight loss, and make one feel more energized.

Coconut Kernel:
The coconut is the edible fruit that is produced by the palm tree species Cocos nucifera, which is also frequently referred to as the coconut palm. Coconut tissue may be dried, eaten fresh, or processed into coconut milk or coconut oil. The coconut kernel powder was used in the present study.

Screening of isolates for L-Glutaminase activity:
After a period of twenty-four hours at room temperature, the plates were examined. The L-glutaminase producers were evaluated by arranging clear pink-colored zones around the provinces in order to compare and contrast the results. Nessler's reagent serving as a marker demonstrated the highest glutaminase activity. A rise in the pH of the filtrates caused the glutaminase-positive sample to exhibit a pink colony.

Assay of L-glutaminase Activity:
L-glutaminase was assayed by direct Nesslerization according to Imada et al.,1973. A reaction mixture, containing 0.5ml of 0.04M L-glutamine, 0.5ml of 0.1M Tris-HCl buffer (pH 8.0), 0.5ml of enzyme solution, and distilled water to a total volume of 2.0ml was incubated at 37oC for 30 min. The reaction was stopped by adding 0.5 ml of 1.5M trichloroacetic acid (TCA).

To 3.5ml distilled water, 0.1ml of the above mixture and 0.2ml of Nessler's reagent were added. After keeping the mixture for 20 min, the absorbance (OD) at 450 nm was measured with a UV-visible spectrophotometer. The ammonium concentration of the reaction was determined by inference from the standard curve of ammonium sulphate.

One unit (U) of L-glutaminase was defined as the amount of enzyme that liberates 1µ mole of ammonia under optimal assay conditions.

Result and Discussion

The present review has been finished on the development of L-glutaminase from a*ctinomycetes* separates using the creation medium containing blended substrates of jowar powder 2.5g and coconut kernel powder 2.5g (1:1) as substrates under SSF. This was done using the production medium containing blended substrates of jowar powder 2.5g and coconut kernel powder 2.5g. The impact of different factors such as the fermentation time, fermentation temperature, pH, inoculum age, Inoculum volume, moisture content, carbon source, nitrogen source, and metal salts was investigated and improved in order to increase the yield of the enzyme.

Optimization Parameters:

Effect of fermentation time on L-Glutaminase:

The influence of fermentation time on the production of glutaminase was investigated by carrying out aging at a variety of different periods, ranging from one day to nine days, employing mixed substrates consisting of jowar powder and coconut part. After a certain amount of time had passed, the concentrates were tested for glutaminase activity. After day seven, glutaminase production begins to decline, which may be due to a reduction in the availability of supplements as well as the accumulation of toxic byproducts of digestion.

From the first to the seventh day, we saw a steady increase in the amount of enzyme production. On the seventh day, glutaminase activity was at its highest, 163.5U/gds shown in Figure 2. After that point, activity began to diminish as a result of a decrease in the production medium, and it reached its lowest point on the ninth day.

Effect Of Temperature on L-glutaminase Production:

To observe the optimum temperature, the temperatures tested were 20oC,28oC,37oC,40oC and 45oC. The formation of the enzyme was investigated at temperatures ranging from 20 to 45oC. The optimal temperature for the formation of l-glutaminase during the hatching process is 175.86U/gds shown in Figure 3. As the temperature continued to rise, a decrease in activity was seen because high temperatures have the potential to denature the molecules that are found in the extracellular space. Therefore, a temperature of 28oC is thought to be the optimal temperature for glutaminase activation.

Effect of pH on L-glutaminase production:

To observe the optimum pH values studies were carried out from 3-11 PH. However, the ideal pH for glutaminase has been observed at 7.5. The amount of enzyme production, 193.8U/gds, was seen when the pH was 7.5, results are shown in Figure 4. The pH affects the transportation of numerous components across the cell membranes, which supports the development of the cell as a whole as well as the item arrangement. This is because the movement of these components is dependent on the pH. The pH is the factor that determines the outcome of each of these processes.

Effect of Inoculum age on L-glutaminase production:

To study of the effective inoculum age on the production of L-glutaminase was carried out ranging from one day to seven days.

On the third day, the level of enzyme production that was observed was 203.54U/gds shown in figure 5. After that, there is a gradual decrease in enzyme production.

Effect of Inoculum Volume on L-glutaminase Production:

To observe the optimum inoculum volume various inoculum quantities ranging from 1 to 7 milliliters were taken.

High production of enzyme was seen for 4 milliliters, with a yield of 225.61 units per gram of dry substrate as shown in Figure 6. As the amount of the inoculum increases, there is a subsequent and steady reduction in the enzyme output.

Effect of Moisture Content on L-Glutaminase Production:

The moisture content was analyzed It was evident that the optimum level of glutaminase production for the combined substrate (Jowar powder and Coconut bit) occurred at a humidity content of 60%, which is equivalent to 247.79U/gds as shown in Figure 7. In addition, the increase in the moisture content causes a decrease in enzyme production.

In the SSF, a higher substrate moisture content will produce substrate molecule aggregation, which will have a negative impact on microbial activity.

Effect of Substrate concentration on L-Glutaminase production:

The substrates that are employed for the growth of organisms are Agro residues. Agro-waste products are the robust substrates that are employed for research, and these substrates are utilized for the production and growth of a*ctinomycetes*. Cassava starch, tea squander, jowar powder, coconut pieces, sugarcane mash, sesame oil, wheat grain, and groundnut oil cake are some of the substrates that are employed.

In spite of the fact that every one of the substrates that are used for the solid-state maturation delivers a high return, when contrasted with other substrates, jowar powder and coconut kernel powder have the highest return, and the results are shown in Figure 8. The examination has been performed using the mixed substrate in this manner.

Fig 1: Isolation and maintenance of pure culture..

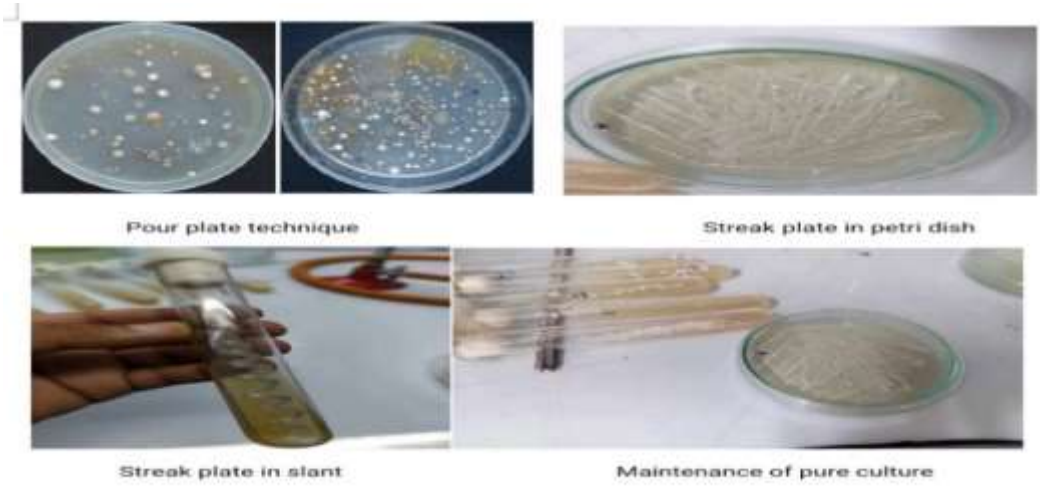


Fig 2. Effect of fermentation time on L-Glutaminase**. Fig3.** Effect of Temperature on L-Glutaminase Production. **Fig 4.** Effect of pH on L-Glutaminase production

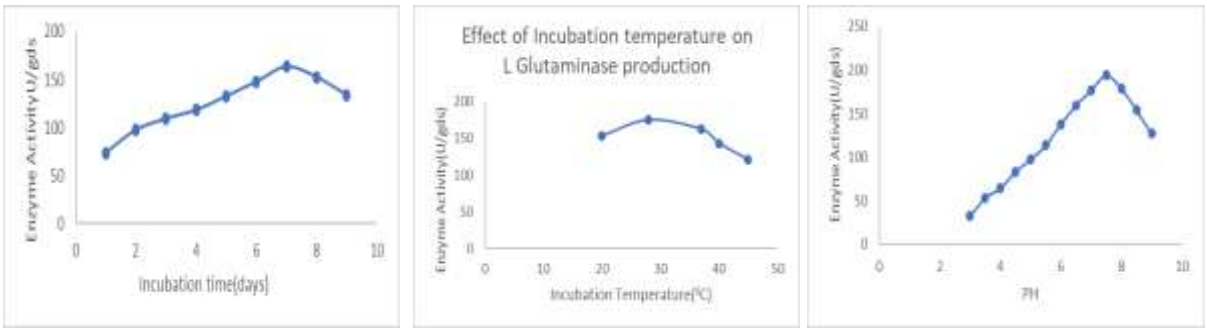
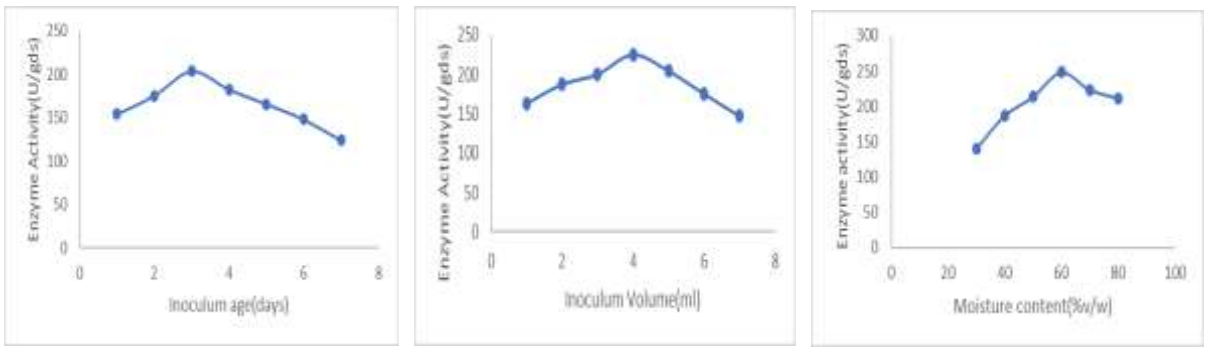


Fig 5: Effect of Inoculum age on L-glutaminase production. **Fig 6:** Effect of Inoculum Volume on L-glutaminase production. **Fig 7:** Effect of Moisture Content on L-Glutaminase production



Effect of Mixed substrate on L-Glutaminase production:

Due to the fact that Jowar Powder and Coconut Kernel powder combination of the two is used as a blended substrate in order to produce more enzymes. The amount of L-Glutaminase that can be produced using jowar powder is 259.92U/gds, whereas the amount that can be produced using coconut kernel powder is 254.75U/gds shown in Figure 9.

Effect of carbon source on L-glutaminase production:

In order to determine the effect that the carbon source has on the production of glutaminase, several different carbon sources were added to the production medium at a concentration of 2% weight per volume. These carbon sources included starch, sucrose, lactose, maltose, mannitol, glucose, fructose, and galactose. The mixture was then incubated at 37 degrees Celsius for five days. After waiting for 5 days, the concentrates were examined to see whether glutaminase activity had occurred. Maltose was a good carbon source that supplied the maximum glutaminase activity out of all of these other carbon sources that were used for the enhancement process. It provided 273.40U/gds and the results are shown in Figure 10.

Effect of Maltose as Carbon Source on L-Glutaminase Production:

Maltose must be taken in different concentrations, which range from 0.5 to 4.5 in percent weight/volume (%W/V), and then it must be included in the production medium. When this is done, the grouping of 3%w/v of maltose produces the highest yield of the enzyme, which is 287.9 units per gram of dry substrate as shown in Figure 11.

Effect of Organic Nitrogen Source on L-glutaminase Production:

The a*ctinomycetes* get their nitrogen from natural sources. The various nitrogen sources, such as urea, peptone, malt extract, yeast extract, hamburger extract, and casamino corrosive, are tested at a concentration of 0.5% weight-to-weight (w/w). It was discovered that among these Malt extract, there is a hotspot for the most intense production of enzyme is 294.65U/gds as shown in Figure 12.

Effect of malt extract as nitrogen supplement at various concentrations on L-glutaminase production:

As Malt extract offers more yield, it must be taken in different concentrations that range from 0.5 to 4.5 in percent weight/volume (%W/V), which must be included in the production medium. The 2.5%w/v of malt extract produces more yield of the enzyme, 298.57 U/gds, and the results are shown in Figure 13.

Effect of Inorganic Nitrogen source on the production of L-glutaminase:

Additionally, inorganic nitrogen sources are employed in the production of the organic entity. There are several inorganic sources of nitrogen, including ammonium nitrate, ammonium sulfate, sodium nitrate, potassium sulfate, ammonium chloride, and calcium nitrate. The highest enzyme production of 307.97U/gds as shown in Figure 14 is achieved with ammonium chloride out of all of these different nitrogen sources.

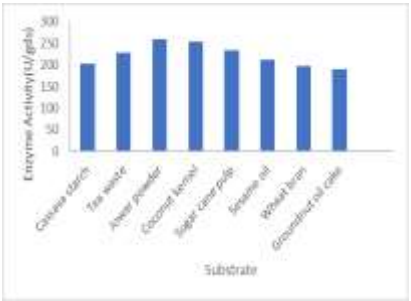
Effect of Ammonium Chloride as nitrogen supplement at various concentrations on L-glutaminase production:

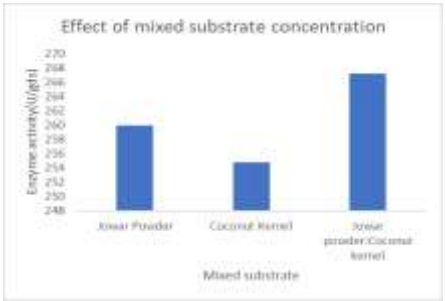
Since ammonium chloride produces a higher yield, it is necessary to use it in a range of concentration from 0.5 to 4.5 in percent weight/volume (%W/V). When this range of ammonium chloride is used in the production medium, 3%w/v of ammonium chloride produces a higher yield of the enzyme, 319.87 U/gds as shown in Figure 15.

Effect of Metal salts on l-glutaminase activity:

In addition, the metal salts are employed in the production of L-glutaminase enzyme by a*ctinomycetes*. Barium chloride, Zinc sulfate, Potassium chloride, Magnesium sulfate, Calcium chloride, Sodium chloride, and Manganese sulfate are some examples of the many types of metal salts that are employed in this process. In comparison to all of these other metal particles, barium chloride produces a higher amount of enzyme, 327.56U/gds, and the results are shown in Figure 16. ”

Fig8: Effect of Substrate concentration on L-Glutaminase production. **Fig 9:** Effect of Mixed substrate concentration. **Fig 10:** Effect of carbon source on L-glutaminase production.





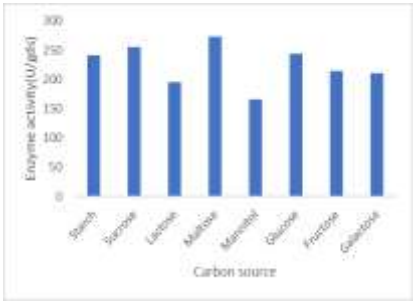


Fig 11: Effect of Maltose as Carbon Source on L-Glutaminase production. **Fig 12:** Effect of Organic Nitrogen Source on L-glutaminase production. **Fig 13:** Effect of malt extract as nitrogen supplement at various concentration on L-glutaminase production

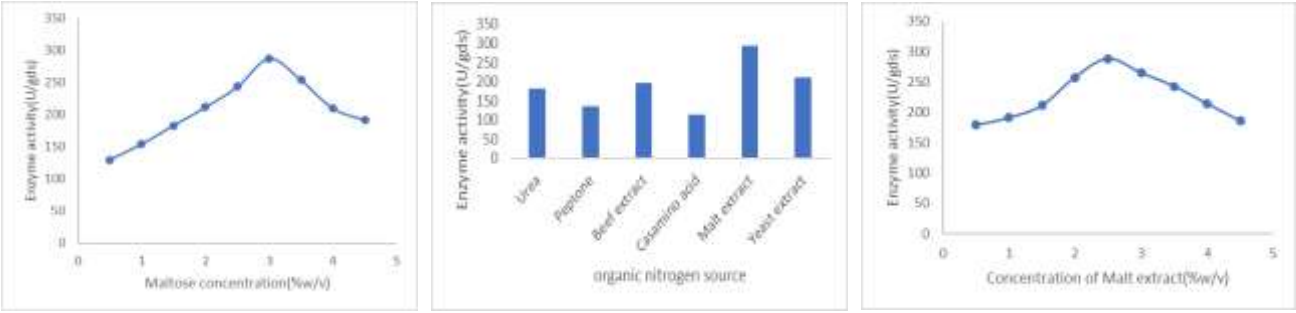
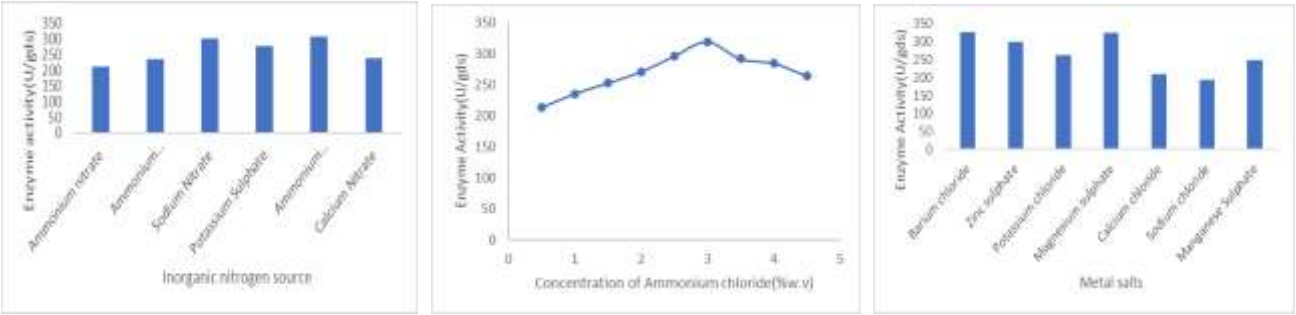


Fig14: Effect of Inorganic Nitrogen source on production of L-glutaminase. **Fig 15:** Effect of Ammonium Chloride as nitrogen supplement at various concentration on L-glutaminase production. **Fig 16:** Effect of Metal salts on l-glutaminase activity



Conclusion

According to the findings of the present study, the environment found in soil is a great hotspot for the isolation of a*ctinomycetes*, which are capable of producing a variety of enzymes. L-glutaminase is an enzyme that is utilized in the treatment of cancer, particularly leukemia. It is also used as a medication to treat HIV/AIDS. The ideal circumstances for the highest production of L-Glutaminase were viewed as at 28°C with a pH of 7.5 when incubated for 5 days with third day old culture and 4ml of the inoculum volume and moisture content of 60% for L-Glutaminase and the expansion of Maltose as a Carbon source and Malt extract as organic Nitrogen source and Ammonium chloride as Inorganic Nitrogen source and Barium Chloride as Metal salt to the production medium. It was observed at these optimised conditions the enzyme activity is 327.56 U/gds. According to this study, the a*ctinomycetes* that were isolated from the soil sample have the potential to be used as a strain for the manufacture of L-glutaminase by using a solid state fermentation strategy with a mixed substrate consisting of jowar powder and coconut kernel powder.

References

Abdallah NA, Amer SK, Habeeb MK (2012) Screening of LGlutaminase produced by a*ctinomycetes* isolated from different soils in Egypt. International Journal of ChemTech Research Vol.4: 1451-1460.

Abdallah NA, Amer SK, Habeeb MK (2013) Production, purification and characterization of L-glutaminase enzyme from Streptomyces avermitilis. African Journal of Microbiology Research Vol.7: 1184-1190.

Abou- Dobara, M. A,M. M. A. Mousa,M. N. A. Hasaneen and Samah M. Nabih (2018) Isolation and Screening of some A*ctinomycetes* from Soil from Damietta and Mansoura and its Antimicrobial Activities Journal of Agricultural Chemistry and Biotechnology Mansoura University. Vol.9(12): 283 – 287.

Afaf Ahmed Elbendary, Ashgan Mohamed Hessain , Mahmoud Darderi El-Hariri , Ahmed Adel Seida , Ihab Mohamed Moussa , Ayman Salem Mubarak , Saleh A. Kabli , Hassan A. Hemeg , Jakeen Kamal El Jakee (2017) Isolation of antimicrobial producing Actinobacteria from soil samples Saudi Journal of Biological Sciences Vol 25 (2017) 44–46.

Anupama, Sapkota, Aishwarya Thapa, Anupa Budhathoki, Muskan Sainju, Prativa Shrestha, and Sagar Aryal (2020) Isolation, Characterization, and Screening of Antimicrobial-Producing A*ctinomycetes* from Soil Samples International Journal of Microbiology Volume 2020, Article ID 2716584, 7 pages.

Ashraf S. A. El-Sayed (2009) L-glutaminase production by Trichoderma koningii under solid state fermentation Indian Journal of Microbiology Vol.49:243–250.

Balagurunathan R, Radhakrishnan M Somasundaram S T (2010) L-glutaminase Producing A*ctinomycetes* from Marine Sediments –Selective Isolation, Semi Quantitative Assay and Characterization of Potential Strain Australian Journal of Basic and Applied Sciences, Vol.4(5):698-705.

Dubey R, Paul A, Prity N (2015) Isolation, production and screening of anti-cancer enzyme L-glutaminase from Bacillus subtilis. International Journal of Pharmacy and Biological Sciences Vol.5: 96- 105.

Ekowati Chasanah ,Tanti Yulianti, and Usman Sumo Friend Tambunan (2012)screening and characterization of l-glutaminase produced by bacteria isolated from sangihe talaud sea Squalen Vol 7 No 3, December 2012: 115-12.

Imada A., Igarasi S., Nakahama K. and Isono M (1973) Asparaginase and glutaminase activities of microrganisms. Journal of General Microbiology Vol.76, 85- 99.

Krishnakumar S., Alexis Rajan R. and Ravikumar S., (2011), Extracellular production of L- glutaminase by marine alkalophilic Streptomyces sp.-SBU1 isolated from Cape Comorin Coast. Indian Journal of Geo-Marine Sciences, Vol.40, 717-721

Magda M. Aly1 , Madeha N. Alsiny1 , Majdah M. AbuRas1 and Samyah D. Jastaniah (2020) Antitumor L-Glutaminase Production by Rare A*ctinomycetes* Obtained from Marine Water Using Submerged Fermentation Biosciences Biotechnology Research Community Vol(3) Pp-1223-1230.

Magda M Aly Roqayah H Kadi , Alia M Aldahlawi, Mayson H Alkhatib , Abdulwahab Noor Wali (2017) Production of the Antitumor L-Glutaminase enzyme from Thermotolerant Streptomyces sp. D214, Under Submerged Fermentation Conditions. Journal of Experimental Biology and Agricultural Sciences vol:5(6)pp:878.885.

Nagwa Ahmed Abdallah, Shaimaa Khairy Amer and Mario Khalil Habeeb(2012) Screening Of L-Glutaminase Produced By A*ctinomycetes* Isolated From Different Soils In Egypt International Journal of Chemtech Research Vol.4, No.4, pp 1451-1460.

Palaniswamy M, K. Nathiya , Sooraj S. Nath, J. Angayarkanni (2011). Optimised production of L-glutaminase: A tumour inhibitor from Aspergillus flavus cultured on agroindustrial residues African Journal of Biotechnology Vol. 10(63), pp. 13887-13894, 17.

Periasamy Dhevagi(2016)Isolation of L-Glutaminase Producing Marine A*ctinomycetes* International Journal of Molecular and Clinical Microbiology Vol.6(1) 635-642.

Prabhu GN, Chandrasekaran M.(1996) L-Glutaminase production by marine Vibrio costicola under solid-state fermentation using different substrates. Journal of Marine Biotechnology Vol 4:176/9.

Rabia Saleem and Safia Ahmed (2021) Characterization of a New L-Glutaminase Produced by Achromobacter xylosoxidans RSHG1, Isolated from an Expired Hydrolyzed L-Glutamine Sample Catalysts 2021, Vol.11, pp1262.

Sabu A., Keerthi T.R., Kumar S.R. and Chandrasekaran M., (1999) L- glutaminase production by marine Beauveria sp. under solid state fermentation. Process. Biochemistry Journal Vol.53, 705- 710

Savitha S. Desai et.al, Sonal J. Chopra, Basavaraj S. Hungund (2016) Production, purification and characterization of L-Glutaminase from Streptomyces sp. isolated from soiL Journal of Applied Pharmaceutical Science Vol. 6 (07) pp. 100-105, July.

Selvamohan.T, N.K.Parameswaran , S. Abisha Felcy (2016) Isolation of A*ctinomycetes* from different soils for analysing the antagonistic activity against pathogens International Journal of Advanced Research in Biological Sciences Volume 3, Issue 8 – 201.

How to Cite This Article:

Douluri V and **Raju KJ**. Studies on the production and optimization of L-Glutaminase by A*ctinomycetes* isolated from soil sample using mixed substrate of jowar powder and coconut Kernel powder under solid-state fermentation. Indian J. Biotech. Pharm. Res. 2023; 11 (3): 1 – 08.