Physicochemical characterization, cytotoxicity and anticancer activity of L-glutaminase from marine Streptomyces luteogriseus

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Abstract

The biological activities of L-glutaminase from bacterial sources have been a subject of significant interest. In this study, we isolated Streptomyces luteogriseus from marine sediment and identified it as a potential producer of L-glutaminase through molecular identification using 16S rRNA sequencing. On SDS-PAGE analysis, the isolated L-glutaminase migrated at a molecular weight of 50 KDa. We optimized L-glutaminase production by investigating various factors such as pH, temperature, metal ions, additives and substrate specificity to enhance production efficiency. Our findings suggest that Streptomyces luteogriseus from marine sediment has the potential to produce L-glutaminase, making it a promising candidate for future research in this field.

Keywords: *Streptomyces luteogriseus*, L-glutaminase, gel filtration chromatography, 2D-PAGE.

Introduction

L-glutaminase is an essential amide molecule in nitrogen metabolism in cells and is present in all known forms of life. Both as a flavor enhancer in the food industry and as a biosensor for measuring glutamine in to mammals to bacteria to actinomycetes to yeast. About all of the L-glutaminase-producing species have been found in either soil or marine environments, according to the literature. The food industry generally agrees that L-glutaminase is a necessary enzyme for enhancing the taste and aroma of fermented foods. As glutaminases catalyze the deamidation of glutamine to glutamic acid, they find applications in a wide variety of industries, including the pharmaceutical and food industries. Several glutaminases, fermented in various ways by various microorganisms, have been reported [9]. Their molecular weight, optimum pH, and substrate specificity all vary. There has been a lot of study on the ideal and stable mammalian and hybridoma cells, eliminating the need for an additional test of glutamic acid, it has found widespread use. L-glutaminase has been discovered in a wide range of organisms, from plants

temperatures for different glutaminases, and it has been shown that several of them work best between 40 and 50 degrees Celsius. Marine microorganisms are generally considered viable options for boosting production of L-glutaminase on a bigger scale due to the enzyme's significant medical and food sector uses. Thus, there is an urgent need to identify untapped marine microbial strains and develop efficient bio-processing protocols to fully harness their potential medicinal applications.

Glutaminases (EC 3.5.1.2) are enzymes that catalyze the hydrolytic deamidation of l-glutamine into l-glutamate and free NH4+, and they are present in most eukaryotic and bacterial species (Cruzat et al., 2019). These

enzymes deamidate l-glutamine but not asparagine or glutamate like glutaminase-asparaginases (EC 3.5.1.1). (Nguyen et al., 2017). Glutaminases are a class of proteins that have been the subject of limited study because of their shared evolutionary background, protein fold, structural motifs, and catalytic mechanism with serine-lactamases and penicillin-binding proteins (Bush K. 2018). These enzymes include the well-known classes of serine-lactamases A, C, and D (Fróes et al., 2016). Penicillin-elucidated, and their respective catalytic molecular processes defined (Bush et al., 2016). Glutaminases are produced by a wide variety of microorganisms, such as yeasts, fungi, Gram-positive and Gram-negative bacteria, and others. Partial descriptions and purifications of enzymes from Micrococcus luteus, Rhizobium etli, Bacillus pasteurii, and Lactobacillus rhamnosus are available.

Since quite some time ago, compound treatment has been utilized to treat malignant growth. Despite the enormous significance of microbial enzymes, it has been discovered that the cultivation and production of enzymes using microorganisms is both economical and good for the environment (Nigam P. S. 2013). According to EC 3.5.1.2, L-glutaminase is an amido hydrolase enzyme that breaks down glutamine into glutamate and ammonia. Reducing the quantity of L-glutamine accessible to cancer cells, and therefore displaying an anticancer influence, has been demonstrated to be useful in the treatment of acute lymphocytic leukemia. This is because cancerous cells consume L-glutamine more avidly than normal cells for their energy requirements and proliferation (Patel et al., 2021). Additionally, it is known that cancerous cells are unable to produce perform insilico characterization of L-glutaminase.

binding proteins catalyze a variety of biosynthetic reactions in bacterial cell walls, such as trans peptidases, transglycosylases, and carboxypeptidases, whereas lactamases (EC 3.5.2.6) hydrolyze the amide bond (N-CO) in the -lactam ring of penicillin and cephalosporin antibiotics. This is the most common way in which bacteria become resistant to beta-lactam antibiotics (ztürk et al., 2016). Each member of the beta-lactamase and dd-peptidase families has had their structural and biochemical features

their own L-glutamine, which is a drawback of these amino acid-depleting anticancer medications (Jiang et al., 2019). As glutaminolysis plays such a significant part in cancer metabolism, it has been considered a possible therapeutic target in the war against cancer (Shen et al., 2021). The first step of glutaminolysis, when glutamine is transformed into glutamate, is catalyzed by glutaminase. Glutaminase is a promising cancer treatment target because of its essential role in glutamine metabolism (Choi, Y. K., and Park, K. G. 2018). The use of glutaminase inhibitors decreases the antioxidant glutathione (GSH) synthesis and increases intracellular reactive oxygen species (ROS) in cancer cells by preventing the conversion of glutamine to glutamate (Cluntun et al., 2017).

Materials and Methods

Physico chemical characterization of purified L-glutaminase

Structural Characterization of Purified Enzyme by In-Silico Studies

The prediction of physiochemical properties, secondary structure, tertiary structure, and gene ontologies for functional activities were used to

Physico-Chemical Characterization

L-glutaminase sequence identified by protein mass fingerprint from MASCOT

server, and the obtained sequence was subjected to further analysis for physico-chemical properties prediction. Physical and chemical properties of the L-glutaminase peptide were predicted from the protein's amino acid sequence. We determined physicochemical properties such molecular weight, isoelectric point, amount of positively and negatively charged amino acid residues, extinction coefficient, instability index, aliphatic index, and grand average hydropathy (GRAVY) using the ProtParam tool on Expasy (Irajie et al., 2016; Cederkvist et al., 2022).

Absorption spectrum of L-glutaminase

Absorption spectroscopy is an effective tool for analysing protein and nucleic acid structural changes as well as their ligand-binding processes and enzymatic reactions. Because the light absorption of a substance or biomolecule is proportional to its concentration, absorption spectroscopy is well suited for both quantitative and qualitative analysis. At a concentration of 0.2 mg/ml and a pH of 7.5, the UV-Visible absorption spectrum of pure L-glutaminase the enzyme's rate of reaction was monitored at temperatures spanning from 10 to 90 degrees Celsius. 50 grams of enzyme and L-glutamine substrate were dissolved in 100 milliliters of Tris-HCl buffer at pH 8 to carry out the experiment. The enzyme substrate combination was then heated in a water bath to the specified temperatures for 2 hours: 20, 30, 40, 50, 60, 70, 80, and 90°C. After incubation, L-glutaminase activity was determined by determining the NH4+ release (Imada et al., 1973; Derst et al., 2000).

Metal ion effects on L-glutaminase activity

For this study, we evaluated the effect of metal ions on enzyme activity by measuring was obtained using a UV-1800 Shimazdu spectrophotometer (Iyer PV et al., 2008; Cederkvist et al., 2022).

L-glutaminase activity and its relation to pH

In order to determine the effect of pH on L-glutaminase activity, the enzyme's activity was measured over a wide range of pH values, from 2.0 to 13.0. Glycine-NaOH (pH 2.0), Tris-HCl (pH 8.0), phosphate (pH 7.0), and citrate-phosphate (pH 4.0-6.0) buffers were used to acquire the pH range (pH 13.0). Dissolving 50 g of L-glutaminase and its substrate L-glutamine in each buffer of known pH enabled analysis of the enzyme's activity. The enzyme substrate combination was then heated to 40 degrees for an hour. After incubation, L-glutaminase activity was determined by determining the NH4+ release (Imada et al., 1973; Derst et al., 2000).

Modification of L-glutaminase Activity by Temperature

In order to determine the effect of temperature on L-glutaminase activity,

L-glutaminase activity in the presence of 20, 40, and 50 mM metal ions. Na+, K+, Mg2+, Cu2+, and Fe2+ were used in this experiment. L-glutaminase activity was measured before and after adding various quantities of metal ions to the enzyme substrate mixture and incubating it at 40 degrees Celsius for 30 minutes (Imada et al., 1973; Derst et al., 2000).

Interaction of ingredients with L-glutaminase activity

By assessing the enzyme's activity in the presence of additives including 2-mercaptoethanol, SDS, EDTA, L-cysteine, and L-histidine, the influence of additives on

L-glutaminase activity was quantified. Several quantities of each component, such as 20, 40, and 50 mM, were added to the enzyme substrate combination in this study. After 30 minutes of incubation at 40 degrees Celsius, the L-glutaminase activity was determined (Imada et al., 1973; Derst et al., 2000).

Specificity of substrates and their influence on L-glutaminase activity

Researchers were able to determine the significance of substrate specificity in the activity of L-glutaminase by measuring the enzyme's activity in the presence of many substrates, including L-glutamine, D-glutamine, and L-asparagine. For this experiment, a 1% substrate solution was prepared by dissolving L-glutamine, D-glutamine, and L-asparagine in 0.1 M Tris-HCl buffer at pH 8.0. The enzyme was then incubated with the three different substrates in a water bath at 40 degrees Celsius for two hours. After incubation, L-glutaminase activity was determined by determining the NH4+ release (Imada et al., 1973; Derst et al., 2000).

Examination of L-Cytotoxic glutaminase's Effects

As detailed by Sudha and Selvam, cytotoxic activity of purified L-glutaminase was evaluated by MTT assay. For the MTT experiment, 5x104 cells were planted in each well of a 96-well plate using a homogenized cell suspension from each of the cell lines. After that, the cells spent 24 hours in a CO2 incubator at 37 °C, 100% humidity (5% CO2), and CO2 concentrations of 5%. A total of 24 hours were spent incubating all cell lines with 0.1 mg/mL of pure purified L-glutaminase in a CO2 incubator at 5% CO2, 37 °C, 95% air, and 100% relative humidity. During the course of four hours, 100 L of MTT reagent was added to each cell line and allowed to rest at room temperature. Without disturbing the cells with pipetting, we removed the surplus reagent and added 100 L of DMSO to the cell lines to dissolve the formazan. A control group lacking L-glutaminase was also introduced to all of the cell lines at the same time. Absorbance at 560 nm from a microplate reader was used to calculate the concentration of purple formazan that was synthesized. L- glutaminases' cytotoxic effect was directly connected to growth suppression (Gwangwa et al., 2019; Krishnan et al., 2012).

The percentage of inhibition was determined using the following equation:

$$\% \text{ inhibition} = 100 - \frac{\text{Mean OD of test sample}}{\text{Mean OD of control}} \times 100$$

L-Cancer-Fighting glutaminase's Properties

The MTT assay was used to determine if L-glutaminase inhibited the growth of a variety of human cancer cell lines in culture. These lines included MCF-7, MDA-MB435S, HeLa, H69PR, COLO 205, and small-cell lung and colon cancers. All the cancer cell lines were procured from Abgenex Pvt. Ltd., Bhubaneswar, India. The chose malignant growth cell lines were refined in different mediums as per the sort of cell line. H69PR and COLO 205 cell lines were cultured in a 1:1 ratio mixture of

DMEM and RPMI 1640 medium, while cultured in DMEM medium. HeLa cell lines were cultured in RPMI 1640 medium. Additionally, 0.37 percent sodium bicarbonate, 7.5% sodium pyruvate, and 4.5 percent glucose were added to the DMEM and RPMI 1640 media. In addition to the salts, the medium contained 10 percent FBS, 2 mM glutamine, and 10 g/mL of penicillin and streptomycin antibiotics. Finally, 0.1N NaOH was used to bring the medium's pH to 7.2. The medium was then put through a membrane filter with 0.22-micron holes to ensure its cleanliness. A CO2 incubator was set at 5% CO2, 37°C, and 70% humidity, and each cell line was injected aseptically into its own medium. All the chosen cell lines had their cultures trypsinized after the media had been discarded. The disaggregated cells were added to 25 mL of the appropriate media in a flask following trypsinization. Using a serological pipette, the homogenized cell lines were suspended in the liquid medium (Gwangwa et al., 2019).

Determination of L-glutaminase dosage on MCF-7 and MDA-MB435S cell lines

In vitro studies using the MCF-7 and MDA-MB435S cell lines shown significant cytotoxic activity for L-glutaminase. from bacterial isolate KLP-08 exhibits a standard protein absorption spectrum in the UV region (Figure 4) due to the charge transitions among energy levels in aromatic amino acids (Durthi et al., 2010). Spectroscopy is a technique for measuring the interaction of molecules with electromagnetic radiation that includes the ultraviolet and visible range of light with an energy of about 150 to 400 KJ/mol.

MCF-7 and MDA-MB435S cell lines were Researchers looked at the MCF-7 and MDA-MB435S cell lines to better understand the effects of L-glutaminase on dosage and cell growth. L-glutaminase was tested for its cytotoxic effects on MCF-7 and MDA-MB435S cells using the MTT assay. L-glutaminase concentrations of 6.25, 12.5, 25, 50, 100, and 200 g/mL were used to determine cytotoxic activity, with a control and standard also present. In this research, methotrexate was utilized as an established method for treating cancer. The plot of L-glutaminase concentration against cytotoxic percentage was used to determine the IC_{50}, or lethal concentration to fifty percent of the cells (Mostafa et al., 2021; Nihal et al., 2014; AlBasher et al., 2018).

Results & Discussion

Absorption spectrum of L-glutaminase

L-glutaminase shows intense absorption in the ultraviolet spectrum, between 260 and 280 nm. Furthermore, the absorption spectrum contains only one peak, indicating that purified L-glutaminase was homogenous, and it was also found that L-glutaminase had maximum absorption at 280 nm and minimum at 260 nm. According to these results, purified L-glutaminase

Aromatic side chains of amino acids like tryptophan, tyrosine, and phenylalanine are responsible for the majority of the absorption between 230 and 300 nm; disulfide bridges have a slight influence at 260 nm. Additionally, the peptide bonds in the proteins exhibit UV light absorption in the range of 180 to 230 nm due to their partial double bond nature (Ashok et al., 2019).

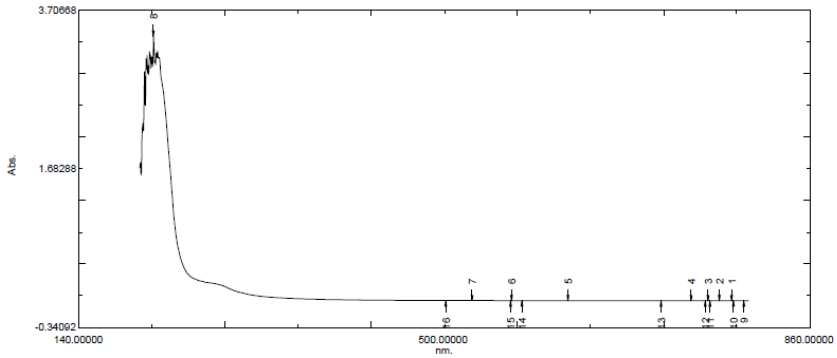


Figure No.4: Absorption spectrum of crude L-glutaminase from bacterial isolate KLP-08.

pH's effect on L-glutaminase activity

By evaluating its activity at different buffer concentrations and pH values, from 4.0 to 13.1, the kinetic properties of pure L-glutaminase were determined. L-glutaminase activity at various pH values isolated from KLP-08 is shown in Figure 5. The generated curve shows how the L-glutaminase activity changes with pH. The findings showed that L-glutaminase from KLP-08 is amidohydrolytically active between pH 4 data. Aljewari et al. (2010) also discovered that L-glutaminase isolated from actinomycetes is most active between pH 7.0 and 8.0. (Dharmaraj et al., 2011). The ideal pH for L-glutaminase activity also depends on the species from which the enzyme was originally isolated. For instance, L-and pH 13. At a pH of 4.0, the enzyme activity began, and at a pH of 7.0, it was at its highest. Activity of the enzyme continued to decrease with time, and by the time the pH reached 13 there was no L-glutaminase left. As a result of these tests, we know that a pH of 7.0 is best for L-glutaminase (Bollineni et al., 2015). Dhevagi, P., and E. Poorani (2006) found that L-glutaminase activity was highest at a pH of 7.5, which is consistent with the current

glutaminase isolated from marine actinomycetes has been shown to be most active at a pH of 8.6 (Dhevagi and Poorani, 2006). Ameena et al. (2010) found that Streptomyces gulbargensis L-glutaminase is most active at a pH of 9.0. The findings were published in 2010 (Amena et al.

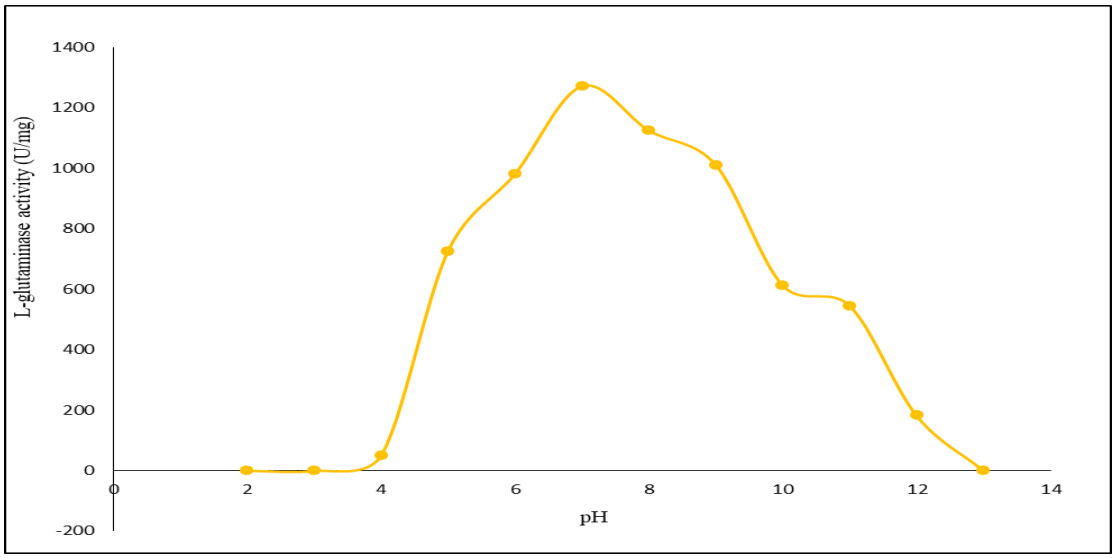


Figure No.5: The influence of pH on the activity of KLP-08 L-glutaminases.

Modification of L-glutaminase Activity by Temperature

L-glutaminase activity was measured at several temperatures, spanning from 20 to 70 degrees Celsius, to establish the impact of temperature on enzyme activity. Figure 6 depicts the purified L-glutaminase activity from KLP-08 at different temperatures. In entirely eliminated at 80°C. These results indicated that 40°C was the optimal temperature for L-glutaminase. L-glutaminase activity was shown to rise monotonically up to 40°C in the current study. Our findings corroborate those of Tiwari et al. (2019) who found that Erwinia this study, we found that L-glutaminase from KLP-08 is most active between 20 and 70 degrees Celsius. On top of that, the optimal temperature for enzyme activity is 40 degrees Celsius. Enzyme activity was shown to decline at temperatures over 40°C and to be

aroideae NRRL B-138 L-asparaginase is most active at a temperature of 45°C (Sohail et al., 2011). Kumar et al. (2011) found that the optimal temperature for Pectobacterium carotovorum MTCC 1428 L-glutaminase activity is 39.3 degrees Celsius. For example: (Wakayama et al., 2005).

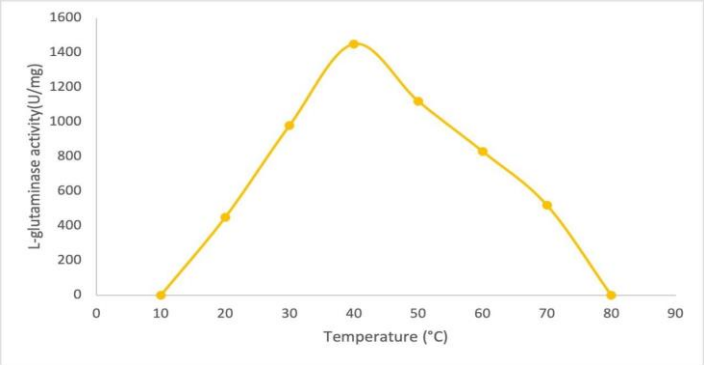


Figure No.6: Temperature's impact on KLP-08 L-glutaminase activity.

L-glutaminase activity and the influence of metal ions

L-glutaminase activity was measured at 5, 10, 15, and 20 mM of metal ions to find out how these concentrations affected the enzyme's performance. The outcomes of metal ion's effect on L-glutaminase activity are shown in (Table No.3 and Figure 7). Our findings indicate that, of the metal ions studied, only Na+ has a positive effect on L-glutaminase activity as ion concentration increases, but K+ has a negative effect. When the enzyme activity in the absence of metal ions (control) was taken as 100%, the increasing concentrations of Na^+ such as 5, 10, 15, and 20 exhibited increased glutaminase activity such as 118, 128, 135, and 144% respectively. In contrast, the increasing concentrations of K^+ such as 5, 10, 15, and 20 exhibited decreased glutaminase activity such as 89, 71, 58, and 47% respectively. In the present study Na^+ enhanced the L-glutaminase activity with increasing concentrations. While the remaining metal ions, including K^+, $Cu^{2+}$and Fe^{2+}, decrease L-glutaminase activity as concentrations increase. This study reveals that the increasing concentrations of Na^+ significantly

enhance the enzyme activity. These results are consistent with those of Raha et al. (1990), who discovered that metal ions such as Zn2+, Fe2+, Cu2+, Hg2+, and Ni2+ significantly increased L-glutaminase activity in Cylindrocarpon obtusisporum MB-10 (Gasteiger et al., 2005). Whereas, studies of Elshafei, Ali & El-Ghonemy, Dina. (2021) revealed that Zn^{2+}, and Cu^{2+} exhibits inhibitory activity against L-glutaminase.

Table No.3: Metallo-L-glutaminase interaction

S. No	Metal Ion	Enzyme activity (%)			
		5 mM	10 mM	15 mM	20 mM
1	Na^{+}	**118**	**128**	**135**	**144**
2	K^{+}	89	71	58	47
3	Mg^{2+}	100	92	105	103
4	Cu^{2+}	105	103	100	97
5	Fe^{2+}	105	100	95	91

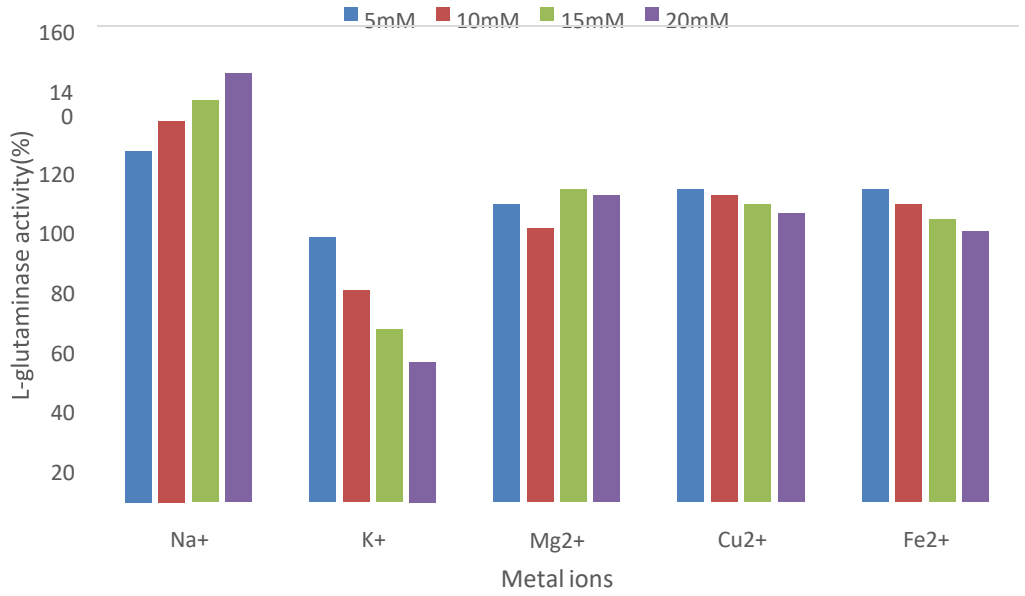


Figure No.7: Metallo-L-glutaminase interaction

Modification of L-glutaminase Activity Caused by Additives

L-glutaminase activity was measured in the presence of several additives, including 2-mercaptoethanol, SDS, EDTA, L-cysteine, activity are shown in [Table No.4 and Figure 8]. From these results, it was observed that, among all the tested inhibitors, L-cysteine, L-histidine, and 2-mercaptoethanol exhibit significant inhibitory effects on L-glutaminase activity at all concentrations, and L-histidine, at 20, 40, and 50 mM concentrations to investigate the influence of additives on enzyme activity. The results of the additives' influence on L-glutaminase

whereas SDS and EDTA can significantly inhibit L-glutaminase activity at 50 mM concentration. L-cysteine and SDS had the most and least inhibitory efficacy, respectively, of all the inhibitors examined. When the enzyme activity in the absence of

additives (control) was taken as 100 %, the increasing concentrations of L-cysteine such as 20, 40, and 50 exhibited decreased glutaminase activity such as 46.91, 19.46, and 0% respectively. This study reveals that the increasing concentrations of L-cysteine significantly inhibit the enzyme activity. As shown above, EDTA is a potent inhibitor of L-glutaminase from marine actinomycetes, as found by Basha et al., 2009. (Idicula-Thomas, S., and Balaji, P. V. 2005).

Table No.4: Effect of additives on L-glutaminase activity

Additive	**Concentration (mM)**	**Residual activity (%)**
Control	--	100
SDS	20	89.7
	40	60.2
	50	16.41
L-cysteine	20	46.91
	40	19.46
	50	0
L-histidine	20	49.45
	40	22.83
	50	16.26
2-mercaptothanol	20	50.95
	40	23.96
	50	14.32
EDTA	20	79
	40	57.67
	50	11.48

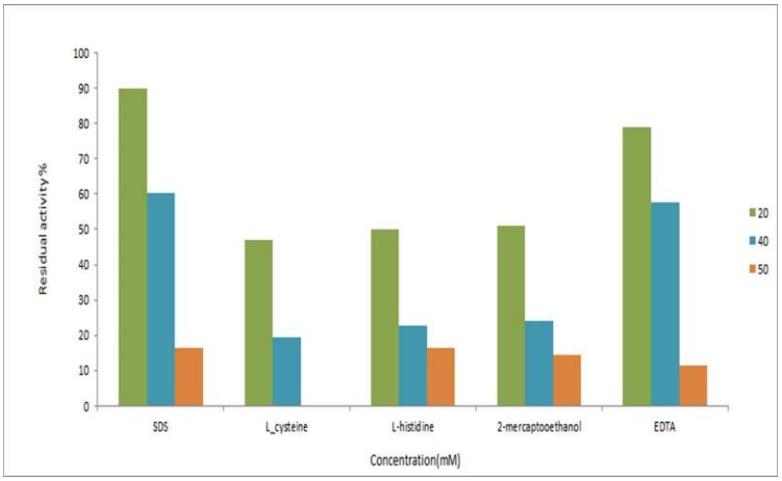


Figure No.8: Modification of L-glutaminase Activity Caused by Additives

L-glutaminase activity as a function of substrate specificity

The effect of substrate specificity on L-glutaminase activity was investigated by measuring enzyme activity in the presence of a variety of substrates, including L-glutamine, D-glutamine, and L-asparagine. In this study, it was discovered that L-glutamine is the best substrate for the purified L-glutaminase enzyme, whereas L-

asparagine produced the least active enzyme (Figure 9). Figure No.8 depicts the impact of substrate specificity on L-glutaminase activity. These data show that L-glutaminase has high hydrolytic activity of 1356, 558, and 412 U/mg with L-glutamine, D-glutamine, and L-asparagine, respectively. This study reveals that the L-glutaminase is highly specific towards the substrate L-glutamine.

L-glutaminase from E. coli showed remarkable selectivity towards the hydrolysis of L-glutamine, as shown in the current study, and in prior work by Campbell and Mashburn (1969). Wakayama et al. (2005) report that L-glutaminase from Stenotrophomonas maltophilia NYW-81 hydrolyzes L-glutamine, D-glutamine, and D-asparagine, but shows highest specific activity with L-glutamine

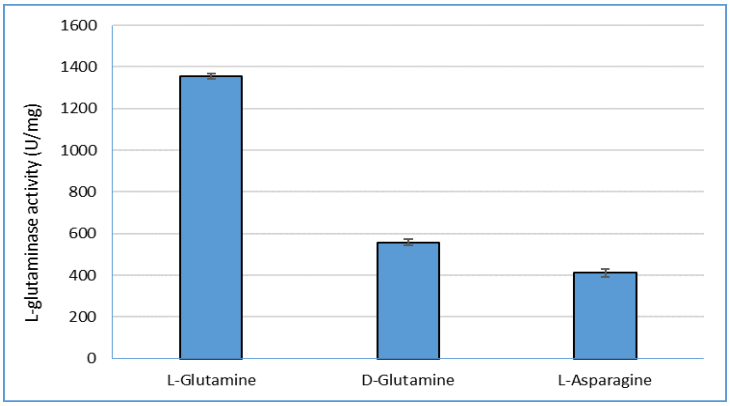


Figure No.9: Substrate specificity's influence on L-glutaminase activity.

L-glutaminase catalyzes the transformation of glutamine into glutamate, with ammonia being the byproduct. This reaction becomes important for cancer treatment, since cancer cells require L-glutamine derived carbon to proceed through the TCA cycle (Vander Heiden et al., 2010). According to the study (Belica et al., 2014), L-glutaminase is an effective inhibitor of HIV and has a significant role in the treatment of malignancies, especially acute lymphocytic leukemia. As a result, it is advantageous to isolate new microbes with enhanced L-glutaminase capabilities.

Anti-Cancer Activity of L-glutaminase

Natural products may act as chemotherapeutic agents and have pharmaceutical uses, and are increasingly being utilized for general health maintenance, immune system enhancement, cancer prevention, treatment, and remission (Talib et al., 2020). It is now understood that normal tissue homeostasis depends on a delicate equilibrium between cellular proliferation and death, and that disruptions in either process may result in the dysregulated clonal growth that is a hallmark of all neoplastic disorders (Fouad, Y. A., and Aanei, C. 2017).

Cytotoxic activity of L-glutaminase

Several studies have demonstrated that L-glutaminase may inhibit tumor growth in a variety of animal and human species by specifically targeting and killing off tumor cells (Fouad, Y. A., and Aanei, C. 2017). Many cancer cell lines, including MCF-7, MDA-MB435S, HeLa, H69PR, and COLO 205, were examined for the cytotoxic effects of purified S. luteogriseus L-glutaminase using the MTT assay. Cancer cell lines MCF-7, MDA-MB435S, HeLa, H69PR, and COLO 205 were subjected to

MTT assays to determine the cytotoxic potential of the L-glutaminase enzyme. The invitro cytotoxic activity of L-glutaminase from S. luteogriseus exhibited significant inhibitory activity on all the five tested cell line. While, the percentage of inhibition was substantially varied to the five tested cell lines. The effects of L-glutaminase on several cell lines in vitro. Based on the results, cytotoxic activity was determined to be greatest in MCF-7 cell lines and lowest in COLO 205 cell lines. L-glutaminase showed cytotoxic activity in vitro (IC50 values) of 73.9% on MCF-7 cells, 65.9% on MDA-MB435S cells, 60.0 on HeLa cells, 55.5% on H69PR cells, and 60.0 on COLO 205 cells, respectively. In addition, L-glutaminase therapy resulted in 26.1, 36.9, 34.1, 40.0, and 44.5 percentage increases in cell viability for their respective cell lines.

The majority of tumour cells are auxotrophic to glutamine; hence they require an external source of L-glutamate to grow and survive. As L-glutaminase depletes tumor cells of L-glutamine, the cells die. As a result, L-glutaminase had received significant interest, which corresponds to its broad applications in the biomedical field as an anti-leukemic drug, as well as, an effective anti-retroviral agent (Xiang et al., 2015). Previous research showed that Aspergillus falvus L-glutaminase had strong cytotoxic activity against MCF-7 cell lines (IC50 = 250 g/mL), supporting the current findings (Abu-Tahon MA et al., 2020). L-glutaminase showed considerable cytotoxic action against MCF-7 and MDA-MB435S, two of the five cell lines evaluated. As a result, researchers analyzed how the addition of L-glutaminase affected dosing and cell proliferation in MCF-7 and MDA-MB435S cell lines.

Dose response of MCF-7 and MDA-MB435S cells to L-glutaminase

Purified L-glutaminase from S. luteogriseus exhibited significant in vitro cytotoxic activity against a variety of cancer cell lines at concentrations of 6.25, 12.5, 25, 50, 100, and 200 g/mL. The results of L-glutaminase dosage effect on MCF-7 cell line was shown in Figure No.13. When tested against MCF-7 and MDA-MB435S cell lines, L-glutaminase was most cytotoxic at a concentration of 200 g/mL. Whereas, lowest cytotoxic activity was found at 6.25 µg/mL concentration of L-glutaminase for both the tested cell lines. The increased dosage of L-glutaminase significantly decreased viability of MCF-7 cells and increased cell inhibition. The cell survival percentages of MCF-7 cells with increasing L-glutaminase dosage such as 6.25, 12.5, 25, 50, 100 and 200 µg/mL were found to be 64.38, 54.65, 46.94, 42.87, 35.17 and 30.37% respectively. Results showed that when L-glutaminase dose was increased, the proportion of viable cells reduced dramatically. As well as, survival percentage of MCF-7 cells showed strong negative correlation (r= -0.9792) with increasing L-glutaminase dosage. The results of L-glutaminase dosage effect on MDA-MB435S cell line was shown in Figure No.15. The increased L-glutaminase dose substantially reduced MDA-MB435S cell viability and increased cell inhibition. MDA-MB435S cell survival percentages were determined to be 84.93, 62.89, 46.38, 44.81, 34.09 and 28.67 with respect to increasing L-glutaminase dosages of 6.25, 12.5, 25, 50, 100 and 200 µg/mL. The current findings indicate that increasing L-glutaminase dose dramatically reduces the proportion of viable cells. As

well as, survival percentage of MDA-MB435S cells shows strong negative correlation (r= - 0.9288) with increasing L-glutaminase dosage. Purified L-glutaminase has an IC50 of 66.53 and 124.8 g/mL when used to treat MCF-7 and MDA-MB435S cell lines, respectively.

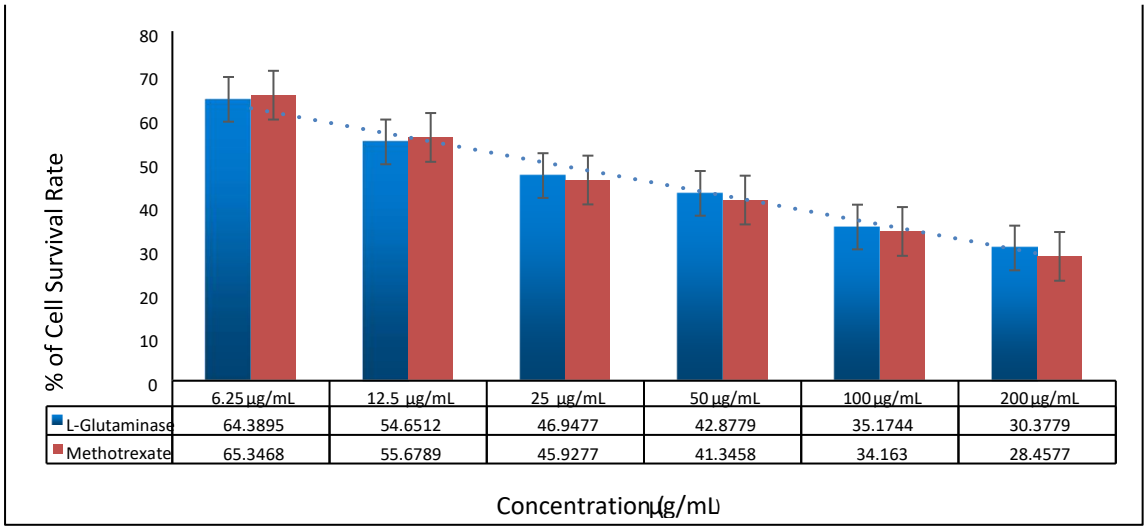
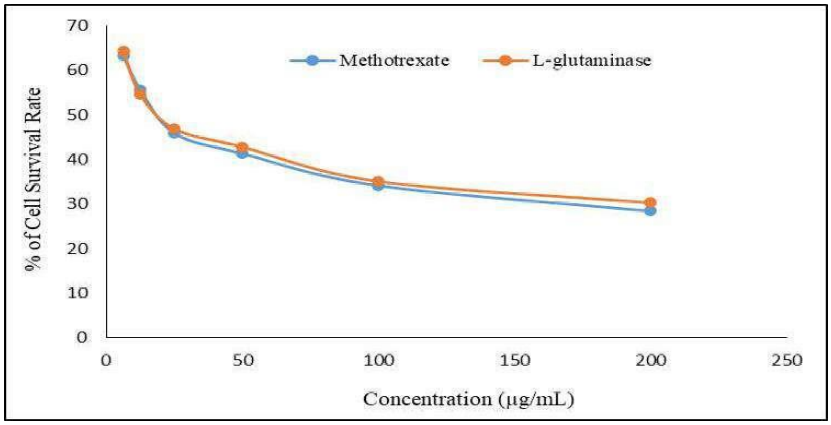


Figure. No.10: Percentage of MCF-7 cell viability with increasing concentration of L-glutaminase.

Figure No.11: Dose-response curve of L-glutaminase on MCF-7 cell line.



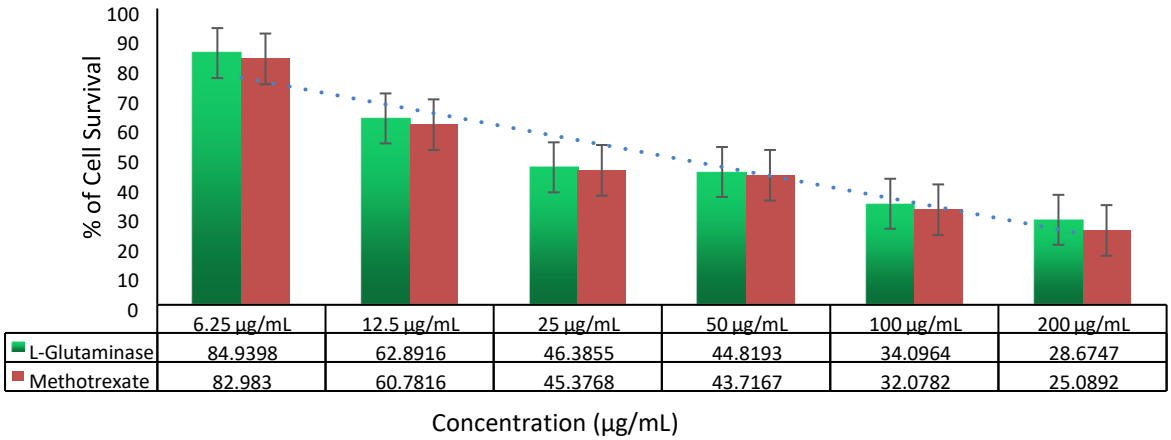


Figure No.12: Percentage of MDA-MB435S cell viability with increasing concentration of L-glutaminase.

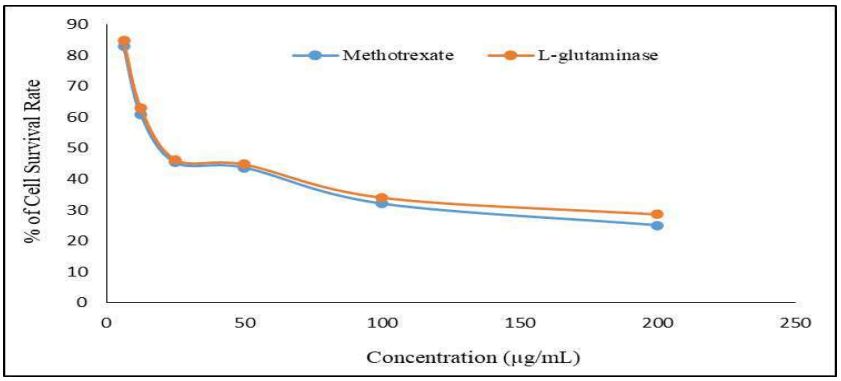


Figure. No.13: L-glutaminase dose-response curve in MDA-MB435S cells.

L-glutaminase was shown to be approximately as effective as the gold standard anticancer medication methotrexate in killing MCF-7 and MDA-MB435S cells in this investigation (Figure No.12 and 14). As compared to MDA-MB435S cell lines, MCF-7 cells responded better to L-glutaminase therapy. Singh and Banik (2013) found that 82.27 g/mL of B. cereus L-glutaminase has substantial anticancer activity against Hep-G2 cell lines. Figure No.14 shows that L-glutaminase significantly inhibited MCF-7 cell growth in a dose-dependent manner, as shown by the MTT experiment. Furthermore, microscopic examination demonstrated that L-glutaminase suppresses MCF-7 cell growth, as evidenced by a substantial difference in cell viability between control and treated cells. It was observed that MCF-7 cells become spherical, tiny, and lose their contact with other cells after being exposed to L-glutaminase.

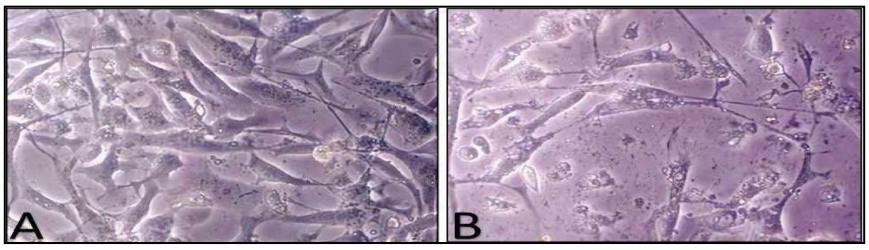


Figure No.14: Photographs of MCF-7 cells A) Control (0 µg/mL) group of MCF-7 cells B) MCF-7 cells under the treatment of 200 µg/mL L-glutaminase.

Microscopic observations of control and treatment group (200 µg/mL) of MDA-MB435S cells with L-glutaminase indicated that L-glutaminase considerably affects the shape of MDA-MB435S cells and thereby reduced cell proliferation (Figure No.15). L-glutaminase therapy also reduced the number of colonies in MDA-MB435S cells, suggesting that it suppressed the formation of mammospheres in these cells.

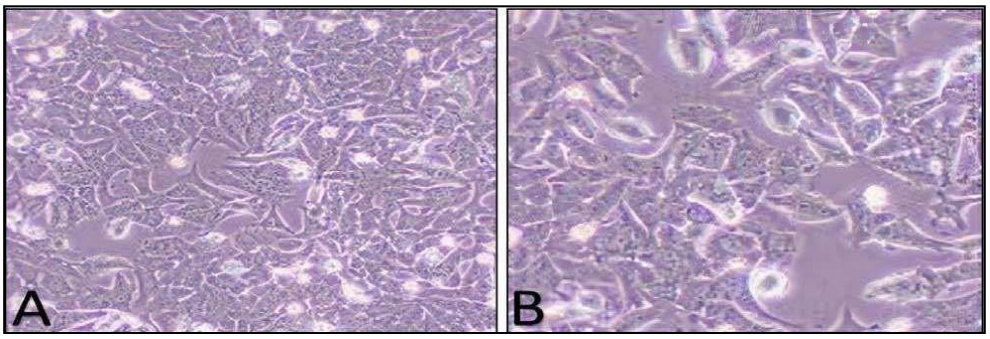


Figure No.15: Photographs of MDA-MB435S cells A) Control (0 µg/mL) group of MDA-MB435S B) MDA-MB435S cells under the treatment of 200 µg/mL L-glutaminase.

Figures 14 and 15 show that L-glutaminase treatment dramatically reduced the proliferation and viability of MCF-7 and MDA-MB435S cell lines at increasing concentrations.

Conclusion

In the treatment of cancer, it has been demonstrated that bacterial L-glutaminase is both efficient and cost-effective. When L- glutaminase concentration and incubation duration were both increased, cell proliferation of MCF-7 and MDA-MB435S was inhibited. The current study found that pure L-glutaminase induces apoptosis in MCF-7 and MDA-MB435S cancer cells, suggesting that it has anti-cancer properties. Our research showed that L-glutaminase has potential applications in biotech, medicine, and the pharmaceutical sectors. Streptomyces luteogriseus-derived L-glutaminase offers numerous therapeutic and industrial applications. Its high specificity towards L-glutamine allows for effective usage in low quantities. Our study's findings suggest that this marine strain of Streptomyces luteogriseus has great potential and is a suitable candidate for large-scale production of L-glutaminase in industries.

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