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Research Article

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Optimization Of Culture Conditions For Maximizing L-glutaminase Production From The Marine Fungus *Aspergillus flavus*

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KEY WORDS

Marine Fungi, L-glutaminase, *A. flavus*, Optimization of Culture Conditions, Molecular identification.

ABSTRACT

Marine fungi have the capacity to extreme environments and live in a highly competitive ecological position make them suppliers of novel metabolites as L-glutaminase which is medically useful in the pharmaceutical industry, as an antioxidant and anticancer and antileukemia. This study aimed to isolate and identified some marine fungi and screened for L-glutaminase production and select the most producing marine fungus to optimize the culture conditions for maximizing production of L-glutamenase. The results showed five isolated fungal species; *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium digitatum*, *P. italicum* which were identified microscopically according to references books on mycology. The Screening for L-glutaminase production from the isolated fungi utilizing solid and liquid media exhibited that *A. flavus* out of the isolated fungi, recorded the highest diameter of pink zone (72.3 mm) with using solid medium and highest L-glutaminase activity (11.489U/ml) and highest mycelial dry weight using liquid medium. The optimization culture conditions of *A. flavus* for maximizing L-glutaminase production, showed that the highest L-glutaminase activity and mycelial dry weight were recorded at 30°C for 5 days of incubation, pH6, L-glutamine and fructose as nitrogen and carbon sources. Molecular identification of *A. flavus* was investigated for confirming the morphomicroscopical identification, Phylogenetic tree based on ITS sequences of rDNA of the isolated fungus closely related to strains accessed from the GenBank. It showed similarity 99.31 -99.83% and 98 - 100% coverage with the strains of *A. flavus* in GenBank with accession no. NR_111041.

Introduction

Exploring efficient Chemotherapy sources of fewer adverse effects is a global challenge because of the rising incidence and mortality rate of cancer. The creation of new and improved chemotherapies, either synthetic or natural, is one of the recent developments in cancer treatment **(Khalil *et al.*, 2020)**. Enzymes for therapeutic purposes are utilized to cure illnesses including severe disorders, but because of their high specificity and affinity, they are most commonly used in treatment for cancer **(Vo *et al.*, 2020)**. The amidohydrolase L-glutaminase (glutamine aminohydr0lase EC 3.5.1.2) hydrolyzes l-glutamine to produce l-glutamic acid and ammonia, which can then be used as an essential chemotherapeutic drug to treat multiple types of cancer **(Ren *et al.*, 2020)**. The most predominant amino acid in human muscles and plasma is glutamine. L-glutamine is converted by the hydrolytic enzyme L-glutaminase into glutamic acid and ammonia which are then further catabolized to yield lipids, nucleotides, amino acids, glutathione, and ATP **(Kim *et al.*, 2021)**. L-glutaminase has several applications in the food, medical industries besides, can be utilized as an antioxidant and anticancer medication to treat acute lymphocytic leukemia **(Sarkar *et al.*, 2020)**. L-glutaminase has a major use in biosensors that monitor the glutamine levels in human and hybridoma cells **(Ahmed *et al.*, 2016)**. Tumor cells require a lot of L-glutamine for cell proliferation, however they are unable to produce it. When immature lymphocytes proliferatet hey use glutamine, synthesized within the human body as a substrate for respiration and as nitrogen to produce proteins, hexosamines, and macromolecules **(Altman *et al.*, 2016)**. Glutamine synthetases use ATP as an energy source to convert glutamate into glutamine in a healthy cell. Because cancer cells require a very high amount of glutamine and lack L-glutamine synthetase enzyme, they are incapable to create enough of these amino acids endogenously and must instead rely on serum levels to proliferate and survive **(Choi and Park, 2018)**. The enzyme L-glutaminase is found in many various parts of nature, including bacteria, actinomycetes, yeast, fungi, plants, and animals **(Lavanya Kothapalli, 2023)**. **Amobonye *et al.*, (2019)** found that thousands of microorganisms had amidase activity. **Saleem and Ahmed, (2021)** studied the isolation and purification of L-glutaminase from different microbiological sources for medicinal uses. Potent manufacturers of glutaminase with the greatest effectiveness and notable anti-tumor effect against several cancer cell lines have been found in a variety species of fungi that are related to the *Acremonium*, *Saccharomyces*, *Penicillium*, *Trichoderma*, and *Aspergillus* genera **(Awad *et al.*, 2021)**. For the fabrication of l-glutaminase by *Fusarium oxysporum*, several endophytic fungal species, *Penicillium digitatum*, *Penicillium* sp., and *Penicillium brevicompactum*, *Trichoderma viride*, *Aspergillus aculeatus*, *Rhizomucor miehei*, and *Cladosporium sp.*, the optimal fermentation method is submerged fermentation used according

to **Khalil *et al., (*2020)**. Many bacterial strains have been shown to secrets glutaminase. Various terrestrial bacteria, involving *Proteus morganii, Bacillus specie Pseudomonas* species, *Escherichia coli* and Acinetobacter species have been found to produce glutaminase **(Neha *et al.,* 2021)**. L-glutaminase synthesis has also been recorded from a few number of marine microorganisms, including *Vibrio costicola, Micrococcus luteus,* and *Pseudomonas fluorescens*, **(Jambulingam and Saranya, 2020)**. Besides bacteria, actinomycetes also exhibit significant variety in the peculiar properties of L-glutaminases; the only one genus mentioned is streptomyces, despite the fact that the possibility of variance noted by the genus According to **Aly *et al.,* (2017)**. *Streptomyces* sp. does not secrete intracellular L-glutaminase; instead, it is primarily extracellular **(Abdelmonem et al., 2023)**. Although there are other alternative sources for *Streptomyces* sp. isolation, the marine environment is the most promising because there aren't many reports about it **(Hassan *et al.,* 2025)**. While the L-glutaminase had not been detectable in drumsticks or ginger, a number of plants, including tomatoes, tamarind, and chillies have significant amounts of the enzyme; onions, potatoes, and lemons have trace amounts **(Yang *et al.,* 2017)**. Reports on marine fungus producing L-glutaminase are somewhat rare. However, a new field of study with great promise is the investigation of marine fungi for the formation of L-glutaminase **(Ahmed *et al.,* 2016)**. On earth, the marine habitat is most diverse and the biggest ecosystem. It is defined by changes in, salinity, pH, pressure, temperature, concentrations of nutrients and minerals, and dynamic interactions between living organisms. Marine-derived biomolecules are the most promising cancer treatment due to their unique features **(Kumar *et al.,* 2021)**. The enzymes of these microorganisms are resistant to changes in temperature, pressure, pH, and salinity and are generally more stable than those of similar terrestrial bacteria (**Nguyen and Nguyen, 2017)**. Enzymes from marine microbes may be appropriate for uses in therapy with minimal side impact since human blood plasma and seawater have a nearer chemical relationship. Unlike enzymes from other sources, those generated from marine microbes are more compatible with human physiology because they have evolved to function in these conditions **(Francis and Chakraborty, 2021)**. So the aim of the current study was to optimize the culture conditions of the isolated marine fungus, *Aspergillus flavus* for maximizing production of L-glutamenase enzyme.

MATERIAL AND METHODS

Isolation and purification of fungi collected from marine water samples

Five marine water samples were obtained from various locations in Mediterranean sea in Alexandria. All water samples were mixed in one sample. Marine fungi were isolated from the mixed water sample directly, one ml from mixed marine water sample was added under aseptic conditions over the surface of plates, each plate contains Czapek’s Dox agar medium. Following seven days of incubation at 30°C, purification was performed on the resulting colonies, which were then maintained as stock cultures on plates and slants with Czapek's Dox

medium and preserved at 4°C for further use.

Identification of the isolated marine fungi

The isolated fungal colonies were recognised using the criteria of morphological and microscopical characters of hypha, and spores according to consult keys, using references books on mycology as:

Domesch *et al.*, (1980). For fungi in general.

Kitch and Pitt, (1992). For common *Aspergillus* species.

Raper and Fennel, (1977). For common *Penicillium* species.

Gilman, (1957). For soil fungi in general.

Screening of L-glutaminase production from the isolated marine fungi using solid medium

A rapid-plate test was used to screen the isolated marine fungi for the synthesis of L-glutaminase, employing glutamine as the only nitrogen source **(Gulati *et al.*, 1997 and Hamed and Al-wasify, 2016)**. Modified Czapek's Dox medium (Glucose 2, L-glutamine 10, $MgSO_4.7H_2O$ 0.52, KCl 0.52, $FeSO_4.7H_2O$ 0.01, KH_2PO_4 1.52, Agar 20.0 g/l) was used for qualitative screening of L-glutaminase synthesis from the isolated fungi, the medium supplemented with phenol red (0.3ml of 2.5% made in ethanol with adjusted pH7.0) as pH indicator. After the medium being autoclaved, were poured into 9 cm-diameter Petri dishes. Control plates were prepared by using $NaNo_3$ as a nitrogen supply (Instead of using glutamine). Separately, the isolated fungus were inoculated to the plates and incubated at 30ºC for 7day. Three replicates were used for each isolated marine fungus. After incubation the pink zone and colony diameters (mm) were measured for each isolated marine fungus.

Screening of L-glutaminase production from the isolated marine fungi using liquid medium

Five mm disc from 7 days old culture of each isolated a marine fungus was grown individually in 100 mL Erlenmeyer flasks each including autoclaved 50 mL of modified Czapek's Dox liquid medium and pH was adjusted to 7.0. Three replicates were used as average values for each isolated fungus. After incubation of culture flasks at 30°C for 7 day on a rotary shaker at 120 rpm, each culture media was filtered using filter paper Whatman no.1, the mycelia were twice washed with distelled water, and then the mycelial dry weight was determined for each the isolated marine fungi after drying at 60°C in a oven at, until the weight remains constant. At 6000 rpm, every culture filtrate was centrifuged (Benchtop Centrifuges) for 15 min. and clear supernatants were utilized for enzyme assay.

Assay of L-glutaminase enzyme

L-glutaminase was assayed using the direct Nesslerization method, which was outlined by **Imada *et al.* (1973)**. In a test tube half mL of 0.01 M solution of glutamine was taken , then 0.5 mL of TRIS hydrochloride buffer (0.05 M) at pH 8.6, 0.5 ml of supernatant containing L-glutaminase and half ml of distilled water was transferred to obtain the volume up to 2.0 ml, then the reaction mixture was incubated for 30 minutes at 37°C . Following incubation, half ml of 1.5 M Trichloroacetic acid was added to pause the interaction. After that, 0.1 ml of the mixture was combined with 0.2 ml of reagent of Nessler and 3.7 ml of

distilled water, and then it was incubated for twenty minutes at 20°C. After the twenty minutes of incubation the optical density (OD) was measured at 450 nm using spectrophotometer (spectrophotometerr, UV-90). Control was prepared by denaturation of enzyme in supernatant by boiling at 100°C for 5 min. in water bath. Then the same of the above assay method was carried for control. International Units, were used for expressing the enzyme activity.

International Unit (IU): is the quantity of enzyme required to release 1 µmol of ammonia per minute.

Units/ml enzyme = (µ mole of NH_3 liberated) (2.5) / (0.1) (30) (0.5)

2.5 = Initial volume of reaction mixture (ml).

0.1 = Volume of enzyme mixture used in the final reaction (ml).

30 = Incubation time (min).

0.5 = Volume of used enzyme extrct (ml).

Optimization of culture conditions of the most potent isolated marine fungus for improving L-glutaminase production

The results of screening for glutaminase activity clearly indicated that the efficiency of *A. flavus* was more than the other screened marine fungal isolates in producing L-glutaminase enzyme. So, the manufacture of L-glutaminase enzyme from *A. flavus* was subjected to evaluate optimization of culture parameters to maximize the productivity of enzyme. The optimal result achieved by each factor was fixed for the subsequent experiment. These parameters included incubation times (1, 2, 3, 4, 5, 6 ,7, 8, 9 and 10 days), pH values (3, 4, 5, 6, 7, 8 and 9), incubation temperatures (25, 30, 35 and 40°C), different nitrogen sources (sodium nitrate, potassium nitrate, ammonium chloride, glutamine, glycine, yeast extract, urea and peptone) and different carbon sources (glucose, fructose, maltose, sucrose, cellulose and starch). All Parameters were carried out separately using 100 ml conical flasks for each one, in every flask fifty milliliters of Modified Czapek's dox liquid media at pH 7 that had been autoclaved. The flakes were inoculated with 1ml from spore suspensin of the most potent selected fungal isolate separately at each factor. All flasks of each factor were incubated. After incubation, each parameter of each culture was filtered and the mycelium was washed by sterile distilled water twicely and dried at 60°C in an oven for determination of the mycelial dry weight (gm /50ml). At 6000 rpm, each culture filtrate was centrifuged for fifteen minutes, and clear supernatants were utilized for l-glutaminase assay as previously mentioned.

Molecular identification of the selected most potent fungal isolate

The selected fungus isolate was grown on sterile petri dishes with 20 milliliters of autoclaved potato sucrose agar media, and it was then incubated for five days at 28 °C for seven days **(Pitt and Hocking, 2009)**. A Patho-gene-spin DNA/RNA extraction kit, supplied by Intron Biotechnology Company, Korea, was used to extract DNA from the prepared culture that had been delivered to Assiut University's Molecular Biology Research Unit. Prior to being shipped to SolGent Company in Daejeon, South Korea, for rRNA gene sequencing and polymerase chain reaction (PCR), the obtained DNA was stored in a 1.5 ml autoclaved Eppendorf

tube. The PCR had been made using forward internal transcribed spacer 1 (ITS1) and reverse internal transcribed 4 (ITS4) primers which were added to the combination of reactions. Where the primers' formula is as follows, ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). The sequencing of the yield of purified PCR was carried out, with the above primers with addition of dideoxynucleoside triphosphates within the mixture of reaction **(White *et al.*, 1990 and Nargesi *et al.*, 2022)**. The Basic Local Alignment Search Tool (BLAST) on the National Center of Biotechnology Information, website had been used to examine the results of sequences. For the phylogenetic tree, MegAlign (DNA Star) software version 5.050 was utilized to carry out phylogenetic analysis of sequences.

Statistical analysis

Analysis of variance, or ANOVA, was employed to examine the measured data. The experiment was subjected in a one-way completely randomised (1WCR), with three duplicates of each parameter assessed (n=3). Using CoStat software, the crucial difference at 5% level of probability was compared with the significant differences between the used treatments. The mean values and standard deviation were computed and expressed as means ± SD.

RESULTS AND DISCUSSION

Isolation and purification of fungi collected from marine water samples

Table (1**)** shows that five fungal species; *Aspergillus flavus, A. niger, A. terreus, Penicillium digitatum, P. Italicum* belonging to two genera of *Aspergillus* and *Penicillin* were isolated from the marine water sample and identified on the basis of morphological and microscopical features of the hyphae and spore structure affording to consult keys given in standard references books on mycology.

Screening for L-glutaminase production from the isolated marine fungi using solid medium

The results in Table (2) revealed that the highest diameter of pink region was recorded with *Aspergillus flavus* (72.3 mm) with colony diameter 12.8 mm followed by *Penicillium italicum* (61 mm) and *Asperillus terreus* (51.3 mm) with colonies diameters 12.00 and 16.7 mm respectively. A pink area surrounding the colonies suggests that the pH was rising, which was the result of ammonium formation in the medium **(Patel *et al.*, 2020)**. According to Many investigators, the isolated marine fungi have been found to generate glutaminase **(Durthia *et al.*, 2019)**.

Table (1) : Isolation of fungal species from the collected marine water sample

Fungal species	Author name
Aspergillus flavus	Link
A. niger	Van Tieghem
A. terreus	Thom
Penicillium digitatum	Saccard
P. italicum	Wehmer

Table (2): Screening for L-glutaminase prduction from the isolated marine fungi using solid medium

Isolated marine fungi	Diameter of fungal colony (mm)	Diameter of pink region (mm)
Aspergillus flavus	12.8	72.3
A. niger	11.5	7.00
A. terreus	16.7	51.3
Penicillium digitatum	12.0	12.0
P. italicum	12.0	61.0

Screening for L-glutaminase production from the isolated marine fungi using liquid medium

The results in Fig. (1) showed that the highest glutaminase activity was recorded with *A. flavus* (11.489U/ml) and the mycelial dry weight was 0.110 g/50ml followed by *P. italicum* which recorded (10.943U/ml) L-glutaminase activity with mycelial dry weight (0.166 g/50ml). While the L-glutaminase activities and mycelial dry weights of *A. terreus*, *P. digitatum*, and *A. niger* recorded 5.872, 3.545 and 1.982, U/ml with mycelial dry weight 0.09, 0.07, and 0.064 g/50ml respectively. The differences in the maximum manufacture of glutaminase by numerous types of marine fungi may be ascribed to two factors: the circumstances of fermentation or to species differences.

The results of screening indicated that *A. flavus* showed the highest level of glutaminase activity in contrast to the other isolates.Therefore, the *A. flavus* was selected for further experimental studies in order to find out the factors which induce the largest production of glutaminase. A new field of study with great promise is the investigation of marine fungi for the manufacturing of glutaminase. According to **Ahmed *et al*., (2016)**, marine isolated fungi such as *Zygosaccharomyces rouxii NRRL-Y 2547*, *Beauveria bassiana*, sp., and *Aspergillus* sp. had been reported to sythesis glutaminase. **Patel *et al*., (2020)** reported that scarcely marine bacteria from Gujarat coasts was screened for glutaminase fabricating. **Vineetha *et al.*, (2024)** reported that *Fusarium solani-melongenea* have the ability to create L-glutaminase by the using liquid media and gave activity of o.86 U/mL.

Optimization of culture conditions of *A. flavus* for maximizing L-glutaminase production

Effect of various incubation times on the mycelial dry weight and L-glutaminase activity of *A. flavus*

The influence of different incubation periods (1,2,3,4,5,6,7,8,9 and 10 days) on the mycelial dry weight and L-glutaminase activity of *A. flavus* was made. The data points in Fig. (2) revealed that gradual increasing in mycelial dry weight and L-glutaminase activity of *A. flavus* with increasing the incubation periods until reached to five days of incubation then decreased gradually until 10 days. The highest mycelial dry weight of *A. flavus* (0.172 g/50 ml) and L-glutaminase activity (15.391 $U.ml^{-1}$). The decline in enzyme activity after it peaked at five days possibly as a result of denaturation through interaction with other components in the medium **(Khalil *et al.*, 2020)**.

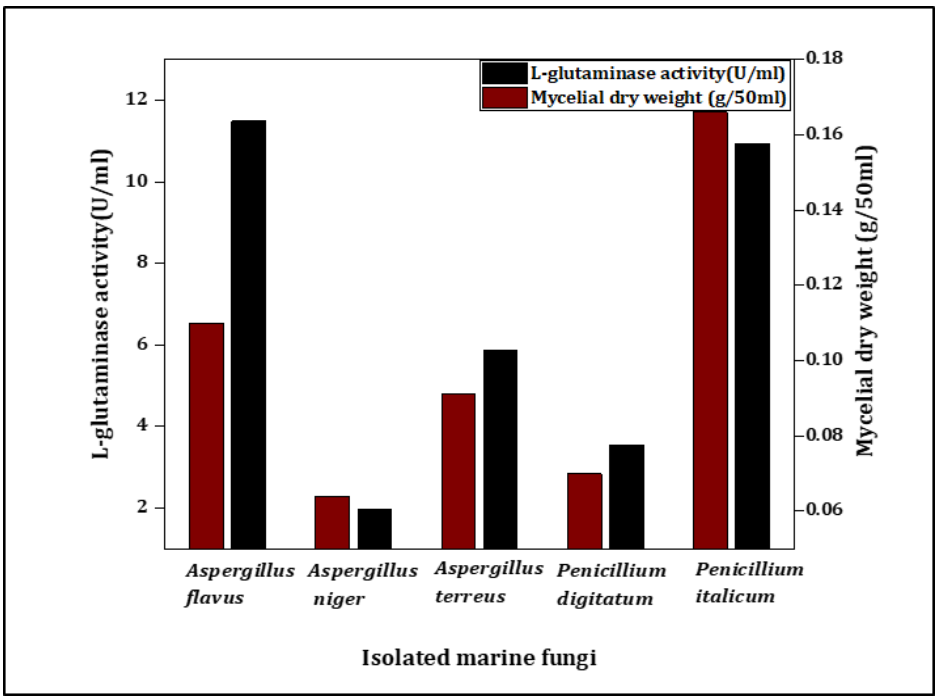

Fig. (1): Screening for L- glutaminase activity from the isolated marine fungi using liquid medium.

Also, the same result was acquired by **Youssef *et al.*, (2024)** who reported that the maximum synthesis of L-glutaminase by *Aspergillus tamarii AUMC* was noted on the fifth day of incubation. Also, **Prasanth *et al.*, (2009)** investigated that *Aspergillus* spp. and *Aspergillus wentii* produced the maximum glutaminase within three days of fermentation. **Hamed and Al-wasify, (2016)** found that, Lglutaminase fabrication by *F. oxysporum* rise gradually till 7 days. Whereas **Mousumi and Dayanand, (2013)** found that *Streptomyces enissocaesilis* produce the highest amount of enzyme after incubating for five days. The outcomes by **Farag *et al.*, (2024)** revealed that Marine *Streptomyces griseorubens* indicated that the highest L-glutaminase activity after 5 days of incubation. According to **Kashyap *et al.*, (2002)** illustrated that the presence of proteolytic activity break down the L-glutaminase in the medium which was most likely the cause of decrease in activity. Additionally, the organism may have reached a point where it was unable to maintain a balance between the availability of nutrient supplies and its steady growth **(Siddalingeshwara *et al.*, 2011)**. According to other references, reported that the ideal time of incubation for *Aspergillus oryzae* was found to be within two days **(Prasanna and Raju, 2011)**. For most fungal strains, the fifth to seventh day of incubation was the best duration to detect glutaminase creation **(Pallem *et al.*, 2010)**.

Effect of different pH values on the mycelial dry weight and L-glutaminase activity of *A. flavus*

Figure (3) revealed that pH 6 was the best pH for mycelial growth weight and L-glutaminase activity of *A. flavus* where the mycelial dry weight was 0.127 g/50ml and enzyme activity was 12.391 $U.ml^{-1}$. Both up and down pH 6 the

activity of glutaminase and growth of *A. flavus* were considerably reduced, where pH value 9 was not suitable for the mycelial growth of *A. flavus* and also L-glutaminase activity. The comparable result was found by **Moorthy *et al*., (2010)**, who illustrated that the ideal enzyme activity of *Aspergillus terreus* and *Aspergillus tamari* was recorded at the optimal pH of 6.2. Also, **Hamed and Al-wasify, (2016)** reported that the maximal L-glutaminase produced by *Fusarium oxysporum* was noticed at pH6.0, enzyme synthesis decreased in response to either a rise or fall in the medium's pH. This probably because of the effect of pH of the medium on the permeability of plasma membrane and the presence of certain metabolic ions, which support the creation of enzymes and cell development. According to **Sabu *et al*., (2000)** *Beauveria* sp. exhibited was two optimal pH values 6.0 and 9.0 for L-glutaminase synthesis under solid state fermentation.

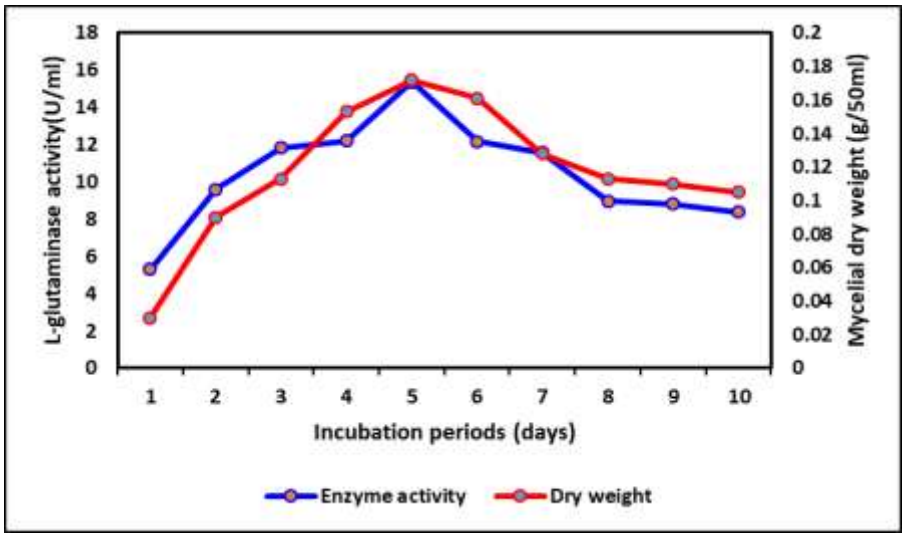

Fig. (2): Effect of various incubation periods on the mycelial dry weight and L- glutaminase activity of *A. flavus.*

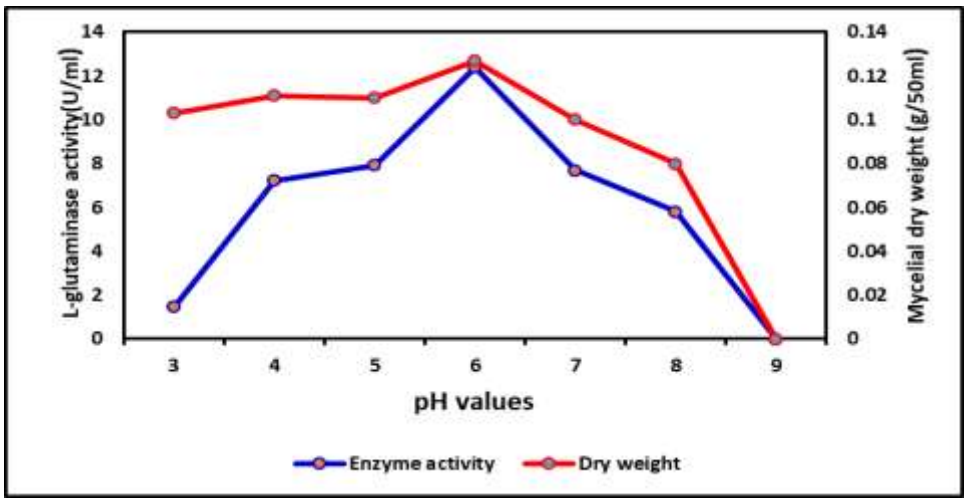

Fig. (3): Effect of different pH values on the mycelial dry weight and L-glutaminase activity of *A. flavus.*

Effect of various temperatures on the mycelial dry weight and L-glutaminase activity of *A. flavus*

The data mentioned in Fig. (4) illustrated that L-glutaminase activity and mycelial dry weight of *A. flavus* raised with increasing incubation temperatures where the greatest enzyme activity, and mycelial dry weight, were 16.455 U/ml and 0.099 g/50ml subsequently at 30°C for 5 days of incubation. In a similar manner, **Kashyap *et al.*, (2002)** showed the largest glutaminase formation by *Zygosaccharomyces rouxii* was found when the fermentation was executed at 30°C. Also, several outhers recorded at 30°C highly production of lglutaminase, by, *Vibrio sp.* **(Prakash *et al.*, 2010)**, *Aspergillus oryzae* **(Prasanna and Raju, 2011)** and *Trichoderma koningii* **(EL-Sayed, 2009).** According to **Bedaiwy *et al.*, (2016)** that 30°C recorded highest mycelial dry weight and enzyme creation *by Aspergillus tamarii***.** Growth temperature has an impact on microbial metabolism in terms of enzymatic reactions and cellular process rates according to **Ibrahim *et al., (*2024)**.

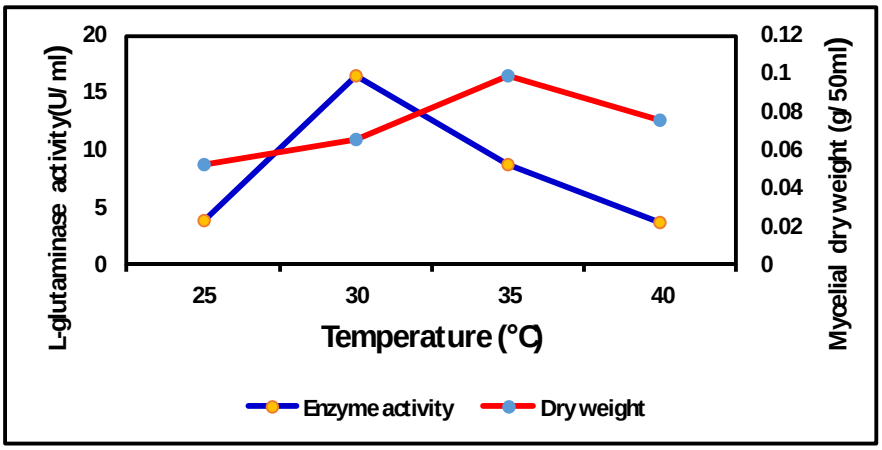

Fig. (4) : Effect of various tempertutes on the mycelial dry weight and L-glutaminase activity of *A. flavus.*

Effect of different nitrogen sources on the mycelial dry weight and L-glutaminase activity of *A. flavus*

Figure (5), illustrated that no relationship between dry weight values of mycelial and L-glutaminase activity of *A. flavus where* KNO_3 recorded the highest dry weight values (0.187 g/50ml) with little L-glutaminase activity (3.169 U/ml), while glutamine supported the maximum enzyme production (24.301U/ml) with mycelial dry weight value (0.135 g/50ml). The results by **Ibrahim *et al.*, (2024)** indicated that l-glutamine stimulates the synthesis of the extracellular l-glutaminase enzyme. Since nitrogen is the most important precursor for protein biosynthesis, it has a significant effect on the generation of enzymes. Additionally, the resource of

nitrogen could affect on the medium's pH, which may have an effect on the enzyme's stability and activity. Similar data was observed by **Prakash *et al.*, (2010)** whom noticed that a significant amount of l-glutaminase was produced when glutamine was applied as the nitrogen source in the culture medium. As stated by **Saleem and Ahmed (2021),** *Achromobacter. xylosoxidans* generated the highest level of glutaminase. When glutamine was utilized as a nitrogen source. In the case of marine *Halomonas meridiana* there is a critical decrease in activity of enzyme found by **Mostafa *et al., (*2021)** which shows the negative influence of inorganic Supplies of nitrogen on L-glutaminase manufacture this is comparable to the present study. Anotherstudy **Ramli *et al.*, (2020)** showed the negative effect of ammonium ions on enzyme production by *Bacillus sp*. The maximal L-glutaminase activity of *Streptomyces. griseorubens* was detected when glutamine was involved as anitrogen source **(Farag *et al.*, 2024)**. Another studies have utilized glutamine as a nitrogen s0urce to increase L-glutaminase production **(Al-Zahrani *et al.*, 2020).**

Effect of different carbon sources on the mycelial dry weight and L-glutaminase activity of *A. flavus*

The present results in Fig. (6) showed that fructose was the most effective carbon source for L-glutaminase activity (58.566U/ml) and mycelial dry weight (0.133 g/50ml) Afterward, Glucose, and maltose where the activity of L-glutaminase were 53.605 and 51.959 U/ml and dry weight were 0.103 and 0.123 g/50ml respectively. Cellulose was the least effective on mycelial dry weight (0.061 g/50ml) and L-glutaminase activity (34.668 U/ml). Several studies were carried out in the use of a single carbon source by different asparaginolytic fungi and bacteria was reported by **Gurunathan and Sahadevan, (2012)** and their results were supportive to our results. According to **Ibrahim *et al.*, (2024),** fructose was determined as most efficient Provider for carbon, resulting in the highest formation of glutaminase by *Klebsiella pneumonia.* Furthermore, in the instance of marine Bacillus subtilis, JK-79, fructose (1% w/v) is the preferred carbon source **(Kiruthika *et al.*, 2018).** Also **, Rao *et al.*, (2023)** stated that the stimulatory effect of fructose on the creation of L glutaminase, in addition to its producers's growth might be because of fructose can be utilized easily as energy source and improved the absorption of amino acids. One possible explanation for the increase in l-glutaminase quantity by the adding of carbon sources, is that glutamine and other carbon sources work well together to promote enzyme formation **(Chitanand and Shete, 2012).** According to **Gomaa, (2022)** that *Bacillus* sp. relied on glucose as a supply of carbon to produce the most L-glutaminase.

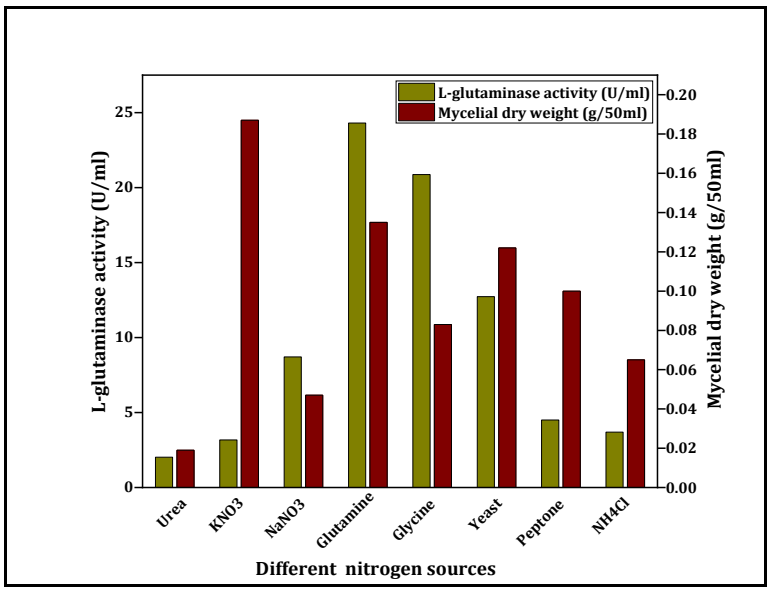

Fig. (5) : Effect of different nitrogen sources on the mycelial dry weight and L- glutaminase activity of *A. flavus.*

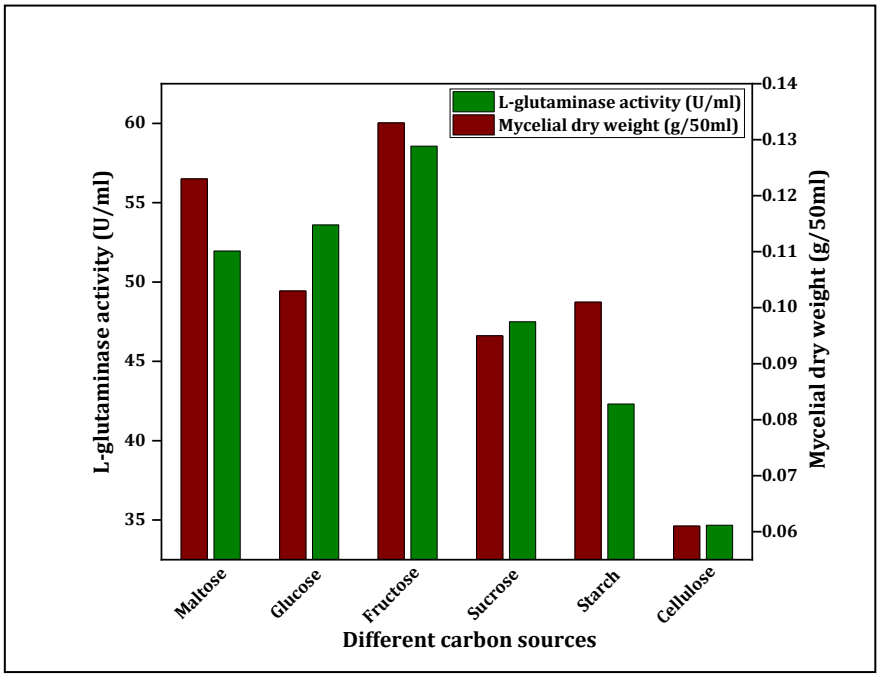

Fig. (6) : Effect of different carbon sources on the mycelial dry weight and L-glutaminase activity of *A. flavus.*

Molecular identification of the selected most potent marine fungal isolate *A. flavus*

Molecular recognition was made for confirming the morphological and microscopical identifying of the isolated *A. flavus*. The data in Fig. (8) showed that phylogenetic tree had been relied on the rDNA gene sequences of Fig. (7) of the used strain *A. flavus* in the this work (*Aspergillus flavus* AUMC15908, arrowed) compared to the comparable

strain in the Genebank. It indicated, 99.31% -99.83% identity and 98% - 100% coverage with numerous strains of *A. flavus* kind of material was included *A. flavus* ATCC16883 with GenBank accession no.NR_111041.

AGGTGAAAACTGCGGAAGGATCATTACCGAGTGTAGGGTTCCTAGCGAGCCCAAC
CTCCCACCCGTGTTTACTGTACCTTAGTTGCTTCGGCGGGCCCGCCATTCATGGCCG
CCGGGGGCTCTCAGCCCCGGGCCCGCGCCCGCCGGAGACACCACGAACTCTGTCTG
ATCTAGTGAAGTCTGAGTTGATTGTATCGCAATCAGTTAAAACTTTCAACAATGGA
TCTCTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAAT
TGCAGAATTCCGTGAATCATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCC
GGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCATCAAGCACGGCTTGTGTGTTG
GGTCGTCGTCCCCTCTCCGGGGGGGACGGGCCCCAAAGGCAGCGGCGGCACCGCG
TCCGATCCTCGAGCGTATGGGGCTTTGTCACCCGCTCTGTAGGCCCGGCCGGCGCT
TGCCGAACGCAAATCAATCTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCC
GCTGAACTTAAGCATATCAAAA.

Fig. (7): ITS sequences of rDNA of the fungal sample isolated in the existing study (*Aspergillus flavus).*

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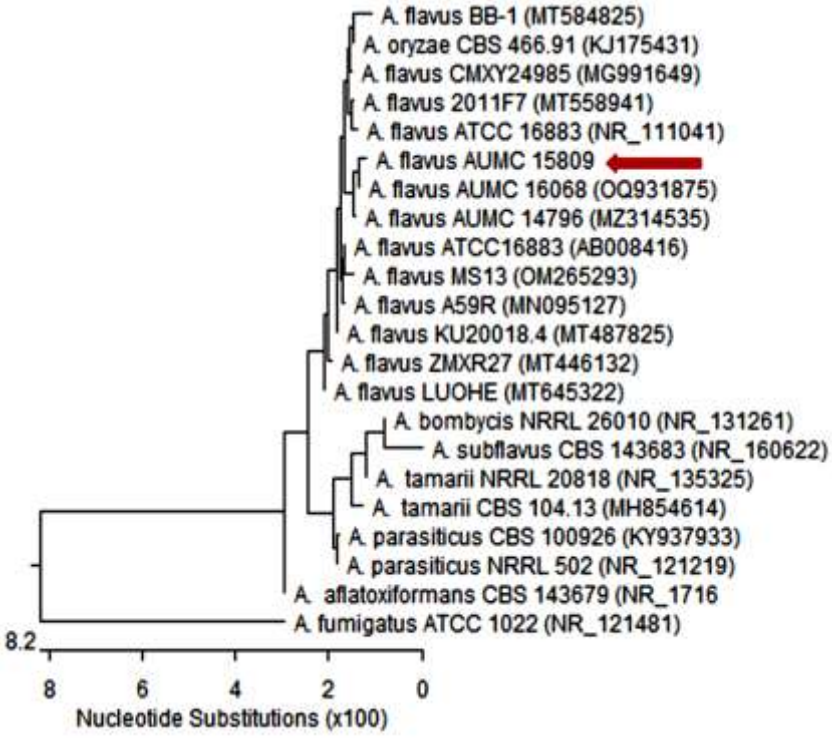

Fig. (8): Phylogenetic tree, based on ITS sequences of rDNA 0f the fungal sample isolated in this study (*Aspergillus flavus* AUMC15908, arrowed) aligned with closely related strains accessed fr0m the GenBank with accession no. NR_111041.

CONCLUSION

The present work concluded that, the isolated marine fungus, *A. flavus* was the most producing glutamenase enzyme and it's optimization processes recorded a promising technique for the maximizing formation of L-glutaminase and it can be applied as an antioxidant, antibacterial, anticancer, and acute leukemia treatment in pharmaceuticals. So, L-glutaminase production from marine fungi, investigating a new field of study with promising possibilities.

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تحسين ظروف زراعة الفطر البحرى اسبرجيلس فلافس لتعظيم إنتاج انزيم جلوتامينيز

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تهدف هذه الدراسه إلي إنتاج إنزيم الجلوتامينيز من بعض الفطريات المعزوله من البحر الأبيض المتوسط في الإسكندريه وقد تم عزل وتنقية وتعريف ٥ عزلات من الفطريات البحريه علي وسط شابكس دوكس وتم تنقية وتعريف هذه الفطريات علي أساس الخصائص المورفولوجية والمجهرية وفقا للمراجع القياسية في تعريف الفطريات ووجد أنها تنتمي إلي الجنسين اسبرجيلس و بنيسليوم. وبدراسة اختبار هذه الفطريات علي إنتاج إنزيم الجلوتامينيز وجد أن فطر أسبرجيلس فلافس (١١.٤٨٩ وحدة / مل)، بنسيليوم إتاليكم (١٠.٩٤٣وحدة/ مل)، اسبرجيلس تيريس (٥.٨٧٢ وحدة / مل) هم الأكثر إنتاجا عند نموهم علي وسط شابكس دوكس صلب أو سائل يحتوي علي الجلوتامين كمصدر للنيتروجين. وقد أظهرت النتائج أن فطر اسبرجيلس فلافس هو الأكثر قدرة علي إنتاج إنزيم الجلوتامينيز، ولذلك تم اختياره ودراسة الظروف المثلي لنموه الميسيلومى فى الوسط السائل والحصول على اعلى وزن جاف للميسيليوم واعلى انتاجية لانزيم الجلوتامينيز ،وقد أظهرت النتائج ان الظروف المثلى لنمو فطر اسبرجيلس فلافس واعلى نشاطية للانزيم هى مدة خمسة أيام تحضين عند درجة حرارة ٣٠ درجة مئوية ورقم هيدروجين ٦ واستخدام الجلوتامين كمصدر نيتروجين والفركتوز كمصدر كربوني. وقد أكد التعريف الجزيئي للفطر الأكثر انتاجا لإنزيم الجلوتامينيز أنه أسبرجيلس فلافس بنسبة تشابه ٩٨-١٠٠%.