IJMCM 12(2) (2022) 1704-1712 Doi: 10.22034/ijmcm.2022.693586

International Journal of
Molecular and Clinical Microbiology

Research Article

Isolation and identification of L-asparaginase producer bacteria from soil and effluent of slaughterhouse in Isfahan

Fereshteh Hozoorbakhsh[1], Mozhgan Ghiasian[1]*, Fereshte Ghandehari[1], Zarrindokht Emami-Karvani[1], Maryam Khademi Dehkordi[2]

1. Department of Microbiology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.
2. Department of Biology, Falavarjan Branch, Islamic Azad University, Isfahan, Iran.

ARTICLE INFO

Article history:
Received 3 April 2022
Accepted 4 july 2022
Available online 1 September 2022

Keywords:
L-Asparaginase,
Screening,
Slaughterhouse,
Phylogenetic tree,
PCR.

ABSTRACT

L-asparaginase is one of the most important treatments for acute lymphoblastic leukemia. The aim of this study was isolation and molecularly identification for L-asparaginase producer bacteria. In this study, samples were taken from the effluent and soil of a slaughterhouse in Isfahan. M9 culture medium was used to screen the L-asparaginase producer bacteria. After screening, qualitative measurement of L-asparaginase was performed based on the color change in the media. The L-asparaginase producer bacteria were identified by biochemical and molecular methods. 114 colonies were isolated from the samples. 5 colonies were able to produce L-asparaginase. The halo diameter of L-asparaginase production in bacteria numbers SS18, SS19, SE64, SE112 and SE114 were 21, 22, 30, 33 and 55 mm, respectively. 16SrRNA sequences showed that strain SS18 was 99% likely to belong to *Stenotrophomonas maltophilia*, strain SS19 was 99% likely to belong to *Chryseobacterium indologenes*, strain SE64 was 99% likely to belong to the genus *Chryseobacterium*, strain SE112 was 99% likely to belongs to *Bacillus safensis* species and strain number SE114 probably 100% belongs to *Bacillus velezensis* species. Since, L-asparaginase from different bacteria has shown different anti-cancer effects, finding the L-asparaginase producer microorganisms is one of the ways that lead us to have an enzyme with ideal medical aspects. One of the best environments for finding these bacteria is slaughterhouse soil and effluent, which are high in protein and can therefore, be a potential source of bacteria that produce L-asparaginase.

1. Introduction

L-asparaginase has different applications. L-asparaginase type II (*AnsB*) with a high affinity produced by *E. coli* and *Erwinia chrysanthemi* is used in the treatment of acute lymphoblastic leukemia by hydrolyzing L-asparagine to aspartate and ammonia. It was used as biosensors to label compounds and the food industry (Ebrahimipour and Ghorban Movahed, 1398). L-asparaginase is effective on other cancer such as lymphoma, gastric cancer, lewis lung carcinoma, breast cancer, ovarian cancer, liver cancer, colon cancer, osteosarcoma and acute myeloid leukemia (Darvishi et al., 2022).

L-asparaginase is found in many microorganisms, plants, animal tissues, and in the serum of some rodents and vertebrates, but it

*Corresponding author: Mozhgan Ghiasian
E-mail address: ghiasian@iaufala.ac.ir
Tel: + 98-31-37420140

is not found in humans. However, isolated enzymes from microorganisms, especially bacteria, are more effective than other L-asparaginases. Recently, *Bacillus australimaris* and *Penicillium citrina* were introduced as new L-asparaginase producer microorganisms (Chakravarty et al., 2021) (Andrade et al., 2021). L-asparaginase has immunological side effects like hypersensitivity and non-immunological side effects such as pancreatitis, liver toxicity, coagulopathy and neurotoxicity (Van et al., 2022). The reports suggest that the kinetic and biochemical properties of the enzyme depend on the genetic nature of the microbial strain. There are several methods to reduce the immunization of therapeutic enzymes, but these methods have disadvantages and reduce the activity of the enzyme. In addition, these methods require a lot of manipulations that are time-consuming and costly. Some of them have side effects and are not completely safe and require further bioequivalence studies to ensure the correct action and low side effects. Therefore, it is better to find a new microorganism that produces the L-asparaginase, which naturally has low immunogenicity and allergenicity. So, it is necessary to identify new sources of L-asparaginase with similar therapeutic effects and more desirable serological properties. The aim of this study was isolation and molecularly identification for L-asparaginase producer bacteria from the soil and effluent of a slaughterhouse in Isfahan.

2. Materials and Methods

2.1. Sampling

Since the existence probability of enzyme producer bacteria is higher in the environments where the substrate (protein) is present, soil and effluent samples were taken from a slaughterhouse in Isfahan province (Figure 1) in this study. Soil samples were taken with a sterile spoon from 10 cm of the soil surface and effluent samples were collected from activated sludge that was entered to aeration pond and sedimentation pond (Mohammadi et al., 2009). Samples were transferred to Falavarjan research laboratory in sterile glass containers and were stored in a refrigerator at 4°C.

2.2. Isolation and purification of bacteria

1 g of 4 soil samples and 1 mL of two effluent samples were added separately to 9 mL of sterile sodium chloride peptone buffer. It was mixed for several hours to be homogenized. Then, 1 mL of the solution was dissolved in 9 mL of sterile sodium chloride peptone buffer. Dilutions (10^{-1} to 10^{-4}) were prepared and 0.1 mL of the dilutions were transferred to the surface of 6 nutrient agar media. After a few minutes, when the water was completely absorbed by the culture medium, the plates were incubated for 24 to 48 hours. Colonies with different appearances were purified on nutrient agar medium and then stored by cryobank (Ramesh et al., 2009).

Figure 1. View of soil sampling sites (right) and effluent from sedimentation pond (center) and aeration pond (left) of slaughterhouse

Table 1. M9 specific culture medium

(g/l)	NaNO3	L-Asparagine	KH_2PO_4	$MgSO_4$	Phenol red	NaCl	$CaCl_2$	Glucose	Agar
M9	-	10	1	0.5	0.009%	0.5	0.013	2	20
Negative control	10	-	1	0.5	0.009%	0.5	0.013	2	20
Negative control	-	10	1	0.5	-	0.5	0.013	2	20

2.4. Qualitative measurement of L-asparaginase

Purified colonies were poured into TS saline to prepare the inoculum. Turbidity was adjusted on 0.5 absorption at 625 nm. Then, a well with a diameter of 7 mm was made with sterile cylinders, and the bottom was closed with sterile agar. In the next step, 100 microliters of inoculum were poured into the well and incubated for 48 hours. After incubation, the color change of the media was examined. Isolates that changed media color to pink were considered as enzyme-producing isolates (Tatari et al., 2012).

2.5. Macroscopic and microscopic identification

Macroscopic characteristics of bacteria were investigated including appearance, color, surface and margin. Microscopic characteristics were evaluated by shape (rod, spherical, spiral), arrangement and the presence of spores.

2.6. Identification of isolates according on biochemical properties

Gram-negative and gram-positive isolates were detected using specific biochemical test includes motility, citrate, H_2S, indole, MR-VP, oxidase, catalase, NaCl resistance, glucose fermentation, lactose fermentation, xylose fermentation and mannitol fermentation (Baldiris et al., 2018; Wang et al., 2020; Tamai et al., 2020; Singh et al., 2019).

2.7. DNA extraction

To molecular identification of bacteria, genomic DNA was first extracted using the Marmur manual method (Ghiasian et al., 2017). Some colonies of bacteria were dissolved in 200 µL of TE buffer. 20 µL of lysozyme (10 mg/mL) was added and incubated at 37°C for 30 minutes. At this stage, 25 µL of sodium dodecyl sulfate (SDS 10%) was added and was incubated for 60 minutes at 37°C. 90 µL sodium chloride solution (5 M) was added to the above mixture and the vial was gently tossed several times and heated in a hot water bath at 65°C for 20 minutes. Then 335 µL of chloroform was added and the vial was shaken gently and centrifuged at 11500 rpm for 15 minutes. The aqueous blue phase was transferred to a new sterile vial. This step was repeated twice. Then, cold isopropanol (0.6 volumes of the previous solution) was added to it and kept overnight at -20°C. The solution was centrifuged at 11500 rpm for 15 minutes and the supernatant was discarded. The precipitate was washed with 70% ethanol. The final precipitate was dried at room temperature. In the last step, 50 µL of TE buffer was added and was kept at -20°C for 5 minutes.

The extracted DNA was electrophoresed on 1% agarose gel for qualitative analysis and ensure the presence of the DNA. The voltage and time were set at 75 volts and 45 minutes.

2.8. 16SrRNA gene analysis by PCR

Phylogenetic analysis of the 16SrRNA gene was performed for the strains, in confirmation of macroscopic and microscopic observations. 530 bp fragment of 16SrRNA gene was amplified using PCR technique and R518 and F8 universal primers (Sinaclon company, Iran). PCR reaction was performed with a final volume of 30 µL including 21.8 µL of deionized water, 3 µL of PCR buffer, 0.9 µL of MgCl2, 0.6 µL of dNTP, 1.2 µL of primer, and 1.2 µL of Taq polymerase (Master mix were prepared from Sinaclon company in Iran).

The PCR program was set to 94°C for 5 min, continued by 35 cycles of 94°C for 30 seconds,

53°C for 35 seconds, 72°C for 40 seconds, and finally 72°C for 5 min.

Amplified DNA was electrophoresed on 1% agarose gel by PCR reaction to qualitative analysis and to ensure the presence of PCR product in the expected range.

2.9. Molecular identification

Sequence analysis was conducted at Macrogen (Seoul, South Korea). Isolated 16S rRNA gene bands were sequenced directly. The sequencing was examined in terms of homology and their similarity with other genes using Chromas 2.1 software and BLAST program in the National Center for Biotechnology Information Database (NCBI). The closest strain was determined based on 16SrRNA sequence. Phylogenetic analysis of these isolates and similarity was carried out using Mega 7 (Kumar et al., 2016). The phylogenetic tree was plotted by applying Maximum likelihood methods.

3. Results

3.1. Isolation, purification and screening of isolates

Among the 114 purified isolates, 14 isolates changed the M9 specific medium to pink. These colonies were cultured in negative control medium without L-asparagine and phenol red. Finally, 5 isolates were selected as L-asparaginase producer bacteria. Pink zone diameter was measured for 5 isolates as shown in Figure 2. Bacteria SS18, SS19, SE64, SE112 and SE114 had 21, 22, 30, 33 and 55 mm red halo, respectively.

Figure 2. Red halo of L-asparagine decomposition

SS18 was isolated from soil. It was gram negative bacilli and it had creamy, round and shiny colonies. SS19 and SE64 were isolated from soil and effluent respectively. Both were gram negative bacilli and yellow similar colonies with a light center, round and mucoid. SE112 and SE114 were gram positive bacilli with spore. They were isolated from effluent. SE112 had creamy-white, round and rough colonies. While, SE114 had creamy-white, lobate and rough colonies.

3.2. Identification of isolates according on biochemical properties

Three isolates were gram negative and 2 were gram positive. The results of biochemical tests of isolates are shown in tables 2 and 3.

Table 2. Biochemical characteristics of gram-negative bacteria

	SS18	SS19	SE64
Motility	+	-	-
Citrate	-	-	-
H_2S	-	-	-
Indole	-	+	+
MR	-	-	-
VP	-	-	-
Oxidase	-	+	+
Catalase	+	+	+
NaCl Resistance 7.5%	-	-	-
Glucose fermentation	-	-	-
Lactose fermentation	-	-	-
Xylose fermentation	-	-	-
Mannitol fermentation	-	-	-

Table 3. Biochemical characteristics of gram-positive bacteria

	SE112	SE114
Motility	+	+
Citrate	-	-
H_2S	-	-
Indole	-	-
MR	+	+
VP	+	+
Lipase	-	+
Lecithinase	-	-
Starch hydrolase	-	+
Gelatin hydrolase	-	-
Oxidase	+	+
Catalase	+	+
NaCl Resistance 7.5%	+	+
Glucose fermentation	+	+
Lactose fermentation	-	+
Xylose fermentation	-	-
Mannitol fermentation	+	+

3.2. 16SrRNA gene analysis and evaluation of DNA qualification

As shown in Figure 3, electrophoresis of PCR products confirms the presence of bands with 530 bp length with the help of a marker (Sinaclon company, Iran).

3.3. Phylogenetic analysis

Sequences and similarity percentage were analyzed with other sequences recorded in NCBI databases using phylogenetic analysis of the 16SrRNA gene. The similarity of the strains with the closest known microorganisms is shown in Table 4.

The phylogenetic tree of 16S rRNA gene sequence for studied strains was plotted by the Neighbor-joining method and bootstrap coefficient of 100 using MEGA7 software. The position of the studied strains on the tree is shown for gram-negative and gram-positive strains in Figures 4 and 5, respectively.

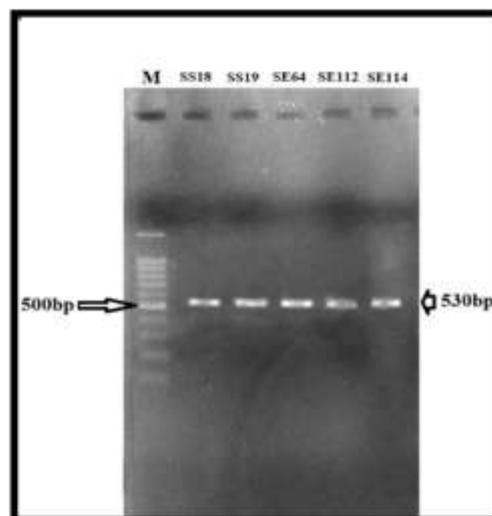

Figure 3. DNA gel electrophoresis for markers and bacteria No SS18, SS19, SE64, SE112 and SE114 (left to right), respectively.

Table 4. Molecular identification of isolates

No.	Strains identification Accession number	Similarity %
SS18	*Stenotrophomonas maltophilia* MG557805.1	99
SS19	*Chryseobacterium indologenes* CP050961.1	99
SE64	*Chryseobacterium* sp. MT516454.1	99
SE112	*Bacillus safensis* MN704543.1	99
SE114	*Bacillus velezensis* MG008635.1	100

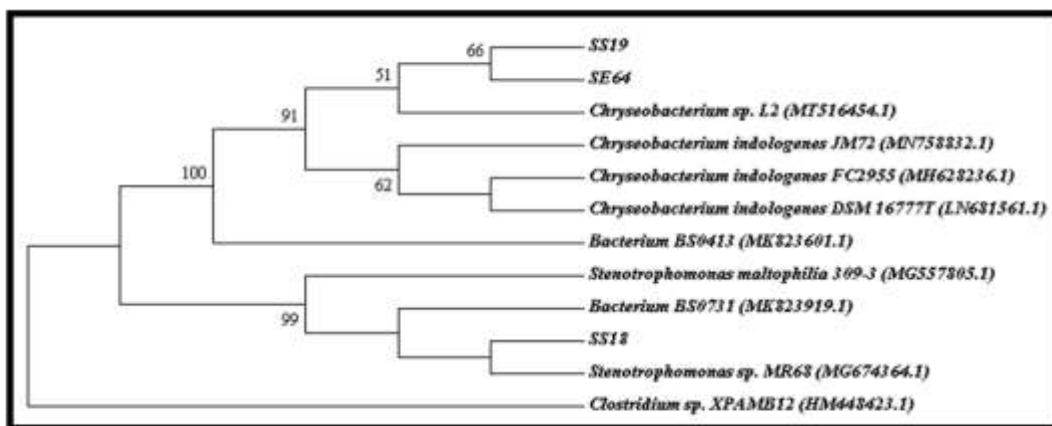

Figure 4. Maximum likelihood phylogenetic tree of 16S rRNA sequences related to gram-negative bacteria in this study using culture method and bootstrap coefficient of one hundred. Bootstraps below 50 have been removed. The accession numbers in the NCBI gene bank are in parentheses. The sequence of *Clostridium* sp. XPAMB12 is listed as an out group.

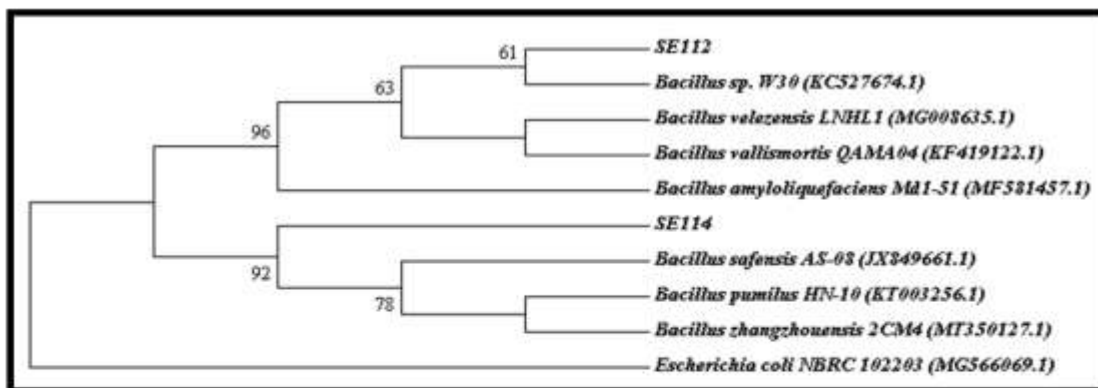

Figure 5. Maximum likelihood phylogenetic tree of 16S rRNA sequences related to gram-positive bacteria in this study using culture method and bootstrap coefficient of one hundred. Bootstraps below 50 have been removed. The accession numbers in the NCBI gene bank are in parentheses. The sequence of *E. coli* NBRC 102203 is listed as an out group.

4. Discussion

Bacteria are an accessible and economical source for the production of various enzymes. Previous research has reported the extraction of the L-asparaginase from various bacteria, such as *Aerobacter*, *Pseudomonas*, *Vibrio*, *Serratia*, *Photobacterium*, *Xanthomonas*, *Streptomyces*,

Proteus, *Bacillus* and *Aspergillus* (Tahira et al., 2016). *E. carotovora* and *E. coli* are used commercially to treat acute lymphoblastic leukemia and other malignant neoplasms in humans. They have side effects like most medicine. Sometimes, these side effects may endanger the patient's life. Therefore, it is necessary to find different microorganisms that produce L-asparaginase with lower side effects.

Badavi Delfard et al. collected water and soil samples from citrus orchards and milk factory sewage of Jiroft in Kerman province. After screening and molecular identification, strains of DJK3 and DJK8 belonging to *Bacillus subtilis*, DJK23 belonging to *Bacillus circulans* and DJK73 belonging to *Bacillus badius* were found (Badoei dalfard et al., 2016). In 1397, Noor Mohammadi et al. isolated six strains belonging to the thermophilic bacterium *Bacillus licheniformis* from hot springs Larijan. These six strains could produce both L-asparaginase and glutaminase (Noormohamadi et al., 2018). Hatamzadeh et al. isolated L-asparaginase producer endophytic fungi from medicinal plants of seven families Asteraceae. L-asparaginase producer endophytes were identified as species of *Plectosphaerella*, *Fusarium*, *Stemphylium*, *Septoria*, *Alternaria*, *Didymella*, *Phoma*, *Chaetosphaeronema*, *Sarocladium*, *Nemania*, *Epicoccum*, *Ulocladium* and *Cladosporium*. This study showed that endophytic fungi from Asteraceae members have a high L-asparaginase producer potential (Hatamzadeh, et al., 2020).

In the present study, samples from a slaughterhouse in Isfahan province were used. Five L-asparaginase producer bacteria were isolated in the soil and effluent of the slaughterhouse (two isolates from the soil and three isolates from the effluent).

SS18 was isolated from the slaughterhouse soil. It is an aerobic, non-fermentative and gram-negative bacterium with 99% similarity percentage to *S. maltophilia* (Brooke, 2012). In 2014, Ashish and Manish isolated *Stenotrophomonas koreensi* from the Lonar Lake in India as a L-asparaginase producer bacteria. In this study, biochemical tests were performed to detect bacteria. Biochemical tests in these two studies illustrated lactose fermentation, citrate, hydrogen sulfide, motility, indole, MR and VP for both *S. maltophilia* and *Stenotrophomonas koreensi* are negative (Ashish et al., 2014). Also, *S. maltophilia* was isolated from soil in 2020 by Abdelrazek et al. as a L-asparaginase producer bacteria. Probably, L-asparagine was abundant in soil in this two studies and *S. maltophilia* was present as a L-asparagine degrading microorganism (Abdelrazek et al., 2020).

SS19 and SE64 are gram-negative bacillus, immobilized with 99% similarity percentage to *Chryseobacterium endogenous* and *Chryseobacterium* sp., respectively. It is found naturally in soil, water, plants and food products (Izaguirre-Anariba et al., 2020). There were no reports of this bacterium as a producer of the L-asparaginase. Probably, because the slaughterhouse soil and effluent are high in protein and a good environment for L-asparaginase producer bacteria, we were able to isolate these bacteria.

SE112, with 99% similarity percentage to *Bacillus safensis*, is a gram-positive bacillus, aerobic, chemotrophs, rod shape that forms spores. It is resistant to salt and UV rays. In 2017, Kanti et al. isolated *B. safensis* from the sediments of the Ganges River in India as a L-asparaginase producer bacteria (Mihooliya et al., 2017). In the present study, *B. safensis* was isolated from the effluent of a slaughterhouse. Since the water of the Ganges River is partially contaminated with industrial and human wastewater, it is an environment similar to slaughterhouse effluent. So, the amount of protein is abundant in both habitats. Therefore, the presence of L-asparaginase producer bacteria in both environments is high.

SE114, with 100% similarity percentage to *Bacillus velezensis*, is an aerobic, gram-positive bacterium that forms an endospore (Rabbee et al., 2019). In 2021, Parmar et al. isolated *B. velezensis* from spoiled pomegranate as a L-asparaginase producer. They observed this bacterium has L-asparaginase, L-arginase and L-glutaminase activities (Parmar et al., 2021). Mostafa et al. isolated *B. velezensis* from sediment samples of the Red sea, Saudi Arabia using the Millipore membrane filter method and asparagine agar medium. They found *B. velezensis* is a producer of glutaminase free asparaginase (Mostafa et al., 2019). *B. velezensis* is a pathogenic bacterium of pomegranate. Most of the constituents of pomegranate are starch and protein. Since, the

L-asparagine is found in proteins, the presence of this bacterium in spoiled pomegranate is quite possible. Large amounts of protein and various bacteria are found in slaughterhouse effluents. Therefore, L-asparagine degrading bacteria are found in abundance.

According to studies, L-asparaginase is produced in a wide range of bacterial, fungi, endophytic fungi and other microorganisms. Therefore, there is a potential source of microorganisms to produce and investigate L-asparaginase for further research.

Conclusion

Slaughterhouse soil and effluent have a high amount of protein. So, they are potential sources of L-asparaginase producer bacteria.

References

Abdelrazek, N. A., Elkhatib, W. F., Raafat, M. M., & Aboulwafa, M. M. (2020). Production, characterization and bioinformatics analysis of L-asparaginase from a new Stenotrophomonas maltophilia EMCC2297 soil isolate. AMB Express. 10(1): 1-16.

Andrade, K. C., Fernandes, R. A., Pinho, D. B., de Freitas, M. M., Pessoa, A., Silva, J. I., & Magalhães, P. O. (2021). Sequencing and characterization of an L-asparaginase gene from a new species of Penicillium section Citrina isolated from Cerrado. Scientific reports. 11(1): 1-13.

Ashish, C., & Manish, B. (2014). Isolation and Characterization of L-asparginase producing isolate from Lonar Lake, Buldhana District, MS, India. Research Journal of Recent Sciences. 3 (ISC-2013): 38-41.

Badoei dalfard, a., & Karami, z., & Ramezani pour, n. (2016). Isolation, molecular identification and production investigation of bacillus-producing l-asparaginase from jiroft microflora. Journal of microbial world. 9(3 (28)), 247-256.

Baldiris, R., Acosta-Tapia, N., Montes, A., Hernández, J., & Vivas-Reyes, R. (2018). Reduction of hexavalent chromium and detection of chromate reductase (ChrR) in Stenotrophomonas maltophilia. Molecules. 23(2), 406.

Brooke, J. S. (2012). Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clinical microbiology reviews. 25(1), 2-41.

Chakravarty, N., Singh, J., & Singh, R. P. (2021). A potential type-II L-asparaginase from marine isolate Bacillus australimaris NJB19: statistical optimization, in silico analysis and structural modeling. International Journal of Biological Macromolecules. 174, 527-539.

Darvishi, F., Jahanafrooz, Z., & Mokhtarzadeh, A. (2022). Microbial L-asparaginase as a promising enzyme for treatment of various cancers. Applied Microbiology and Biotechnology. 1-13.

Ebrahimipour G H, Ghorban Movahed M. (2020). An Overview of Asparaginase Enzyme Features: An Anticancer Enzyme with High Potency. JSSU, 27 (12): 2132-2148.

Ghaderi, f., & Ghezelbash, g. (2017). Isolation and optimization of glutaminase-free l-asparaginase enzyme producing serratia marcescens isolated from natural sources. Journal of microbial world. 10(1 (30)): 49-58.

Ghiasian, M., AKHAVAN, S. A., Amoozegar, M. A., Saadatmand, S., & Shavandi, M. (2017). Bacterial diversity determination using culture-dependent and culture-independent methods., Global J. Environ. Sci. Manage. 3(2): 153-164.

Hatamzadeh, S., Rahnama, K., Nasrollahnejad, S., Fotouhifar, K. B., Hemmati, K., White, J. F., & Taliei, F. (2020). Isolation and identification of L-asparaginase-producing endophytic fungi from the Asteraceae family plant species of Iran. PeerJ, 8, e8309.

Izaguirre-Anariba, D. E., & Sivapalan, V. (2020). Chryseobacterium indologenes, an emerging bacteria: a case report and review of literature. Cureus. 12(1).

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution. 33(7): 1870-1874.

Mihooliya, K. N., Nandal, J., Swami, L., Verma, H., Chopra, L., & Sahoo, D. K. (2017). A new pH indicator dye-based method for rapid and efficient screening of l-asparaginase producing micro organisms. Enzyme and Microbial Technology. 107, 72-81.

Mohammadi-Sichani, M. E., Mazaheri Assadi, M., Farazmand, A., Kianirad, M., Ahadi, A. M., & Hadian-Ghahderijani, H. (2019). Ability of Agaricus bisporus, Pleurotus ostreatus and Ganoderma lucidum compost in biodegradation of petroleum hydrocarbon-contaminated soil. International Journal of Environmental Science and Technology. 16(5): 2313-2320.

Mostafa, Y., Alrumman, S., Alamri, S., Hashem, M., Al-izran, K., Alfaifi, M., ... & Taha, T. (2019). Enhanced production of glutaminase-free L-asparaginase by marine Bacillus velezensis and cytotoxic activity against breast cancer cell lines. Electronic Journal of Biotechnology. 42, 6-15.

Noormohamadi E, Farahmand M, Esmaeili Rastaghi A R. (2018). Identification of extermophilies producing l- glutaminase and l-asparaginase from Larijanhotspring. NCMBJ. 8(31): 29-38.

Parmar, F. A., Patel, J. N., & Upasani, V. N. (2021). Screening of microorganisms for production of therapeutic enzymes. Screening. 3(6): 39.

Rabbee, M. F., Ali, M. D., Choi, J., Hwang, B. S., Jeong, S. C., & Baek, K. H. (2019). Bacillus velezensis: a valuable member of bioactive molecules within plant microbiomes. Molecules, 24(6), 1046.

Ramesh, S., & Mathivanan, N. (2009). Screening of marine actinomycetes isolated from the Bay of Bengal, India for antimicrobial activity and industrial enzymes. World Journal of Microbiology and Biotechnology. 25(12): 2103-2111.

Singh, A., Mukhopadhyay, K., & Ghosh Sachan, S. (2019). Biotransformation of eugenol to vanillin by a novel strain Bacillus safensis SMS1003. Biocatalysis and Biotransformation. 37(4): 291-303.

Tahira B, Essam A. M, Muna J, Mashitah, MY. (2016). A Comprehensive Review on L-Asparaginase and Its Applications. Appl Biochem Biotechnol. 178:900–923.

Tamai, I. A., Pakbin, B., Kafi, Z. Z., & Brück, W. M. (2021). Oral abscess caused by Chryseobacterium indologenes in ball python (Python regius); A case report. Antibiotics, 10(6), 686.

Tatari, M., Ghazvini, R. F., Etemadi, N., Ahadi, A. M., & Mousavi, A. (2012). Analysis of antioxidant enzymes activity, lipid peroxidation and proline content of Agropyron desertorum under drought stress. Biology and Environment, 3(1), 9-24.

Van Trimpont, M., Peeters, E., De Visser, Y., Schalk, A. M., Mondelaers, V., De Moerloose, B., ... & Van Vlierberghe, P. (2022). Novel Insights on the Use of L-Asparaginase as an Efficient and Safe Anti-Cancer Therapy. Cancers. 14(4): 902.

Wang, S., Sun, L., Zhang, W., Chi, F., Hao, X., Bian, J., & Li, Y. (2020). Bacillus velezensis BM21, a potential and efficient biocontrol agent in control of corn stalk rot caused by Fusarium graminearum. Egyptian Journal of Biological Pest Control. 30(1): 1-10.