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REGULAR ARTICLE

CYTOTOXIC POTENTIAL OF L-ASPARAGINASE FROM *BACILLUS* SP. *IN VITRO*

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ABSTRACT

This study reports the cytotoxic potential of L-Asparaginase isolated from *Bacillus* sp. *Bacillus* sp. was isolated from local soil/water samples and identified by rapid plate assay and further confirmed by phenotypic characterization. Extracellular L- Asparaginase was isolated from broth culture of *Bacillus* sp. and purified by ammonium sulfate precipitation, followed by dialysis and ion exchange and gel filtration chromatography techniques. The purified enzyme was used to study the *in vitro* cytotoxic potential. Varying concentrations (31.25, 62.5, 125, 250, 500 µg/mL) of purified L-Asparaginase was tested on MCF7, HeLa, HepG2 and 3T3L1cell lines by MTT assay. Curcumin was maintained as a positive control. The results revealed that the enzyme showed a significant cytotoxic activity and a dose dependent effect. The minimum inhibitions exhibited at a concentration of 32.25 µg/mL was 19.44% (MCF-7), 10.04% (HeLa), 7.45% (HepG2) and 4.4% (3T3L1) , while the maximum inhibition at a concentration of 500 µg/mL was 71.14% (MCF-7), 68.92% (HeLa), 68.28% (HepG2) and 47.4% (3T3L1). The positive control, curcumin (5 µM) showed an inhibition of 48.13% (MCF-7), 54.42 (HeLa), 64.94% (HepG2) and 44.5% (3T3L1).

Keywords: L-Asparaginase, MCF-7, HeLa, HepG2, 3T3L1, ion exchange, gel filtration

INTRODUCTION

L-asparaginase (L-asparagine amidohydrolase EC 3.5.1.1) is a hydrolase that plays a major role in the metabolism of all living organisms specifically catalyzing the hydrolysis of L-asparagine to L-aspartic acid and ammonia **(Lincoln and More, 2014**). The reaction is irreversible when maintained at physiological conditions. The enzyme is found widespread in nature and has been isolated from various sources such as plant tissues, bacteria, fungi and the serum of rodents (**Batool *et al.*, 2016**). L-asparaginases havebeen isolated and purified from several different bacteria such as *E. coli* **(Roberts *et al.*, 1966**), *Serratia marcescens* **(Whelan and Wriston, 1974)**, *Vibrio succinogens* **(Saxena and Sinha, 1981)**, *Pseudomonas acidovorans* **(Davidson *et al.*, 1977)** *Pseudomonas geniculata* **(Kitto *et al.*, 1979)**, *Corynebacterium glutamicum* **(Mesas *et al.*, 1990)**, *Pseudomonas stutzeri* MB-405 **(Manna *et al.*, 1995**), and *Pseudomonas aeruginosa* **(El-bessoumy *et al.*, 2004).**
The enzyme is proven to have cytotoxic activity and is being used in the treatment of acute lymphoblastic leukemia. This is due to the fact that the leukemic cells do not have the property to produce L-asparagine, which is a non-essential amino acid, whereas the normal cells can produce their own. Therefore, the leukemic cells are deprived of L-asparagine which is their source of nutrition and they are prevented from malignant growth **(El-Naggar *et al.*, 2018).** ELSPAR, ONCASPAR, ERWINASE and KIDROLASE are some of the brands of L-Asparaginase approved by FDA for the treatment of acute lymphoblastic leukemia and lymphosarcoma **(Noura El-Ahmady *et al.*, 2014)**
With reports suggesting difficulties in the bulk preparation of L-asparaginase from pig serum, focus has been shifted to alternate sources where sufficient amount of enzyme can be extracted **(Verma *et al*., 2007**). The objective of our research was to screen local soil and water isolates for L Asparaginase producers, purify and study the cytotoxic potential of the L-Asparaginase isolated.

MATERIAL AND METHODS

Screening and isolation of L-Asparaginase producing organisms

The screening and isolation of L-Asparaginase producing organisms was carried out as described earlier **(Gulati *et al.*, 1997**); (**D'Souza *et al*., 2018**) with some modifications. The media employed was modified M-9 media. The organism giving maximum yield of L-Asparaginase was identified by enzyme assay **(Imada *et al.*, 1973**).

Identification of the L-Asparaginase producing bacterial isolate

All the isolates positive for L-Asparaginase in the screening were subjected to primary identification according to Bergey's Manual of Systematic Bacteriology **(Sneath *et al.*, 1986)**
The isolate giving maximum enzyme activity $B10^{-1}C_2$ (B represents Bandhar sample, 10^{-1}– represents the dilution and C_2- represents the colony number was sent for phenotypic characterization to Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Scale up of the *Bacillus* sp. by suspension culture

***Bacillus* sp.** positive for L-asparaginase by plate assay technique **(Gulati *et al.*, 1997)** and maintained on M-9 agar slant pH 7 at 4ºC, revived at regular intervals, was used for L-asparaginase production from bacteria.The bacterial isolate was transferred to a freshly prepared M-9 agar plate with a sterile loop and incubated at 37ºC for 24 hours. A loop full of culture was aseptically inoculated to 50 mL of M-9 broth in a 100 mL Erlenmeyer flask and incubated at 37ºC in an incubator for 24 hours. This was used as a starter culture for large scale production of the enzyme. M-9 media (1 litre) was taken in a 2000 mL conical flask and 100 mL of 24 hours old inoculum was inoculated into the media and left in static conditions in the incubator at 37ºC for 3 days.

Purification of L-Asparaginase

The purification of the crude extract was carried out at 4°C according to the method of **(Distasio *et al*., 1976).** Finely powdered ammonium sulfate was added to 70% saturation. The mixture was left overnight for 12 hours at 4°C, followed by centrifugation at 8000 rpm for 20 min at 4°C. The precipitate was dissolved in 0.05M Tris HCl buffer pH 8.5, and dialyzed overnight against the same buffer at 4°C. The dialyzed sample was further purified by passing through a column of DEAE cellulose previously equilibrated with 0.05M Tris HCl buffer, pH 8.5. A total of 55 fractions were collected at the flow rate of 3 mL/15min. Fractions showing high activity were pooled together and dialysed against the same buffer. The dialysed sample was lyophilized and concentrated. Lyophilized sample (1 mL)was loaded on to the Sephacryl S-200 gel filtration column and was eluted by using the Tris HCl buffer pH 8.5. A total of 45 fractions were collected at the rate of 3mL/15min.Fractions showing high activity were pooled together dialyzed, lyophilized and used for further studies.

Evaluation of Cytotoxic potential of L-Asparaginase

The cytotoxic potential of L-Asparaginase was carried out according to the procedure described by **(Mosmann, 1983**) . Human Cervical Cancer cell line,

Human Breast Cancer Cell line, Human Liver Cancer Cell line and Mouse Embryo Fibroblast Cell lines were obtained from NCCS, Pune. The cells were maintained in DMEM medium supplemented with 10% FBS and Penicillin (100U/ mL) in a humidified atmosphere of 50µg/ mL CO_2 at 37°C. The cytotoxicity of the sample on the cell lines was determined by MTT assay. Cell suspension (200µL) was seeded in a 96 well plates at required cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 24 hours. The various concentrations of the sample (31.25, 62.5, 125, 250, 500 µg/mL) were added. The plates were incubated at 37°C for 48 hours in a 5% CO_2 atmosphere.

After the incubation period, the spent media was removed and MTT reagent was added to a final concentration of 0.5 mg/mL of the total volume. The plates were returned to the incubator and incubated for 3 hours. The MTT was removed and 100 µl of solubilisation solution (DMSO) was added. The absorbance was read on an ELISA reader (ELX800, bioteck) at 570 nm and 630 nm was used as reference wavelength. The IC_{50} value was determined graphically. All experiments were performed in triplicates.

RESULTS AND DISCUSSION

Screening and isolation of L-Asparaginase producing organisms

Strains with pink colour zones around the colonies were considered as positive result for L-asparaginase producing strains

Figure 1 *Bacillus* sp. Colonies showing pink zone on M-9 media supplemented with 0.09% phenol red.

The morphological and cultural characteristics of the isolate $B10^{-1}C_2$ (B represents Bandhar sample , 10^{-1}– represents the dilution and C_2- represents the colony number) are appended herewith. The colonies of isolate were circular, with entire margin, flat elevation and smooth surface. The isolate was found to be gram positive bacilli. It was motile, sporulating bacteria (Table 1). It was exposed to different temperatures 10-55ºC (Table 2), pH range 4-9 (Table 3) and varying concentrations of sodium chloride 2-9% (Table 4), biochemical tests (Table 5) and acid production from various carbohydrates (Table 6). It exhibited growth at pH range of 5-9 and tolerated sodium chloride concentrations of 2-9%, respectively. Phenotypic characterization of the isolate $B10^{-1}C_2$ identified it as *Bacillus* sp.

Table 1 Colony Morphology of the isolate

Colony Morphology	
Character	Observed feature
Configuration	Circular
Margin	Entire
Elevation	Flat
Surface	Smooth
Density	opaque
Gram reaction	+ ve Rods
Spore	+
position	Central
Shape	Oval
Motility	positive

Table 2 Effect of temperature on the growth of the isolate

Temperature	Growth
10ºC	-
15 ºC	-
25 ºC	+
30 ºC	+
37 ºC	+
42 ºC	+
50 ºC	+
55 ºC	-

'+' indicates presence of growth;'-' indicates absence of growth

Table 3 Effect of pH on the growth of the isolate

pH	Growth of the isolate
pH 4	-
pH 5	+
pH 6	+
pH 7	+
pH 8	+
pH 9	+

'+' indicates presence of growth;'-' indicates absence of growth

Table 4 Growth of the isolate at different NaCl (%) concentration

Concentration of NaCl (%)	Growth of the isolate
2.0	+
3.0	+
4.0	+
5.0	+
6.0	+
7.0	+
8.0	+
9.0	+

'+' indicates presence of growth;'-' indicates absence of growth

Table 5 Biochemical tests of the isolate

Biochemical tests	
Growth on Mac Conkey agar medium	-
Indole test	-
Methyl red test	+
Voges Proskauer test	-
Citrate test	+
Casein test	+
Starch hydrolysis	-
Gelatin hydrolysis	-
Nitrate reduction	-
catalase	+
oxidase	+
Esculine hydrolysis	+
H2S gas production	-
Urease test	-

'+' indicates positive result for the test;'-' indicates negative result for the test

Table 6 Acid production from various carbohydrates by the isolate

Acid production from carbohydrates	
Glucose	+
Fructose	+
Salicin	+
Mannitol	+
Raffinose	-
Sucrose	+
Rhamnose	-
Galactose	-
Inositol	-
Xylose	-

'+' indicates production of acid;'-' indicates no production of acids

Purification of L-Asparaginase

Table 7 represents the purification profile of L-Asparaginase from *Bacillus* sp., which shows that there was 14.82 fold purification of the enzyme. The purified enzyme had a specific activity of 124.52 IU/mL/mg.

Table 7 Purification profile of L-Asparaginase from *Bacillus* sp.

Steps	Total activity (IU/mL)	Total protein Conc. (mg)	Specific activity (IU/mL/mg)	Fold purification	Yield
Crude enzyme	19,219.6	2285.76	8.40	1	100
Ammonium sulphate (80%) Precipitation	10,271.9	729.3	14.08	1.676	53.44
Dialysis	5,291	148.10	35.72	4.252	27.52
DEAE cellulose chromatography	1150	16.12	71.33	8.49	5.98
Sephacryl S-200 gel filtration chromatography	327.5	2.63	124.52	14.82	1.70

Evaluation of Cytotoxic potential of L-Asparaginase

Table 8 represents the cytotoxic potential of L-Asparaginase against various cell lines. The IC_{50} value of L-Asparaginase for HeLa cells was found to be 263.18 µg/mL, MCF-7 it was 277.40 µg/mL, for HepG2 it was found to be 273.16 µg/mL and showed resistance against 3T3L1 normal cell line.

Table 8 Cytotoxic effect of *Bacillus* sp. L-Asparaginase on HeLa, MCF-7, HepG2 and 3T3L1 cell lines by MTT assay

Cell lines	Concentration (µg/mL)	cell death (%)	cell viability (%)	IC_{50} (µg/mL)
HeLa	31.25	10.04 ± 0.02	89.96 ± 0.02	263.18
	62.5	22.95 ± 0.00	77.05 ± 0.00	
	125	31.71 ± 0.01	68.29 ± 0.01	
	250	43.51 ± 0.00	56.49 ± 0.00	
	500	68.92 ± 0.01	31.08 ± 0.01	
MCF-7	31.25	19.44 ± 0.02	80.56 ± 0.02	277.40
	62.5	24.44 ± 0.0	75.56 ± 0.0	
	125	37.80 ± 0.0	62.20 ± 0.0	
	250	52.52 ± 0.0	47.48 ± 0.0	
	500	71.14 ± 0.01	28.86 ± 0.01	
HepG2	31.25	7.45 ± 0.00	92.55 ± 0.00	273.16
	62.5	15.86 ± 0.00	84.14 ± 0.00	
	125	28.23 ± 0.00	71.77 ± 0.00	
	250	45.52 ± 0.01	54.48 ± 0.01	
	500	68.28 ± 0.01	31.72 ± 0.01	
3T3L1	31.25	4.40 ± 0.02	95.60 ± 0.02	> 500
	62.5	11.2 ± 0.01	88.80 ± 0.01	
	125	14.3 ± 0.02	85.70 ± 0.02	
	250	30.2 ± 0.00	69.80 ± 0.00	
	500	47.4 ± 0.01	52.60 ± 0.01	

Table 9 Cytotoxic effect of Curcumin (positive control) on HeLa, MCF-7, HepG2 and 3T3L1 cell lines by MTT assay

cell lines	Curcumin concentration (µM)	cell death (%)	cell viability (%)
HeLa	5	54.42	45.58
MCF-7	5	48.13	51.87
HepG2	5	64.94	35.06
3T3L1	5	44.5	55.5

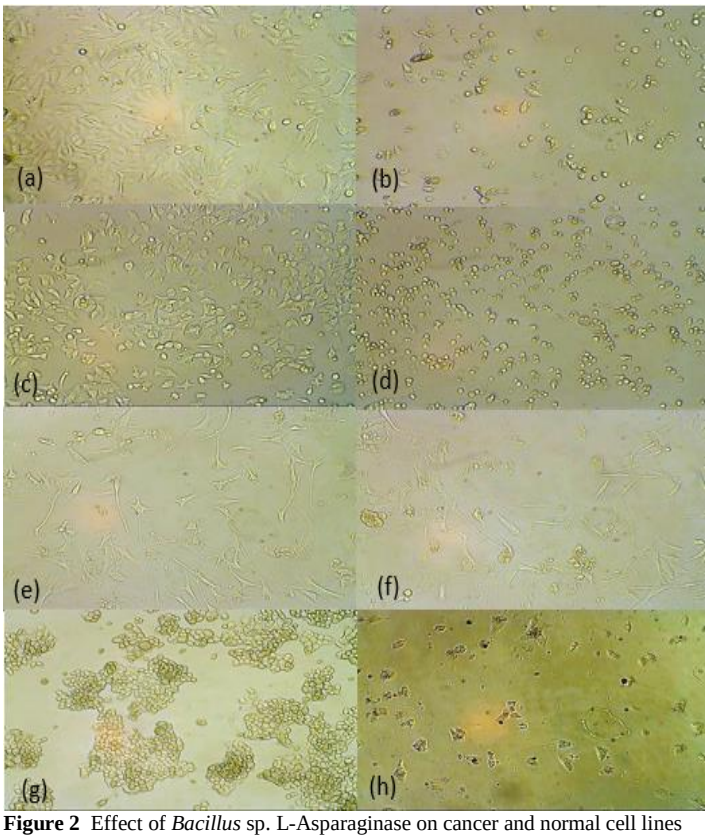

Figure 2 Effect of *Bacillus* sp. L-Asparaginase on cancer and normal cell lines HeLa cell lines (a) untreated (b) treated ; MCF cell lines (c) untreated (d) treated; 3T3L1 cell lines (e) untreated (f) treated ; HepG2 cell lines (g) untreated (h) treated .

Morphological analysis revealed that the number of cells were reduced significantly upon treatment and the treated cells undergoing apoptosis were characterized by cellular rounding up, shrinkage, membrane blebbing and loss of cell adhesion. However 3T3L1 cells showed less resistance to the enzyme treatment.

DISCUSSION

In the present study we observed that the L-Asparaginase from *Bacillus* sp. showed varied levels of inhibition of HeLa, MCF-7, HepG2 cancer cell lines and resistance towards 3T3L1 normal cell lines. The purified L-asparaginase from *Bacillus licheniformis* showed cytotoxic effect against Jurkat clone E6-1, MCF-7 and K-562 **(Mahajan *et al.*, 2014)** with an IC_{50} value of 0.78 IU against MCF-7 cell lines.

Antitumor activity of L-Asparaginase from *Erwinia caratovora* has been studied on Jurkat, Molt 4, human chronic myloid leukemia K562 cells, HL 60 and other human soild tumors. L-Asparaginase significantly increased the number of apoptotic cells to 40% (Jurkat cells) and 99% (HL60 cells) suggesting that the enzyme cytoxicity is associated with only L- asparagine deficiency **(Abakumova *et al.*, 2012)**. *In vitro* cytotoxicity of L-Asparaginase from pathogenic strain *Helicobacter pylori* against different cell lines reported that AGS and MKN 28 gastric epithelial cells being the most affected **(Scotti *et al.*, 2010)**. L-asparaginase is reported to show sensitivity against fibrosarcoma and liposarcoma **(Tardito *et al.*, 2007)**. L-Asparaginase from *Salinicoccus* KJ997975 also exhibited cytotoxicity against Jurkat and HeLa cell lines with an IC_{50} value of 0.171 IU/mL and 0.096 IU/mL respectively **(Bhat and Marar, 2015)**. L-Asparaginase also causes selective death of asparagine dependent tumor cells and induces apoptosis in tumor cells **(Kelo *et al.*, 2009)**. Human Asparaginase enzyme inhibits the growth of Leukemic cells. Lymphoid cells (NALM-6 and MOLT-4) are more sensitive than myeloid cells (K562) to L-Asparaginase treatment (**Belviso *et al.*, 2017**). L-Asparaginase showed cytotoxicity and high selectivity to three leukemic cell lines with a IC_{50} value of 1.16 µg/mL against Daudi cell line, 1.38 µg/mL against Jurkat cell line and 1.08 µg/mL against Molt 4 cell line **(Aljewari *et al.*, 2010).**The differences in the IC_{50} values might be due to the differences in the level of purity of the enzymes. We could achieve 14 .84 fold purity in the present study. Further fold purification of L-Asparaginase might prove to be useful.

CONCLUSION

L-Asparaginase producing bacterial isolate was screened and purified. The *in vitro* cytotoxic effect of L-Asparaginase isolated from *Bacillus* sp. was tested on human cancer cell lines, namely, MCF-7, HeLa, HepG2, and normal cell line 3T3L1. In this study it showed cytotoxic effect on MCF-7, HeLa and HepG2 cell lines. Purified enzyme did not show any effect on normal 3T3L1 cell lines indicating that it may be used for chemotheraputic purpose.

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REFERENCES

Abakumova, O.Yu., Podobed, O.V., Karalkin, P.A., Kondakova, L.I., Sokolov, N.N., 2012. Antitumor activity of L-asparaginase from Erwinia carotovora against different human and animal leukemic and solid tumor cell lines. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry 6, 307–316. https://doi.org/10.1134/S1990750812040026

Aljewari, H.S., Nader, M.I., Alfaisal, A.H.M., Natthida, W., Sahapat, 2010. High Efficiency, Selectivity against Cancer Cell Line of Purified L-Asparaginase from Pathogenic Escherichia coli. International Journal of Medical, Health, Biomedical, Bioengineering and Pharmaceutical Engineering 4.

Batool, T., Makky, E.A., Jalal, M., Yusoff, M.M., 2016. A Comprehensive Review on l-Asparaginase and Its Applications. Applied Biochemistry and Biotechnology 178, 900–923. https://doi.org/10.1007/s12010-015-1917-3

Belviso, S., Iuliano, R., Amato, R., Perrotti, N., Menniti, M., 2017. The human asparaginase enzyme (ASPG) inhibits growth in leukemic cells. PLOS ONE 12, e0178174. https://doi.org/10.1371/journal.pone.0178174

Bhat, M., Marar, T., 2015. Cytotoxic Effect of Purified L-asparaginase from Salinicoccus sp. M KJ997975. International Journal of Current Microbiology and Applied Sciences 4, 701–712.

Davidson, L., Brear, D.R., Wingard, P., Hawkins, J., Kitto, G.B., 1977. Purification and Properties of an L-Glutaminase-L- Asparaginase from Pseudomonas acidovorans. J. BACTERIOL. 129, 1379–1386.

Distasio, J.A., Niederman, R.A., Kafkewitz, D., Goodman, D., 1976. Purification and characterization of L-asparaginase with anti-lymphoma activity from Vibrio succinogenes. J. Biol. Chem. 251, 6929–6933.

D'Souza, R.M., Pinto, S.K., Abraham, A., 2018. Identification of a Novel Strain of Extracellular L- Asparaginase Producing Scopulariopsisbrevicaulis (Sacc.) Bainier using Molecular Techniques. International Journal of Scientific Research in Computer Science Applications and Management Studies 7, 1–5.

El-bessoumy, A.A., Sarhan, M., Mansour, J., 2004. Isolaton, and Purification of L-Asparaginase from Pseudomonas Aeruginosa 50071 Using Solid-state Fermentation. Journal of Biochemistry and Molecular Biology 387–393.

El-Naggar, N.E.-A., Deraz, S.F., El-Ewasy, S.M., Suddek, G.M., 2018. Purification, characterization and immunogenicity assessment of glutaminase free L-asparaginase from Streptomyces brollosae NEAE-115. BMC Pharmacol Toxicol 19. https://doi.org/10.1186/s40360-018-0242-1

Gulati, R., Saxena, R.K., Gupta, R., 1997. A rapid plate assay for screening l-asparaginase producing micro-organisms. Letters in Applied Microbiology 24, 23–26. https://doi.org/10.1046/j.1472-765X.1997.00331.x

Imada, A., Igarasi, S., Nakahama, K., Isono, M., 1973. Asparaginase and Glutaminase Activities of Micro-organisms. Journal of General Microbiology 76, 85–99. https://doi.org/10.1099/00221287-76-1-85

Kelo, E., Noronkoski, T., Mononen, I., 2009. Depletion of L-asparagine supply and apoptosis of leukemia cells induced by human glycosylasparaginase. Leukemia 23, 1167–1171. https://doi.org/10.1038/leu.2008.387

Kitto, G.B., Smith, G., Thiet, T.-Q., Mason, M., Davidson, L., 1979. Tumor Inhibitory and Non-Tumor Inhibitory L-Asparaginases from Pseudomonas geniculata. Journal of Bacteriology 137, 204–212.

Lincoln, L., More, S.S., 2014. Isolation and production of clinical and food grade L- Asparaginase enzyme from fungi. Journal of Pharmacognosy and Phytochemistry 3, 177–183.

Mahajan, R.V., Kumar, V., Rajendran, V., Saran, S., Ghosh, P.C., Saxena, R.K., 2014. Purification and Characterization of a Novel and Robust L-Asparaginase Having Low-Glutaminase Activity from Bacillus licheniformis: In Vitro Evaluation of Anti-Cancerous Properties. PLoS ONE 1–8, e99037. https://doi.org/10.1371/journal.pone.0099037

Manna, S., Sinha, A., Sadhukhan, R., Chakrabarty, S.L., 1995. Purification, characterization and antitumor activity of l-asparaginase isolated from Pseudomonas stutzeri MB-405. Current Microbiology 30, 291–298. https://doi.org/10.1007/BF00295504

Mesas, J.M., Gil, J.A., Martin, J.F., 1990. Characterization and Partial Purification of L-Asparaginase from Corynebacterium Glutamicum. Journal of General Microbiology 136, 515–519. https://doi.org/10.1099/00221287-136-3-515

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. Journal of Immunological Methods 65, 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Noura El-Ahmady, E.-N., El-Ewasy, S.M., El-Shweihy, N.M., 2014. Microbial L-asparaginase as a Potential Therapeutic Agent for the Treatment of Acute Lymphoblastic Leukemia: The Pros and Cons. International Journal of Pharmacology 10, 182–199. https://doi.org/10.3923/ijp.2014.182.199

Roberts, J., Prager, M.D., Bachynsky, N., 1966. The Antitumor Activity of Escherichia coli L-Asparaginase. Cancer Research 26, 2213–2217.

Saxena, R.K., Sinha, U., 1981. L-asparaginase and glutaminase activities in the culture filtrates of *Aspergillus nidulans*. Current Science 50, 218–219.

Scotti, C., Sommi, P., Pasquetto, M.V., Cappelletti, D., Stivala, S., Mignosi, P., Savio, M., Chiarelli, L.R., Valentini, G., Bolanos-Garcia, V.M., Merrell, D.S., Franchini, S., Verona, M.L., Bolis, C., Solcia, E., Manca, R., Franciotta, D., Casasco, A., Filipazzi, P., Zardini, E., Vannini, V., 2010. Cell-Cycle Inhibition by Helicobacter pylori L-Asparaginase. PLoS ONE 5, e13892. https://doi.org/10.1371/journal.pone.0013892

Sneath, P.H.A., Mair, N.S., Sharpe, M.E., Holt, J.G., 1986. Bergey's manual of systematic bacteriology. Volume 2. Bergey's manual of systematic bacteriology. Volume 2.

Tardito, S., Uggeri, J., Bozzetti, C., Bianchi, M.G., Rotoli, B.M., Franchi-Gazzola, R., Gazzola, G.C., Gatti, R., Bussolati, O., 2007. The inhibition of glutamine synthetase sensitizes human sarcoma cells to l-asparaginase. Cancer Chemotherapy and Pharmacology 60, 751–758. https://doi.org/10.1007/s00280-007-0421-z

Verma, N., Kumar, K., Kaur, G., Anand, S., 2007. L-Asparaginase: A Promising Chemotherapeutic Agent. Critical Reviews in Biotechnology 27, 45–62. https://doi.org/10.1080/07388550601173926

Whelan, H.A., Wriston, J.C., 1974. Purification and properties of l-asparaginase from Serratia marcescens. Biochimica et Biophysica Acta (BBA) - Protein Structure 365, 212–222. https://doi.org/10.1016/0005-2795(74)90266-9