Saudi Journal of Biological Sciences 26 (2019) 1146–1153

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

L-asparaginase produced from soil isolates of *Pseudomonas aeruginosa* shows potent anti-cancer activity on HeLa cells

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ARTICLE INFO

Article history:
Received 11 February 2019
Revised 28 April 2019
Accepted 1 May 2019
Available online 2 May 2019

Keywords:
HeLa cells
ALL
L-asparaginase
Pseudomonas aeruginosa

ABSTRACT

Among cancers, acute lymphoblastic leukemia (ALL) occurs in the children <15 years of age. L-asparaginase is an important therapeutic enzyme used for treating ALL. Owing to its therapeutic use and demand, microorganisms have been in use for many years to produce L-asparaginase on an industrial scale. Gram-negative bacteria (*Serratia, Erwinia and Escherichia coli*) species were used in L-asparaginase. However, earlier studies have documented that the long-term use of enzymes produced from these commercial strains induces hypersensitivity in patients. Therefore, there is a need to discover novel microbial strains producing L-asparaginase with anti-cancer properties, which can be employed for the commercial production of the enzyme. In this study, three strains of *Pseudomonas aeruginosa* (accession numbers LC425424 (P31), LC425425 (P32), and LC425426 (P34)) isolated from garden soil were screened for the invention of L-asparaginase. Fermented production media was dialyzed to attain the purified enzyme, thus showed a dose-depended cytotoxic effect on HeLa cells, as determined by MTT assay. The IC_{50}s of the different isolates were 86.73, 57.65, and 40.34 μg/mL. These results indicate that pseudomonal L-asparaginase may be used for cancer treatment.

1. Introduction

Acute lymphoblastic leukemia (ALL) is a blood cancer commonly ripens in children. Treatment with L-asparaginase enzyme was shown to achieve complete remission in ALL (Ashok et al., 2019; Brumano et al.,2018) which was shown to be mediated by hydrolysis of aspartic acid-ammonia in the blood (Pike et al.,2019). When L-asparaginase is administered to ALL patients, it depletes asparagine levels in the blood. Following the depletion of asparagine, the regular cells recompense via synthesizing L-asparagine from aspartic acid-glutamine using the enzyme asparagine synthetase. Still, the cancer cells lack asparagine synthetase, causing asparagine deficiency in these cells ultimately leading to the non-availability of asparagine for protein synthesis and normal cell functioning resulting in their death (De Koning, 2017; Zhang et al., 2014). Systemic depletion of asparagine with asparaginase was shown to induce cell death in leukemia (Fernandez et al., 2013). At present pegylated L-asparaginase (Oncaspar®) and other L-asparaginase preparations like Spectrila and Kidrolase are approved for the treatment of ALL (Horvath et al., 2019). Many studies have documented that usage of *E. coli* asparaginase for treatment of ALL induces the development of side effects such as allergies, disturbances in the central nervous system, and liver dysfunction. Subsequently, *E. chrysanthemi* asparaginase was considered for therapeutic use but failed to achieve complete remission (Duval et al., 2002; Egler et al., 2016; Haskell et al., 1969; Oettgen et al., 1970; Pieters et al., 2011). Therefore, further studies on novel bacterial strains producing L-asparaginase, enzyme purification strategies, and formulations to reduce allergenic reactions and to increase enzyme stability are essential so to overcome the disadvantages associated with *E. coli* and *E. chrysanthemi* asparaginases. The present study aims to isolate novel bacteria species particularly *Pseudomonas aeruginosa* producing L-asparaginase from the soil and to optimize enzyme synthesis. In this study, the enzyme produced from the pseudomonal isolates where tested for its anti-cancer activity on HeLa cells using MTT assay. All enzyme preparations showed anti-cancer activity against HeLa cell lines. This study is a first of

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.05.001
1319-562X/

Table 1
Diameter of pink zones representing L-asparaginase production by soil isolates of *Pseudomonas aeruginosa.*

S. No	Strain code	Pink zone diameter (cm)
1	P31	5.2
2	P32	5.5
3	P33	3
4	P34	5.8
5	P35	2.5
6	P36	3.5
7	P37	2.6
8	P38	3.4

Table 2
Amount (μg/mL) of enzyme produced at pH 7.0 & 8.0.

S. No	Strain code	Protein concentration (μg/ml) at pH 7.0	Protein concentration (μg/ml) at pH 8.0
1	P31	325	425
2	P32	225	625
3	P34	550	800

its kind to report the anti-cancer activity of pseudomonal L-asparaginase.

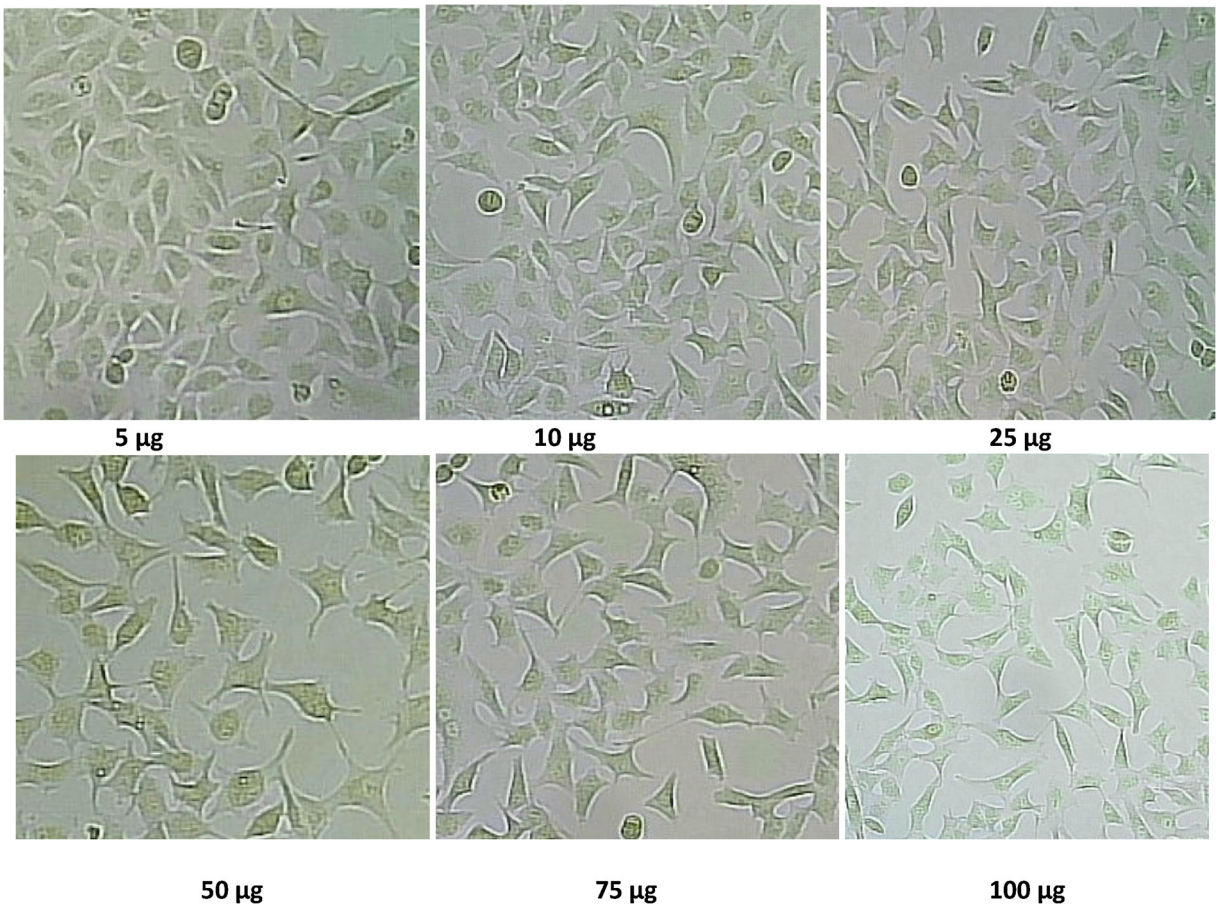
2. Materials and methods

2.1. Isolation of Pseudomonas aeruginosa from the soil

Soil samples from a depth of 30 to 40 cm were collected from gardens and fields (G1, G2, F1, and F2) in and around Hyderabad, Telangana, India. The samples were collected in sterile screw-capped tubes, for the microbial analysis. One gram of soil sample was suspended in 9 mL physiological saline in a flask and incubated in an orbital shaker incubator at 100 rpm at 37 °C for 28 ± 2 h. Toward the end of the incubation period, the flasks were taken off the shaker, and the suspended particles were allowed to settle down. The supernatants were collected and serially diluted with physiological saline. Aliquots of 1 mL from the 10^{-4}–10^{-9} dilutions were plated on separate cetrimide agar plates by the spread plate technique under sterile conditions for isolating *P. aeruginosa*. The colonies were purified by repeated subculturing.

Table 3
Enzyme activity of L-asparaginase isolated from *P. aeruginosa* at pH 7.5 & 8.0.

S. No	Strain code	Concentration of ammonia (μg/ml) at pH 7.5	Concentration of ammonia (μg/ml) at pH 8.0	Enzyme Activity (unit/ml) at pH 7.5	Enzyme Activity (unit/ml) at pH 8.0
1	P31	13.1	61.7	13.1/15 * 0.5 = 1.75	61.7/15 * 0.5 = 8.22
2	P32	66.5	145.9.	66.5/15 * 0.5 = 8.87	145.9/15 * 0.5 = 0.19.45
3	P34	63.8	143.55	63.8/15 * 0.5 = 8.51	143.55.1/15 * 0.5 = 19.1



Graph 1. Cytotoxic effect of L-asparaginase produced from *P. aeruginosa* (LC425424).

Pure cultures were identified by Gram staining, colony morphology, and oxidase test (Devi and Ramanjaneyulu, 2016; Peterson and Ciegler, 1969).

2.2. Screening for L-asparaginase using the plate method assay (primary screening)

Pure cultures of *P. aeruginosa* were inoculated on modified brain heart infusion (BHI) media. One liter of BHI media was modified by supplementing with 6 g KH_2PO_4, 10 g asparagine, 4 mL 1 M $MgSo_4$, 2 mL 0.1 M $CaCl_2$, and 0.4 mL 0.009% phenol red indicator and the pH was adjusted to 7.0 using 1 N HCl. Control plates containing BHI medium without the dye and asparagine were also prepared. The samples were inoculated with the plates and incubated at room temperature for 1 d. Colonies exhibiting pink zones formed by the deamination of asparagine to yield aspartate and ammonia (detected by change in the color of the pH indicator phenol red) were considered as L-asparaginase positive colonies. The phenol red pH indicator was used to detect the release of ammonia. The positive colonies were isolated and were subjected to secondary screening by the agar well diffusion (AWD) assay (Badoei-Dalfard, 2016).

2.3. Agar well diffusion assay

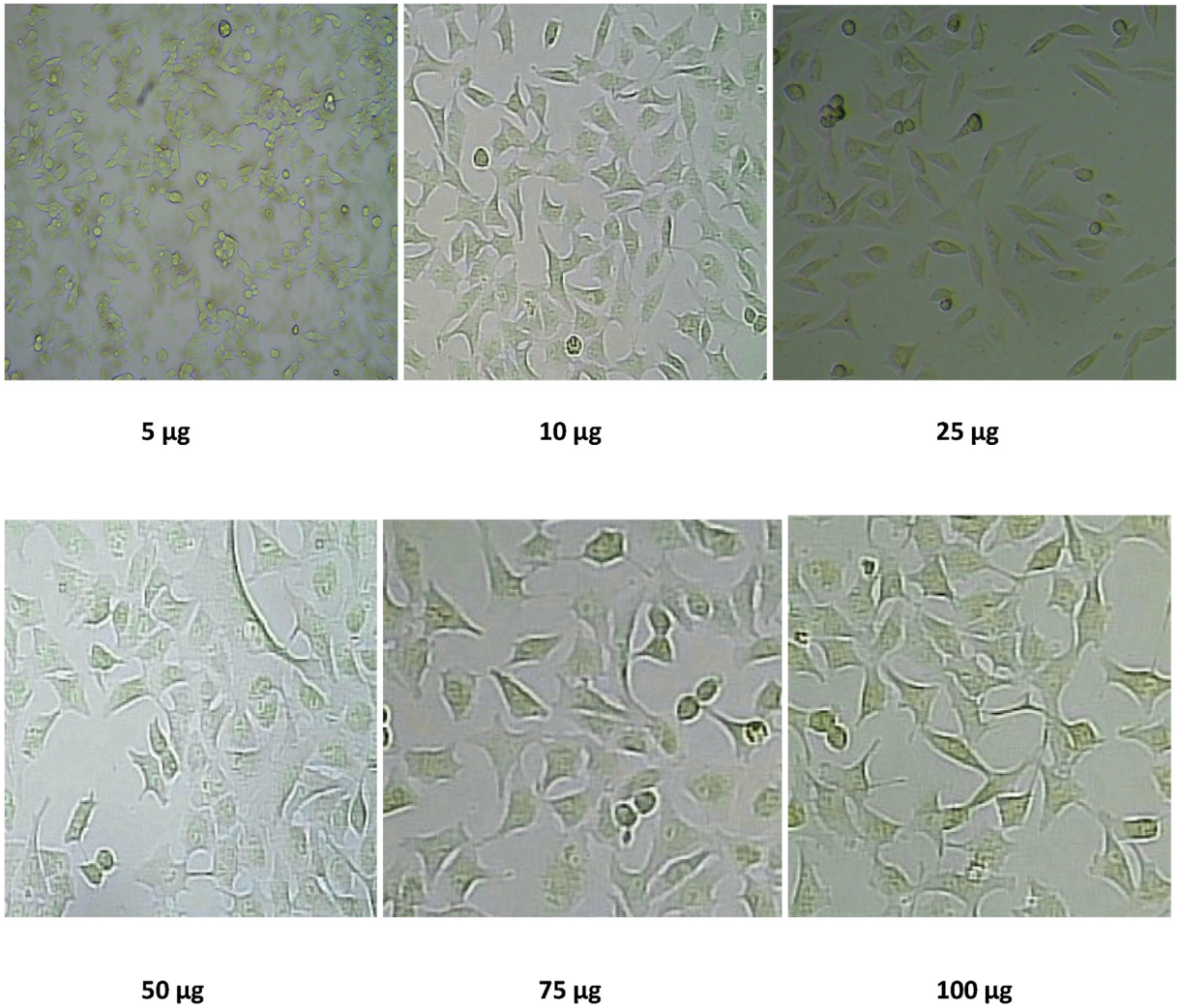
Culture filtrates from selected pseudomonal isolates showing L-asparaginase production (primary screening) were screened qualitatively for L-asparaginase. Asparagine tryptone glucose yeast extracts broth medium (50 mL) was sterilized in a 250 mL conical flask (Erlenmeyer). Subsequently, the media was inoculated with pure cultures of selected *P. aeruginosa* strains and incubated in a shaker incubator for 48 h at 37 °C and 150 rpm.

After incubation, 50 µL of the culture broth was dispensed in each well of an AWD assay plate containing modified BHI medium supplemented with 1% asparagine and 0.009% phenol red indicator. Uninoculated media served as negative control. Plates were incubated for 1 d on 37 °C and diameter of the pink zone around the wells was measured. The cultures showing wider pink zones indicated strains that showed promise of enzyme production, and these strains were selected for inoculum preparation (El-Naggar et al., 2015; Gulati et al., 1997).

2.4. Enzyme production through submerged fermentation method

2.4.1. Inoculum preparation

The strains that had larger pink zones in the AWD assay were used for inoculum preparation. One lite inoculum media contain-

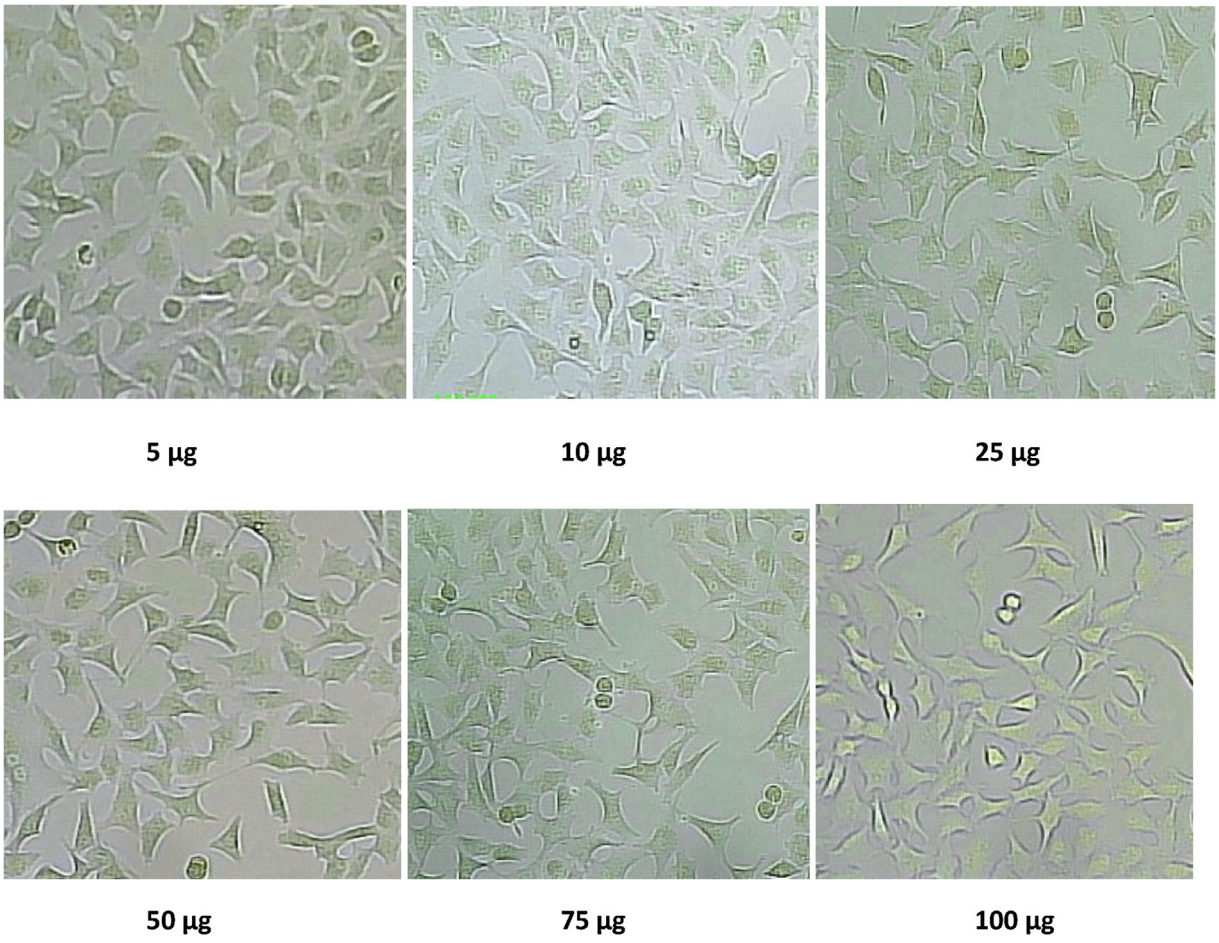


Graph 2. Cytotoxic effect of L-asparaginase isolated *P. aeruginosa* (LC425425) on HeLa Cells.

ing 6 g Na_2HPo_4, 3 g KH_2Po_4, 0.5 g NaCl, 1 g glucose, 2 mL 1 M $MgSo_4$, and 1 mL 0.1 M $CaCl_2$ was prepared and the pH was adjusted to 7.2. A loopful of culture from the selected strains were first inoculated into 50 mL inoculum media that was incubated at 37 °C for 16–18 h.

2.4.2. Production of L-asparaginase

Two sets of flasks containing sterile 50 mL tryptone glucose yeast extract broth supplemented with 1% asparagine (production media) were prepared. The pH of one set of flasks was adjusted to 7.0 and the pH of the other set was adjusted to 8.0 using sterile 1 N



Graph 3. Cytotoxic effect of L-asparaginase isolated *P. aeruginosa* (LC425426) on HeLa Cells.

Table 4
Cytotoxic properties of different concentrations of L-asparaginase isolated from *P. aeruginosa* (LC425424).

Concentration (µg)	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (µg)
100	0.855	0.857	0.859	0.857	0.854	46.087	86.73
75	0.995	0.997	0.998	0.996	0.993	53.588	
50	1.113	1.115	1.117	1.115	1.112	60.01	
25	1.209	1.211	1.213	1.211	1.208	65.191	
10	1.246	1.248	1.249	1.247	1.244	67.134	
5	1.363	1.365	1.367	1.365	1.362	73.502	
Untreated	1.856	1.857	1.856	1.856	1.853	100	
Blank	0.003	0.004	0.003	0.003	0		

Table 5
Cytotoxic effect of different concentrations of L-asparaginase isolated from *P. aeruginosa* (LC425425).

Concentration (µg)	Absorbance at 570 nm			Average	Average-Blank	% Viability	IC_{50} (µg)
100	0.597	0.599	0.601	0.599	0.594	28.339	57.655
75	0.926	0.928	0.93	0.928	0.923	44.036	
50	1.239	1.241	1.243	1.241	1.236	58.969	
25	1.295	1.297	1.299	1.297	1.292	61.641	
10	1.398	1.4	1.402	1.4	1.395	66.555	
5	1.584	1.586	1.588	1.586	1.581	75.429	
Untreated	2.101	2.102	2.101	2.101	2.096	100	
Blank	0.005	0.006	0.005	0.005	0		

Table 6
Cytotoxic effect of different concentrations of L-asparaginase produced from *Pseudomonas aeruginosa* (LC425426).

Concentration (µg)	Absorbance at 570 nm		Average	Average-Blank		% Viability	IC_{50} (µg)
100	0.696	0.697	0.699	0.697	0.692	37.425	40.349
75	0.781	0.783	0.784	0.782	0.777	42.022	
50	0.843	0.845	0.846	0.844	0.839	45.375	
25	0.993	0.995	0.997	0.995	0.99	53.542	
10	1.029	1.031	1.033	1.031	1.026	55.489	
5	1.126	1.128	1.129	1.127	1.122	60.681	
Untreated	1.854	1.856	1.854	1.854	1.849	100	
Blank	0.005	0.006	0.005	0.005	0		

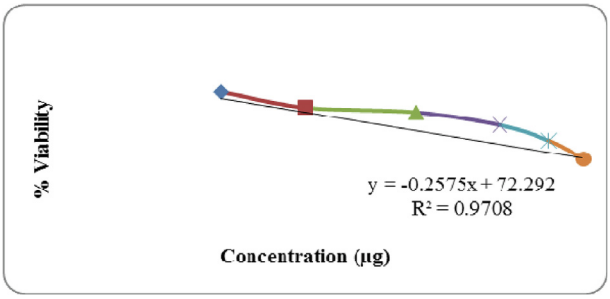


Image 1. Cytotoxic effect of L-asparaginase isolated from *P. aeruginosa* (LC425424) on HeLa cells.

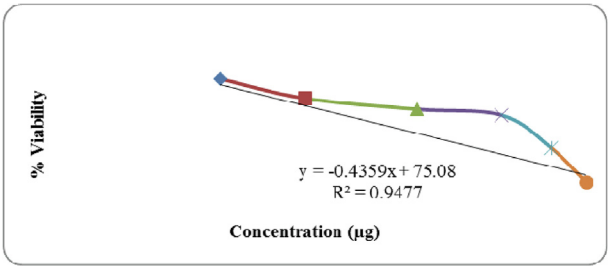


Image 2. Cytotoxic effect of L-asparaginase isolated from *P. aeruginosa* (LC425425) on HeLa cells.

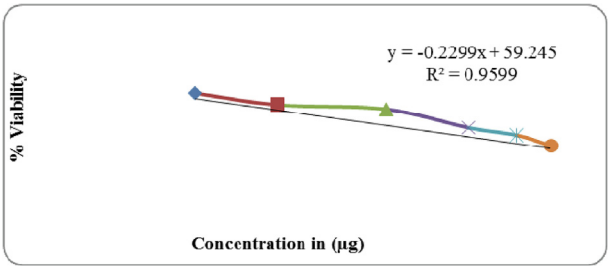


Image 3. Cytotoxic effect of L-asparaginase isolated from *P. aeruginosa* (LC425426) on HeLa cells.

NaOH. Two milliliters of the seed or inoculum culture was inoculated into each flask containing 50 mL production media. The flasks were incubated in an orbital shaker incubator at 37 °C and 150 rpm for 48 h. Flasks with uninoculated medium served as negative controls (Peterson and Ciegler, 1969; Yadav et al., 2014).

2.5. Protein purification

The crude supernatant containing L-asparaginase was partially purified by dialysis using 8 cm nitro-cellulose dialysis bags (Amena et al., 2010).

2.5.1. Quantitative estimation of the dialyzed protein

Lowry's method was used to determine the amount of protein content in the crude and dialyzed supernatant.

2.5.2. Determination of enzyme activity

Dialyzed protein (0.5 mL), 50 mM asparagine (0.5 mL), and 0.02 M potassium phosphate buffer (1 mL) was taken in a tube and the pH was adjusted to 7.5 or 8.0. The tubes were mixed well and incubated in a water bath at 37 °C for 15 m. After incubation, 1 mL 1.5 M TCA was added to stop the reaction and then centrifuged at 12,000 rpm for 10-min. The collected supernatant was used for direct Nesslerization method (El-Bessoumy et al., 2004; Wriston and Yellin, 1973).

2.5.3. Direct Nesslerization

Concentration of liberated ammonia was determined by adding 4 mL double distilled water to 0.5 mL sample. Afterward, 0.5 mL of Nessler's reagent was added to the sample and was mixed well. The mixture was then incubated for 15 min at room temperature. After incubation, the absorbance was recorded at 450 nm against the blank (9 parts of double distilled water to 1 part Nessler's reagent) (Badoei-Dalfard, 2016; Wriston and Yellin, 1973).

2.6. Anti-cancer activity

Anti-cancer activity of the purified L-asparaginase on HeLa cells was determined using the MTT assay.

2.6.1. Maintenance of cells

The human cervical cancer HeLa cell line was achieved from the National Centre for Cell Sciences, Pune. Cells were well maintained in DMEM supplemented with 10% FBS and specific antibiotics in a 5% CO_2 atmosphere at 37 °C.

2.6.2. Procedure

Suspended cells were stained with trypan blue to distinguish between live and dead cells. Live cells were counted using a hemocytometer, and 5×10^3 live cells in 100 µL culture media were plated in each well of a 96-well plate. After overnight incubation at 37 °C, the culture media was removed and fresh media with different concentrations of purified L-asparaginase was added to the wells and incubated for 48 h at 37 °C. Cisplatin was used as a positive control and untreated cells served as negative controls (Moharib, 2018).

$$\%\,\text{Inhibition} = \frac{100(\text{Control} - \text{Treatment})}{\text{Control}}$$

2.6.3. 16S rRNA typing

The strains of *P. aeruginosa* exhibiting maximum L-asparaginase activity, and those with the highest yield of the enzyme was subjected to 16S rRNA analysis. The sequences obtained were deposited in the DNA Data Bank of Japan (DDBJ).

3. Results

3.1. 2 & 3: P. Aeruginosa producing L-asparaginase

Twelve *P. aeruginosa* strains were isolated from the soil samples of which eight strains showed L-asparaginase production. Of the eight strains showing L-asparaginase production, only three strains exhibited promising enzyme activity on AWD analyses as shown in Table 1.

3.2. Enzyme concentration

L-asparaginase produced by each strain at different pH conditions was purified by dialysis and the amount of enzyme in each sample was quantified. The values are presented in Table 2. An increase in enzyme production was observed at pH 8.0, indicating that pH plays an important role in L-asparaginase production.

3.3. Enzyme activity &3.3

The activity of the purified enzyme was assayed at pH 7.0 & 8.0 and the results are presented in Table 3. From the table, it can be noticed that the enzyme activity was higher at pH 8.0.

3.4. The anti-cancerous activity of the purified enzyme

The samples showing the highest enzyme activity were studied for anti-cancer activity on HeLa cells. The IC_{50} results obtained for each sample are shown in Graphs 1–3, Tables 4–6, & Images 4–6.

The phylograms for the three *P. aeruginosa* strains with good enzyme yield, enzyme activity, and potent anti-cancer activity are presented in (Figs. 1–3). The sequences were submitted to DDBJ under the accession numbers LC425424 (P31), LC425425 (P32), and LC425426 (P34).

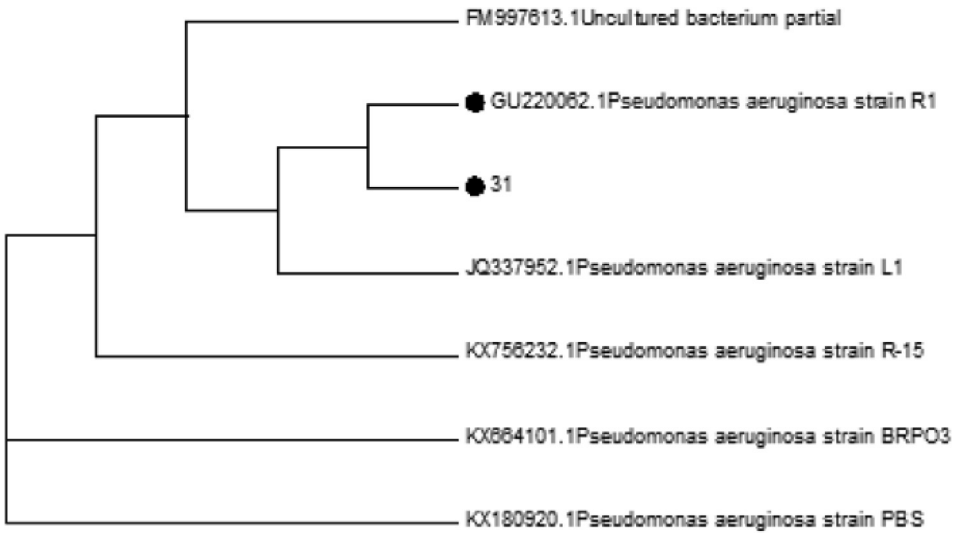


Fig. 1. Phylogram of *Pseudomonas aeruginosa* (LC425424).

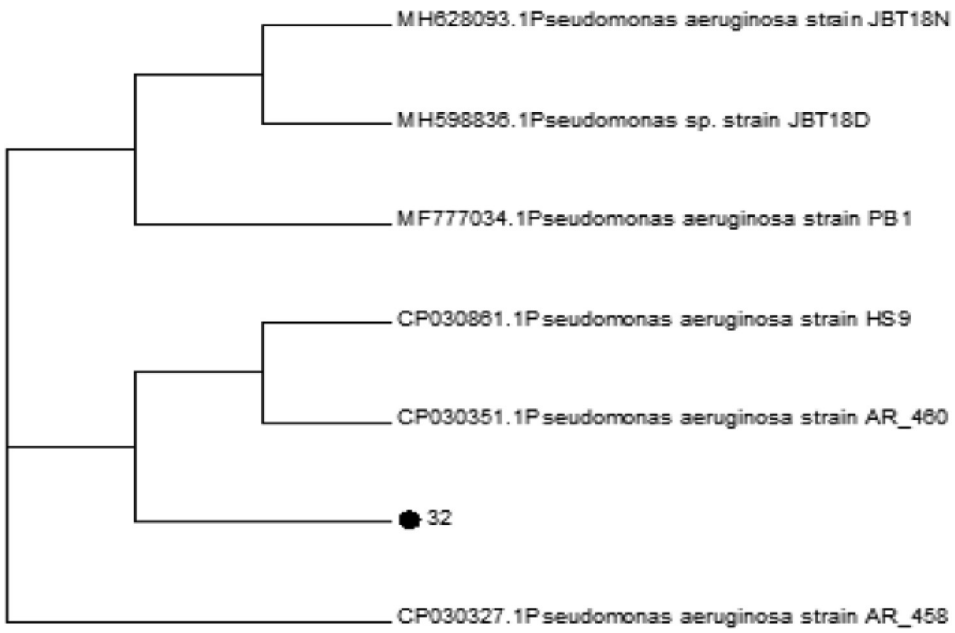


Fig. 2. Phylogram of *Pseudomonas aeruginosa* (LC425425).

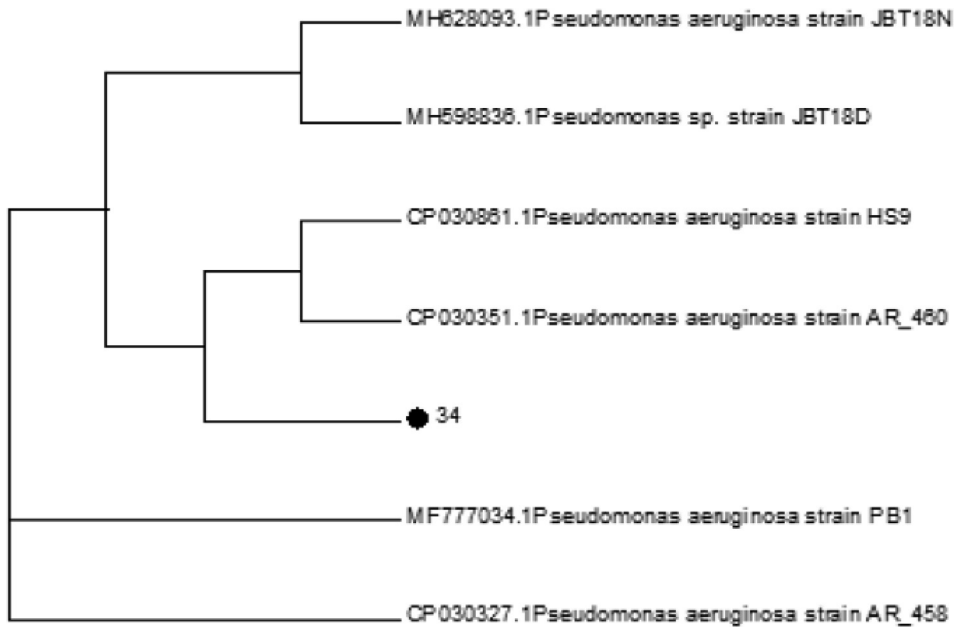


Fig. 3. Phylogram of *Pseudomonas aeruginosa* (LC425426).

4. Discussion

The activity of the enzymes in our study was in accordance with that was reported earlier (Ashraf and Foolad, 2007). It has been reported that the L-asparaginase synthesis in *P. aeruginosa* increased with increase in pH until pH 9 (El-Bessoumy et al., 2004). In addition, enzyme activity below pH 8 is considered to be therapeutically not effective in cancer patients (Yadav et al., 2014). In the present study, the enzyme activity recorded at pH 8 which were 8.22, 19.45, and 19.14 (Unit/mL) for the three strains P31, P32, and P34, respectively. The specific activity of commercially available *E. coli* asparaginase is at least 85 IU/mg of protein. The enzyme activity in our study is in a similar range with another study in which the activity of asparaginase isolated from seven strains of *P. alcaligenes* ranged from 20 to 240 units/mL (Badoei-Dalfard, 2016). However, the enzyme activity can further be increased by incorporating glucose and metal ions like Cobalt/thiol protecting reagents (Badoei-Dalfard, 2016; Manna et al., 1995; Trilokchandran et al., 2016). A positive effect of pH on enzyme production and activity was documented with the used three isolates in this study. The produced amount of L-asparaginase ripens vice versa with an increase in pH. This study has showed a growth in protein concentration and enzyme activity at pH 8 with the used strains (*Pseudomonas aeruginosa*; Tables 2 and 3). Higher pH increases enzyme activity, but this enzyme may become inactive when it evolves pH at 11.8 (Yadav et al., 2014).

The *in vitro* cytotoxicity of L-asparaginase produced from microorganisms and plants against many types of cell cultures have been demonstrated (Asthana and Azmi, 2003). For instance, L-asparaginase from plant sources was found to effectively inhibit the growth of HEPG2 and HCT-116 cell lines; while it was not effective on HeLa cell lines (Moharib, 2018). In contrast, many studies have demonstrated that L-asparaginase produced from microbial sources had cytotoxic effects on HeLa cell lines (Bhat and Marar, 2015; Rani et al., 2012; Sudarkodi and Sundar, 2018). A previous reported an IC_{50} of 0.171 IU of L-asparaginase on HeLa cells (Bhat and Marar, 2015) while another study demonstrated a dose-dependent anti-cancer activity of L-asparaginase produced by *Aspergillus oryzae* up to a maximum concentration of 2 μg/mL on HeLa cells (Sudarkodi and Sundar, 2018). A similar study on fungal asparaginases reported that a concentration of 131.25 μg/ml of L-asparaginase produced from *Aspergillus flavus* inhibiting 50% of HeLa cell growth (Rani et al., 2012). In the current study 86.7, 40.3, and 57.6 μg/mL of L-asparaginase isolated from P31, P32, and P34, respectively were found to kill 50% HeLa cell lines. In addition, the cytotoxicity increased with increase in enzyme concentration (5, 10, 25, 50, 75, and 100 μg/mL) with maximum viability of 46.08% and minimum viability of 28.33%. The enzyme activity of present study pH8 suggested to use in anticancer therapies and if the enzyme activity was found to be below pH8 is not suggested in the tumor patients (Yadav et al., 2014). Furthermore, this study also demonstrates that pseudomonal L-asparaginase to be effective against HeLa cells. Our study also shows that L-asparaginase can prove to be an effective therapeutic agent in treating cervical cancer; although *in vivo* trials are needed for further validation of therapeutic efficacy.

5. Conclusion

L-asparaginase is well known for effectively usage in ALL. Many other invitro studies have suggested L-asparaginase can be used in cervical cancer. Therefore, new treatment strategies and new drugs are required for effective cancer treatment and management in the clinic. Our results demonstrate that L-asparaginase isolated from *P. aeruginosa* can effectively inhibit the growth of human cervical cancer cells *in vitro*, supporting the potential use of L-asparaginase for the treatment of cervical cancer in the clinic. However, additional *in vivo* studies and trials are required to determine the therapeutic efficacy of L-asparaginase.

Acknowledgement

The author extends their sincere appreciation to the Deanship of Scientific Research, College of Applied Medical Sciences Research Center at King Saud University.

Conflict of interest

None.

References

Amena, S., Vishalakshi, N., Prabhakar, M., Dayanand, A., Lingappa, K., 2010. Production, purification and characterization of L-asparaginase from *Streptomyces gulbargensis*. Brazilian J. Microbiol. 41 (1), 173–178.

Ashok, Anup, Doriya, Kruthi, Rao, Jyothi Vithal, Qureshi, Asif, Tiwari, Anoop Kumar, Kumar, Devarai Santhosh, 2019. Microbes Producing L-asparaginase free of glutaminase and urease isolated from extreme locations of antarctic soil and moss. Sci. Rep. 9 (1). PubMed PMID: 30723240; PubMed Central PMCID: PMCPMC6363723 http://www.nature.com/articles/s41598-018-38094-1. https://doi.org/10.1038/s41598-018-38094-1.

Ashraf, M., Foolad, M., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59 (2), 206–216.

Asthana, N., Azmi, W., 2003. Microbial L-Asparaginase: A Potent Antitumour Enzyme.

Badoei-Dalfard, A., 2016. L-asparaginase production in the pseudomonas pseudoalcaligenes strain JHS-71 isolated from Jooshan Hot-spring. Molecul. Biol. Res. Commun. 5 (1), 1.

Bhat, M., Marar, T., 2015. Cytotoxic effect of purified L-asparaginase from *Salinicoccus* sp. M KJ997975. Int. J. Curr. Microbiol. Appl. Sci. 4 (4), 701–712.

Brumano, Larissa Pereira, da Silva, Francisco Vitor Santos, Costa-Silva, Tales Alexandre, Apolinário, Alexsandra Conceição, Santos, João Henrique Picado Madalena, Kleingesinds, Eduardo Krebs, Monteiro, Gisele, Rangel-Yagui, Carlota de Oliveira, Benyahia, Brahim, Junior, Adalberto Pessoa, 018. Development of L-Asparaginase Biobetters: Current Research Status and Review of the Desirable Quality Profiles. Front. Bioeng. Biotechnol. 6. PubMed PMID: 30687702; PubMed Central PMCID: PMCPMC6335324 https://www.frontiersin.org/article/10.3389/fbioe.2018.00212/full. https://doi.org/10.3389/fbioe.2018.00212.

De Koning, T., 2017. Amino acid synthesis deficiencies. J. Inherit. Metab. Dis. 40 (4), 609–620.

Devi, A.L., Ramanjaneyulu, R., 2016. Isolation of L-asparaginase producing microbial strains from soil samples of Telangana and Andhra Pradesh States, India. Int. J. Curr. Microbiol. Appl. Sci. 5 (10), 1105–1113.

Duval, M., Suciu, S., Ferster, A., Rialland, X., Nelken, B., Lutz, P., et al., 2002. Comparison of Escherichia coli–asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer—Children's Leukemia Group phase 3 trial. Blood 99 (8), 2734–2739.

Egler, R.A., Ahuja, S.P., Matloub, Y., 2016. L-asparaginase in the treatment of patients with acute lymphoblastic leukemia. J. Pharmacol. Pharmacotherap. 7 (2), 62.

El-Bessoumy, A.A., Sarhan, M., Mansour, J., 2004. Production, isolation, and purification of L-asparaginase from Pseudomonas aeruginosa 50071 using solid-state fermentation. BMB Rep. 37 (4), 387–393.

El-Naggar, N.E.-A., Moawad, H., El-Shweihy, N.M., El-Ewasy, S.M., 2015;2015.. Optimization of culture conditions for production of the anti-leukemic glutaminase free L-asparaginase by newly isolated Streptomyces olivaceus NEAE-119 using response surface methodology. BioMed Res. Int. 2015.

Fernandez, C.A., Cai, X., Elozory, A., Liu, C., Panetta, J.C., Jeha, S., et al., 2013. High-throughput asparaginase activity assay in serum of children with leukemia. Int. J. Clin. Exp. Med. 6 (7), 478.

Gulati, R., Saxena, R., Gupta, R., 1997. A rapid plate assay for screening l-asparaginase producing micro-organisms. Lett. Appl. Microbiol. 24 (1), 23–26.

Haskell, C., Canellos, G., Leventhal, B., Carbone, P., Block, J., Serpick, A., et al., 1969. L-asparaginase: therapeutic and toxic effects in patients with neoplastic disease. N. Engl. J. Med. 281 (19), 1028–1034.

Horvath, T.D., Chan, W.K., Pontikos, M.A., Martin, L.A., Du, D., Tan, L., et al., 2019. Assessment of l-asparaginase pharmacodynamics in mouse models of cancer. Metabolites 9 (1), 10.

Manna, S., Sinha, A., Sadhukhan, R., Chakrabarty, S., 1995. Purification, characterization and antitumor activity of L-asparaginase isolated from Pseudomonas stutzeri MB-405. Curr. Microbiol. 30 (5), 291–298.

Moharib, S.A., 2018. Anticancer activity of L-asparaginase produced from Vigna unguiculata. World Sci. Res. 5 (1), 1–12.

Oettgen, H.F., Stephenson, P.A., Schwartz, M.K., Leeper, R.D., Tallal, L., Tan, C.C., et al., 1970. Toxicity of E. coli L-asparaginase in man. Cancer 25 (2), 253–278.

Peterson, R., Ciegler, A., 1969. L-asparaginase production by various bacteria. Appl. Microbiol. 17 (6), 929.

Pieters, R., Hunger, S.P., Boos, J., Rizzari, C., Silverman, L., Baruchel, A., et al., 2011. L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer 117 (2), 238–249.

Pike, Meghan, Kulkarni, Ketan, MacDonald, Tamara, 019. Asparaginase activity monitoring experience from the Maritimes, Canada. Leuke. Lymph., 1–4 PubMed PMID: 30721104 https://www.tandfonline.com/doi/full/10.1080/10428194.2019.1571196. https://doi.org/10.1080/10428194.2019.1571196.

Rani, S.A., Sundaram, L., Vasantha, P.B., 2012. A study on in vitro antioxidant and anticancer activity of l-asparaginase. J. Pharm. Res. 5, 1463–1466.

Sudarkodi, C., Sundar, S., 2018. Anticancer Activity of L-Asparaginase from Aspergillus oryzae against Hep G2 and Hela Cell Lines.

Trilokchandran, B., Agarwal, P., Krishna, V., 2016. Media optimization and growth parameters for the production of L-asparaginase enzyme from bacterial isolate. J. Chem. Pharm. Res. 8 (8).

Wriston, J.C., Yellin, T.O., 1973. L-asparaginase: a review. Adv. Enzymol. Relat. Areas Mol. Biol. 39, 185–248.

Yadav, S., Verma, S.K., Singh, J., Kumar, A., 2014. Industrial production and clinical application of L-asparaginase: a chemotherapeutic agent. Stroke 76, 41.

Zhang, J., Fan, J., Venneti, S., Cross, J.R., Takagi, T., Bhinder, B., et al., 2014. Asparagine plays a critical role in regulating cellular adaptation to glutamine depletion. Mol. Cell 56 (2), 205–218.