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Research Article

ISOLATION, OPTIMIZATION, AND ANTITUMOR ACTIVITY OF L-ASPARAGINASE EXTRACTED FROM *PECTOBACTERIUM CAROTOVORUM* AND *SERRATIA MARCESCENS* ON *HUMAN BREAST ADENOCARCINOMA* AND HUMAN HEPATOCELLULAR CARCINOMA CANCER CELL LINES

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ABSTRACT

Objectives: The objective of this research was to obtain isolates capable of producing a high yield of L-asparaginase enzyme and to evaluate the antitumor activity of the purified enzyme against different cancer and normal cell lines.

Methods: Isolation of bacteria was performed by the serial dilution technique of soil samples collected from Cairo, Egypt, using modified M9 agar plates. Culture filtrates of selected isolates were quantitatively screened for L-asparaginase production using well-diffusion and direct nesslerization techniques. Factors influencing L-asparaginase activity were optimized by studying the effect of physical and nutritional conditions on the enzyme activity. The purification of L-asparaginase extracted from both the isolates was achieved using chilled acetone (−20°C), followed by gel filtration on Sephadex G-100. The anticancer activity of the purified enzyme against human breast adenocarcinoma (*MCF-7*), human hepatocellular carcinoma (*HepGII*) and homo sapiens human (*WISH*) cell line was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide assay.

Results: Two L-asparaginase producers were identified by Biolog identification system as *Pectobacterium carotovorum* and *Serratia marcescens*. Optimization increased the production of L-asparaginase to 4.835 and 5.221 U/ml for *P. carotovorum* and *S. marcescens,* respectively. L-asparaginase was extracted, purified, and tested *in vitro* for cytotoxic activity using 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT assay) against *MCF-7, HepGII,* and *WISH* cell line. L-asparaginase from *P. carotovorum* and *S. marcescens* was neutral to normal epithelial *WISH* cells. On the other hand, L-asparaginase from both isolates was cytotoxic to *MCF-7* and *HepGII* cancer cell lines with an half maximal inhibitory concentration of 15 µg/ml and 26 µg/ml and 26 µg/ml and 25 µg/ml, respectively.

Conclusion: L-asparaginase extracted from *P. carotovorum* and *S. marcescens* showed remarkable anticancer activity. Further studies on hypersensitivity action need to be carried out to recommend the use of L-asparaginase as an alternative to commercially available preparations.

Keywords: L-asparaginase, Optimization, Cytotoxicity, *Pectobacterium, Serratia.*

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INTRODUCTION

L-asparaginase is relatively widespread enzyme found in bacteria, yeast, fungi, and plants. It catalyzed the hydrolysis of the amide group of asparagine to yield aspartate and ammonia [1]. Many reports demonstrated the anti-leukemic activity of L-asparaginase [2-4]. Today, L-asparaginase is an essential drug that is used in the treatment of acute lymphoblastic leukemia in children all over the world. ELSPAR, ONCASPAR, KIDROLASE, and ERWINASE are the brand name of L-asparaginase used as drug [5]. The antitumor activity of L-asparaginase was believed to be because most neoplastic tissues require their exogenous supply of L-asparagine from circulating pools because of the absence of L-asparagine synthetase. On the other hand, normal cells synthesize L-asparagine in sufficient quantities for protein synthesis within the cell by L-asparagine synthetase from aspartic acid and glutamine or can be absorb available L-asparagine from the cell surrounding. L-asparaginase breakdowns the circulating asparagine to L-aspartate and ammonia and prevents protein synthesis of neoplasms. Therefore, the most common therapeutic practice to treat this condition is to intravenously administer L-asparaginase to deplete the blood L-asparagine level and exhaust its supply to selectively affect the neoplastic cells [6].

Microbial sources are very common and convenient for L-asparaginase production since they can be easily cultured and produced compounds that could be extracted and purified, as a result facilitating the industrial scale production [7]. The most commonly used microorganisms to produce L-asparaginase were *Escherichia coli*, *Bacillus* sp., *Streptomyces albidoflavus,* and actinomycetes from the rhizosphere of medicinal plants [8]. L-asparaginase from *E. coli* and *Erwinia chrysanthemi* has a perfect action to inhibit the activity of tumor cells. They were clinically used as effective drugs in the treatment of Hodgkin's disease, acute myelocytic leukemia, acute myelomonocytic leukemia, chronic lymphocytic leukemia, lymphosarcoma, reticulosarcoma, and melanosarcoma [9]. However, L-asparaginase from these two bacterial sources caused hypersensitivity in the long term leading to mild allergic reactions to anaphylactic shock [10]. *E. chrysanthemi* L-asparaginase exhibited less allergic reactions compared to the *E. coli* enzyme. However, *Erwinia* (synonym: *Pectobacterium*) [11,12] L-asparaginase had a shorter half-life than *E. coli* [13]. This advocated the need to discover new L-asparaginases with less adverse effects but has similar therapeutic effects. Therefore, the aim of this work was to obtain isolates capable of producing a high yield of L-asparaginase enzyme and to evaluate the antitumor activity of the purified enzyme against different human cell lines.

METHODS

Detection and screening L-asparaginase-producing bacteria

Isolation of bacteria was performed by the serial dilution technique of two soil samples collected from two sites from Cairo, Egypt, into sterile bottles at a depth of 30–40 cm, using modified M9 agar plates

containing 5 µg/ml of nystatin as an antifungal agent and supplemented with 0.09% phenol red (prepared in ethanol) as indicator. The pH was adjusted to 6.2 using 1 N HCl. M9 medium plates without both indicator and asparagine served as controls (instead containing $NaNO_3$ as nitrogen source) were also prepared. After incubation at 37°C for 48 h, colonies with pink zones were considered as L-asparaginase-producing bacteria.

Culture filtrates of selected isolates were semi-quantitatively screened for L-asparaginase production using well diffusion technique. Sterile Erlenmeyer flasks (250 ml) containing 50 ml sterile modified asparagine M9 broth medium were inoculated with 1 ml (10^6 colony-forming unit [CFU]) of each isolate that showed production of L-asparaginase. Flasks were incubated at 37°C for 24 h in a rotatory shaking incubator at 120 revolutions per minute (rpm) [14].

Wells (5-mm diameter) were made using sterilized cork borer in the center of modified M9 agar plate, and then, 50 µl of broth culture was transferred to each well. After incubation at 37°C for 24 h, the diameter of pink zones around the wells was measured and recorded [14].

Identification of bacterial isolates

The preliminary identification of potential isolates was performed according to Bergey's Manual of Systematic Bacteriology [15]. Identification of the potent isolates was carried out by Biolog System (GEN III identification) EGYVAC - VACSERA, Giza, Egypt.

Assay of L-asparaginase

An overnight inoculum (1 ml) of bacterial suspension (10^6 CFU) was transferred to 250 ml Erlenmeyer flasks with 50 ml broth modified M9 medium and incubated in a shaking incubator (120 rpm) at 37°C for 24 h. After incubation, bacterial cells were removed by centrifugation at 10,000 rpm for 10 min at 4°C [16]. The supernatant was used to assay extracellular L-asparaginase activity which was measured by Nessler's reaction (Direct Nesslerization of ammonia) according to Mashburn and Wriston [17]. The blank was prepared by mixing distilled water with Nessler's reagent. The concentration of ammonia was estimated and the absorbance was measured at 450 nm, using spectrophotometer (Thermo scientific). A standard curve was drawn with various concentrations of ammonia.

Optimization of L-asparaginase production

Factors influencing L-asparaginase activity were optimized by studying the effect of physical and nutritional conditions on the enzyme activity. The optimal condition identified for each parameter was applied for optimizing the next one.

Optimization of different environmental conditions (incubation temperature, pH, and incubation period) for L-asparaginase production

The optimum temperature for maximum L-asparaginase production was determined by incubating the cultures at different temperatures from 25 to 40°C with an interval of 5°C on M9 media for 24 h. To detect the optimum pH for the production of L-asparaginase, media were adjusted at different pH values (6.5, 7.0, 7.5, and 8.0) using 1 N NaOH/1N HCl. The effect of incubation period on L-asparaginase production by the bacterial cultures was determined by incubating for 24, 48, 72, and 120 h. For each parameter, bacterial isolates were grown on M9 broth medium and the cultures supernatants were assayed as described (2.3) [18]. The optimal condition identified for each parameter was applied for optimizing the next one.

Optimization of different nutritional factors (carbon and nitrogen sources) for L-asparaginase production

The addition of different carbon and nitrogen sources other than glucose and L-asparagine was made separately in the medium, to enhance enzyme production such as sucrose, maltose, and lactose and yeast extract, peptone, and ammonium nitrate at 1% (w/v) according to Indira *et al*. [19].

Purification of L-asparaginase

The culture filtrate was filtered through Whatman No. 1 filter paper (0.22 µ) and centrifuged at 8000 rpm for 10 min at 4°C. Chilled acetone (−20°C) was added to the culture filtrate (crude enzyme) with constant stirring at 4°C in the gradient concentration of 4:1, for proteins to precipitate. The precipitated proteins were centrifuged and air dried then dissolved in 0.01 M phosphate buffer (pH 8.5).

The concentrated enzyme was applied to diethylaminoethyl cellulose column (4 cm × 60 cm) equilibrated with 50 mM Tris-HCl (pH-8.6). The column was washed with two volumes of starting buffer, and the protein was eluted with linear gradient of NaCl (0–0.5 M) prepared in phosphate buffer pH 7.4 at the rate of 60 ml/h. Fractions showing L-asparaginase activity were pooled together dialyzed against 50 mM Tris-HCl (pH8.6) and concentrated with bench top protein concentrator at 4°C.

The concentrated enzyme solution was added on the top of Sephadex G-100 column (4 m × 60 cm) equilibrated with 50 mM Tris-HCl (pH8.6) and eluted with the same buffer at the flow rate of 0.5 ml/min. Fractions showing L-asparaginase activity were pooled and dialyzed against the same buffer and lyophilized with bench top lyophilizer [20].

Determination of the molecular weight of the purified enzyme by sodium dodecyl sulfate polyacrylamide gel electrophoreses (SDS-PAGE)

The molecular weight of the extracted enzyme was determined by performing SDS-PAGE according to the method of Laemmli [21], using protein marker of 10–260 KDa (Thermo Fischer Scientific).

Cytotoxicity assay

Cell lines

Three cell lines: Human breast adenocarcinoma (*MCF-7*; ATCC number: HTB-22), human hepatocellular carcinoma (*HepGII*; ATCC number: HB-8065), and Homo sapiens human (*WISH*, HeLa contaminant; ATCC number: CCL-25) were obtained from EGYVAC-VACSERA, Giza, Egypt.

Cell viability determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) method

Each of the three cell lines, *MCF-7, HepGII,* and *WISH,* were seeded at 2×10^4 cells/well in tissue culture plates and incubated at 37°C for 24 h. For background absorption, some wells were remained cell free as blank control. The L-asparaginase was added to the cells at serial concentrations of 100, 50, 25, 12.5, and 6.25 µg/ml, and the control was also included (without L-asparaginase). The final volume was adjusted to 100 µl/well. The plates were incubated overnight at 37°C, 5% CO_2. 25 µl of (0.5 mg/ml) MTT stain was added to each well, and the plates were incubated at 37°C for 4 h. 100 µl of dimethyl sulfoxide (DMSO) stop solution was added to each well. The plates were shaken at room temperature for 30 min–1 h. The plates were then read using ELISA Microplate reader at 570 nm. The percentage of viable cells was calculated from the formula:

Survival fraction=OD of treatment cell/OD of control cell.

The half maximal inhibitory concentration (IC_{50}) was calculated by fitting the survival curve using GraphPad Prism software in corporate [22].

Morphological characteristics of cell lines

MCF-7 and *HepGII cells* were cultured in 6-well plates. L-asparaginase extracted from the selected isolates was added for medium at a concentration of IC_{50} (15 µg/ml and 26 µg/ml and 26 µg/ml and 25 µg/ml, respectively). After treatment, all the cultures were incubated at 37°C, 5% CO_2 for 24 h, then were washed well with phosphate buffered saline, and fixed in 10% neutral formalin for 24 h. Photographs were taken under an inverted Leica fluorescence 40 × 10 microscope [23].

Statistical analysis

All statistical analyses in this study were carried out using Microsoft Excel 2000, Analysis Tool pack (Microsoft Corporation). All data were

calculated from at least 3 replicates, and the standard error for each datum was plotted on the graph.

RESULTS AND DISCUSSION

Selection and identification of potent L-asparaginase-producing bacteria

Fifty-three colonies randomly selected were purified and subcultured on modified M9 agar media supplemented with 0.09% phenol red to confirm their production of L-asparaginase. Only nine isolates which showed pink color around the colonies confirming their production of L-asparaginase were selected. The ability of the production of L-asparaginase was based on the color change due to a change in pH of the medium. Asparaginase causes the hydrolysis of L-asparaginase into L-aspartic acid and ammonia. The release of ammonia changes pH due to phenol red indicator; it has a pale yellow color with the acidic condition, as the pH changes to alkaline it turns to pink [24]. According to well diffusion agar assay, two bacterial isolates, FS-4 and GS-7, that showed larger zone of pink color (5.2 cm±0.005 and 4.5 cm±0.005, respectively) were selected (Fig. 1). Similar diameters were recorded by Upadhyay *et al*. [25]. Smaller diameter in the range of 1.9 cm, 1.0 cm, and 0.8 cm was reported by Jain *et al*. [26] and Patil and Jadhav [27].

The two isolates FS-4 and GS-7 were identified using the Biolog identification System as *Pectobacterium carotovorum* and *Serratia marcescens*, respectively**.**

Production of L-asparaginase by *P. carotovorum* and *S. marcescens* isolates

P. carotovorum and *S. marcescens* were used to produce L-asparaginase enzyme on modified M9 broth media. The enzyme activities were 4.497 U/ml±0.009 and 4.238 U/ml±0.007, while the specific activities were found to be 6.424 U/ml and 8.476 U/ml for *P. carotovorum* and *S. marcescens,* respectively.

Effect of incubation temperature on L-asparaginase production

The bacterial isolates were incubated at four different temperatures (25, 30, 35, and 40°C). The results showed thst the best incubation temperature was 35°C for *P. carotovorum* and 30°C for *S. marcescens* which gave activity 4.494 U/ml±0.09 and 4.503 U/ml±0.09, respectively, as shown in Fig. 2. Similar temperature range was reported by previous studies carried out by Jha *et al*. [28] who reported that 37°C was the optimal temperature for maximum activity by *Pseudomonas fluorescens.* Prakasham *et al*. [29] and Ghosh *et al*. [30] produced L-asparaginase at 30°C by *S. marcerens* and *Staphylococcus* sp., respectively, Similarly, Manna *et al*. [31] isolated L-asparaginase from *Pseudomonas stutzeri* under the same temperature. Temperature is a critical factor which has to be controlled and has to be optimized. Previous studies have validated that the enzyme metabolism of microorganisms represented an important pathway for survival and in turn depends on the incubation temperature [32].

Effect of initial pH on L-asparaginase production

Four initial medium pH values (6.5, 7.0, 7.5, and 8.0) were used to increase the L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates. The results indicated that the *P. carotovorum* and *S. marcescens* isolates preferred the neutral pH value 7 and 7.5 and gave an activity of 4.497 U/ml±0.08 and 4.443 U/ml±0.04, respectively (Fig. 3). This result is similar to that obtained by Narayana *et al*. [33] for maximum L-asparaginase production from *S. albidoflavus* at pH 7.0. Kumar *et al.* [34] also reported maximum L-asparaginase production from *S. marcescens* at pH 7.5. On the other hand, Akilandeswari *et al*. [35] and Neelima *et al.* [36] found that the maximum L-asparaginase production from *Streptomyces ginsengisoli* was at pH 9.0.

Effect of incubation period on L-asparaginase production

The bacterial isolates were incubated at four different periods (24, 48, 72, and 120 h). The results showed that the best incubation period was 48 h for both *P. carotovorum* and *S. marcescens* which gave activity 4.44 U/ml±0.05 and 4.49 U/ml±0.04, respectively (Fig. 4). These findings were comparable to that described by Kumar *et al.* [34] who reported that the maximum L-asparaginase activity was observed at 48 h. After 48 h, it was decreased due to the depletion of nutrient materials.

Effect of carbon sources on L-asparaginase production

Four carbon sources, namely glucose, sucrose, maltose, and lactose, were applied to enhance the L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates. The results concluded that the lactose was the best carbon source for L-asparaginase production by *P. carotovorum* (4.802 U/ml±0.03), while the sucrose sugar was the best one for *S. marcescens* (5.00 U/ml±0.06) (Fig. 5). Stanbury *et al*. [37]

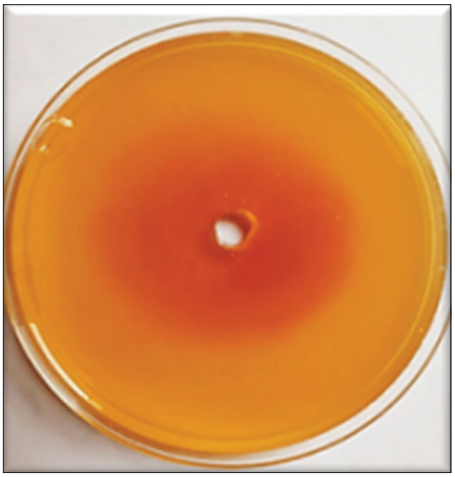

Fig. 1: Field study-4 isolate showed highest L-asparaginase production

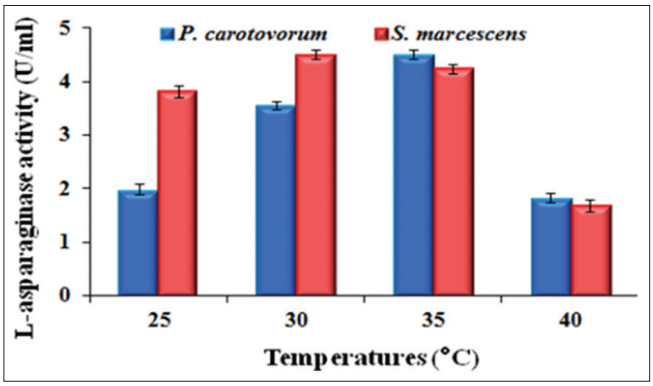

Fig. 2: L-asparaginase production by *Pectobacterium carotovorum* and *Serratia marcescens* isolates under different incubation temperatures

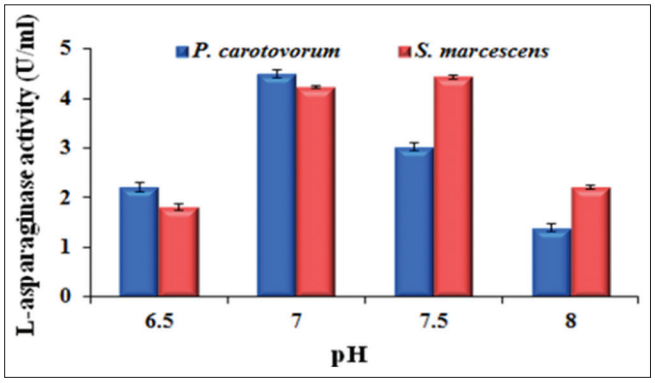

Fig. 3: L-asparaginase production by *Pectobacterium carotovorum* and *Serratia marcescens* isolates under different pH values

stated that carbohydrates are used as carbon sources in the microbial fermentation processes. Kumar *et al*. [34] observed higher titers of L-asparaginase by *S. marcescens* when the medium was supplemented with 1.5% sucrose. Susmita and Mandal [38] reported sucrose as the best carbon source for L-asparaginase production. This may be due to the inductive effect of sucrose and its efficiency in asparaginase production, and it also helps in stabilizing the enzyme [39]. In contrary, El Shobaky *et al*. [40] indicated that the maximum L-asparaginase production was recorded in the presence of lactose as carbon source followed by maltose and fructose than sucrose. Lactose was reported as the best carbon source under aerobic conditions for the synthesis of L-asparaginase by *Staphylococcus* sp. [29]. However, Varalakshmi [41] reported a decline in L-asparaginase production on supplementation with fructose and a highest L-asparaginase activity by *Staphylococcus* sp. when supplemented with maltose.

Effect of nitrogen sources on L-asparaginase production

In this study, asparagine as the main nitrogen source was changed by yeast extract, ammonium nitrate, and peptone to enhance the L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates. The results showed that the best nitrogen source was ammonium nitrate for *P. carotovorum* (4.839 U/ml±0.03) and asparagine for *S. marcescens* (4.238 U/ml±0.03) (Fig. 6). Our results agreed with El Shobaky *et al*. [40] who showed that ammonium nitrate was the most suitable nitrogen source for optimum L-asparaginase production followed by urea and sodium nitrate. Maximal production of L-asparaginase varied by varying the nitrogen source. Athira *et al*. [42] indicated sodium nitrate as best nitrogen source. However, Narayana *et al*. [33] indicated yeast extract to be best nitrogen source and Neelima *et al*. [36] used peptone as the best nitrogen source.

Production of L-asparaginase by *P. carotovorum* and *S. marcescens* isolates under optimum conditions

Optimization of conditions yields an increase in the production of L-asparaginase enzyme of 7.5% (from 4.497 U/ml to 4.835 U/ml) by *P. carotovorum* and 23% (from 4.238 U/ml to 5.221 U/ml) by *S. marcescens,* and the specific activity was reached to 6.9 and 10.4 U/ml with *P. carotovorum* and *S. marcescens*, respectively (Fig. 7).

Isolation and purification of L-asparaginase enzyme

L-asparaginase enzyme produced by *P. carotovorum* and *S. marcescens* isolates under optimum conditions was isolated from free cells supernatants by precipitation. After purification, the molecular weight and purity of the L-asparaginase enzyme was assessed by running through electrophoresis SDS-PAGE. The purified L-asparaginase enzyme had a molecular weight of 35 kDa for *P. carotovorum* and 36 kDa for *S. marcescens* isolates (Fig. 8). L-asparaginase from different sources showed close molecular weights: Mohamed [43] reported that the molecular weight of L-asparaginase from *E. coli* was 33 kDa. Prista and Kyridio [44] indicated that *E. coli* L-asparaginase had molecular weight value 33 kDa. On the other hand, Jain *et al*. [26] found that the molecular weight of purified L-asparaginase obtained from *E. coli* VRY-15 was 56 KDa which was different from that of commercially available L-asparaginase having a molecular weight of 31.73 KDa.

***In vitro* cytotoxicity assay of *P. carotovorum* and *S. marcescens* produced L-asparaginase on *MCF-7*, *HepGII*, and *WISH* cell lines**

Cell viability determined by MTT assay

Cell viability results of L-asparaginase enzyme obtained from *P. carotovorum and S. marcescens* isolates are summarized in Tables 1 and 2. Cell viability of *WISH* cell line treated with L-asparaginase extracted from both isolates increased by the increase of enzyme concentration. No cytotoxic effect was observed for normal epithelial *WISH* cell line when treated with purified L-asparaginase. Bhat and Marar [45] reported that the purified enzyme did not exhibit any effect on normal human lymphocytes, implying that it may prove to be a novel source for L-asparaginase isolated for chemotherapeutic purpose.

The incubation of *MCF-7* cell line with the increasing concentration of L-asparaginase enzyme caused a gradual inhibition of cell growth as observed from its low IC_{50} value 15 µg/ml and 26 µg/ml with L-asparaginase from *P. carotovorum* and *S. marcescens*, respectively. However, the L-asparaginase purified from *S. marcescens* isolate showed better toxicity on *HepGII* cell line (7.53% survival) in comparison to L-asparaginase produced by *P. carotovorum* isolate (29.81% survival). The sensitivity of the cell line to both L-asparaginases (purified from both the isolates) appeared to be dose dependent, resulting in the significant decrease in viable cells.

Similarly, it was reported that the purified L-asparaginase from *Erwinia caratovora* significantly increased the number of apoptotic cells to 40% (*Jurkat cells*) and 99% (*HL60 cells*), suggesting that the enzyme

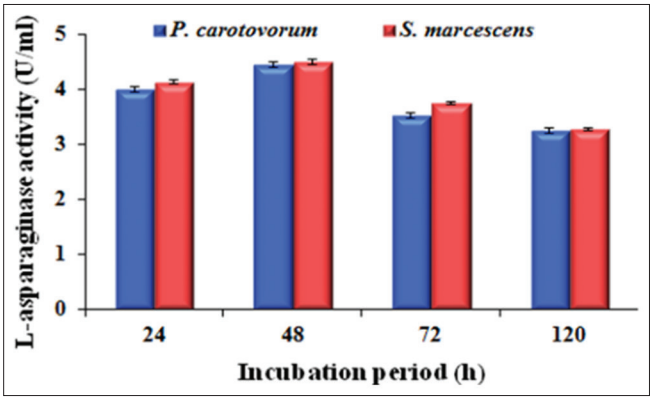

Fig. 4: L-asparaginase production by *Pectobacterium carotovorum* and *Serratia marcescens* isolates under different incubation periods

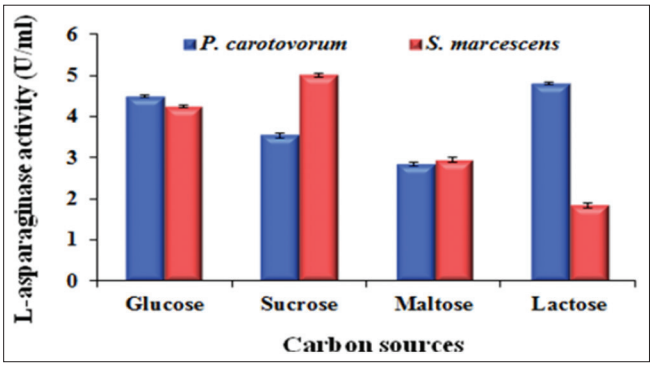

Fig. 5: L-asparaginase production by *Pectobacterium carotovorum* and *Serratia marcescens* strains under different carbon sources

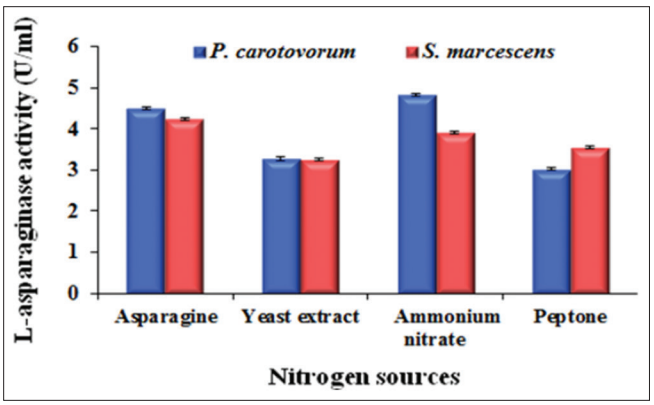

Fig. 6: L-asparaginase production by *Pectobacterium carotovorum* and *Serratia marcescens* isolates under different nitrogen sources

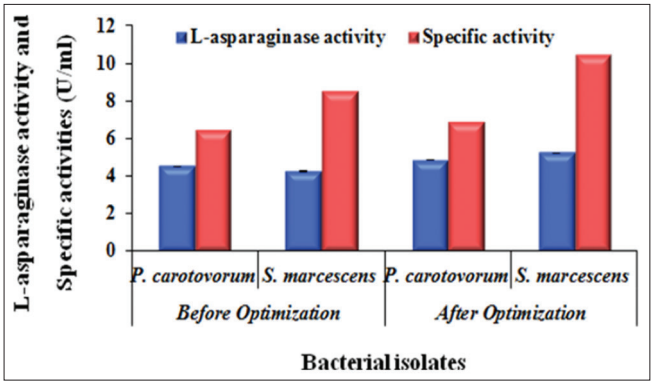

Fig. 7: L-asparaginase activity and specific activities (U/ml) produced by *Pectobacterium carotovorum* and *Serratia marcescens* isolates before and after optimization

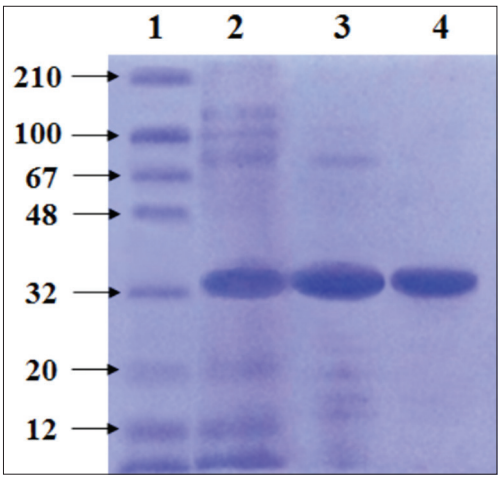

Fig. 8: Sodium dodecyl sulfate polyacrylamide gel electrophoreses of purified L-asparaginase enzyme from *Serratia marcescens* isolate. Lane 1: Protein marker (10–260 KDa, Thermo Fischer Scientific), 2: Ammonium sulfate precipitation, 3: Diethylamino ethyl cellulose purification, and 4: Sephadex purification

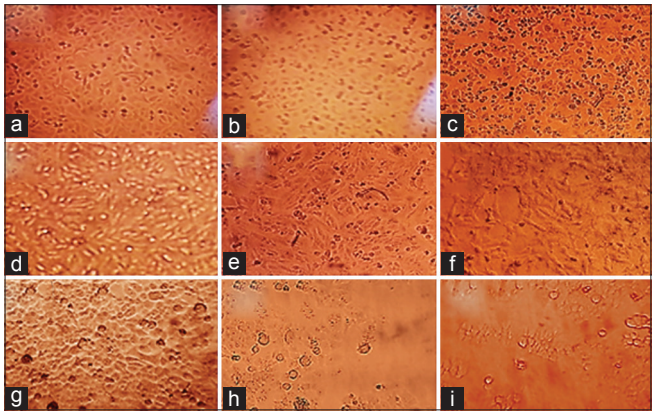

Fig. 9: Photomicrographs of morphological changes for human breast adenocarcinoma (*MCF-7*), human hepatocellular carcinoma (*HepGII*), homo sapiens human cells in response to L-asparaginase extracted from *Serratia marcescens* and *Pectobacterium carotovorum* at ×40 10 magnification microscopy. (a, d, and g) *MCF-7*, *HepGII*, and *WISH* cells (control); and (b, e, and H): *MCF-7*, *HepGII*, and *WISH* cells treated with L-asparaginase from *S. marcescens*, respectively. (c, f, and i) *MCF-7*, *HepGII*, and *WISH* cells treated with L-asparaginase from *P. carotovorum*, respectively

Table 1: Efficacy of *P. carotovorum* L-asparaginase on cell viability relative to its concentration

Concentration (µg/ml)	% of cell viability after the application of *P. carotovorum* L-asparaginase		
	MCF-7	*HepGII*	*WISH*
0	100±0.008	100±0.002	100±0.002
6.25	82.253±0.005	58.201±0.030	100.301±0.001
12.5	55.310±0.009	56.790±0.007	98.979±0.002
25	32.124±0.005	51.146±0.025	96.396±0.004
50	31.735±0.001	32.275±0.001	90.090±0.001
100	26.683±0.004	29.806±0.001	80.829±0.005
IC_{50}	15 µg/ml	26 µg/ml	100 µg/ml

MCF-7: Human breast adenocarcinoma, *HepGII*: Human hepatocellular carcinoma, *WISH*: Homo sapiens human, *P. carotovorum: Pectobacterium carotovorum*, IC_{50}: Half maximal inhibitory concentration

Table 2: Efficacy of *S. marcescens* L-asparaginase on cell viability relative to its concentration

Concentration (µg/ml)	% of cell viability after application of *S. marcescens* L-asparaginase		
	MCF-7	*HepGII*	*WISH*
0	100±0.124	100±0.284	100±0.002
6.25	81.122±0.077	80.607±0.189	100.541±0.002
12.5	59.777±0.099	60.846±0.068	99.879±0.006
25	50.805±0.105	49.329±0.074	93.213±0.003
50	45.372±0.058	38.993±0.094	81.802±0.015
100	46.135±0.037	7.532±0.066	64.865±0.004
IC_{50}	26 µg/ml	25 µg/ml	100 µg/ml

S. marcescens: Serratia marcescens, *MCF-7*: Human breast adenocarcinoma, *HepGII*: Human hepatocellular carcinoma, *WISH*: Homo sapiens human

cytotoxicity is associated with only L-asparagine deficiency [46]. L-asparaginase enzyme was tested against three different cell lines for its anticancerous activity, human cancer colon *Caco-2*, human breast cancer *MCF-7,* and human cancer prostate *pc-3*. The enzyme showed anticancer activity against *MCF-7*and *pc-3*, where it was non-effective to the cell line *Caco-2* [43].

Morphological changes of *MCF-7*, *HepGII*, and *WISH* cell lines induced by L-asparaginase

The effect of L-asparaginase extracted from *S. marcescens and P. carotovorum* was evaluated on *MCF-7, HepGII,* and *WISH* cell morphology using the calculated IC_{50} values previously reported. The morphological changes were observed using inverted phase-contrast microscopy. The control *MCF-7, HepGII,* and *WISH* cells (treated only with DMSO) showed the normal morphology of cobblestone-like appearance with strong cell-cell adhesion, monotonous spindle-shaped cells containing single round nuclei with flattened cytoplasm, and epithelioid amnion cells that grow in a closely apposed monolayer, respectively (Fig. 9a,d and g).

Results demonstrated that both *MCF-7 and HepGII* cells treated with L-asparaginase extracted from *S. marcescens* and *P. carotovorum* showed shrinkages, dispersing, and irregularity in shape, rounding cells, and complete detachments of cells from the surface and loss of cytoplasmic vacuole (Fig. 9b,c,e and f), while *WISH* cell have no morphological changes induced by L-asparaginase extracted from both *P. carotovorum* and *S. marcescens* isolates (Fig. 9h and i).

AUTHORS' CONTRIBUTIONS

The majority of the experimental work was performed by Noha E. Abdel-Razik. All authors contributed equally to conduct this work, idea, protocol, consultancy, writing, and review the article.

CONFLICTS OF INTEREST

All authors have none to declare.

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