Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(3):2802-2812. **OPEN ACCESS**

Isolation, screening and optimization of L-asparaginase producing bacterial strains inhabiting agricultural soils

Osama M. Darwesh, Mohamed F. Eida and Ibrahim A. Matter*

Agricultural Microbiology Department, National Research Centre, Cairo**, Egypt**

***Correspondence:** ibrahimmatter@gmail.com **Accepted: 18** Aug. 2018 **Published online:** 30 Sep. 2018

This study aimed at isolation, screening and characterization of indigenous L-asparaginase producing bacteria from Egyptian agricultural soils. Several soil bacterial isolates were screened, and plate assayed for their abilities to produce, biotechnological and environmental important enzyme, L-asparaginase. Nine isolates belong to seven genera were proven to produce L-asparaginase *i.e. Escherichia* (FS-2), *Pectobacterium* (FS-4)*, Pseudomonas* (FS-6 and GS-2), *Bacillus* (FS-7 and GS-4), *Aeromonas* (GS-3), *Proteus* (GS-5) and *Serratia* (GS-7). The most potent L-asparaginase producing isolates, *Pectobacterium* (FS-4) and *Serratia* (GS-7), were identified as *P. carotovorum* and *S. marcescens* following biochemical (BIOLOG) and molecular based methods with GeneBank accession numbers of MG808385 and MG808384, respectively. The optimization of enzyme productivity results under submerged fermentation conditions indicated that, lactose and ammonium nitrate as C and N sources, pH 7 and incubating temperature at 35 ºC were the optimum for *P. carotovorum*. Furthermore, *S. marcescens* was optimized utilizing sucrose and asparagine as C and N sources, respectively, pH 7.5 and 30 ºC for incubation. Optimized growth parameters improved L–asparaginase activities by 7.51% and 23.19% for *P. carotovorum* and *S. marcescens*, respectively. Partially purified L–asparaginase produced from *P. carotovorum* and *S. marcescens* at optimized conditions displayed molecular weights of approximately 34 and 40 kDa, respectively. Many environmental, medical and industrial bioprocesses could benefits from the isolated L–asparaginase producing bacteria as well as the purified enzyme.

Keywords: L-asparaginase, *P. carotovorum*, *S. marcescens*, isolation and optimization, soil bacteria

INTRODUCTION

Soil is considered as the richest microbial habitat on the Earth. The bacterial population surpasses all other microbial groups in soil ecosystem. The importance of soil bacteria relies on its vital roles in the environmental processes including N-fixation, nutrient cycling (Prashar et al., 2014; Eida et al., 2013), plant growth promoting, degradation of pollutants e.g. pesticides and toxic dyes (Abou-Shanab et al., 2012; Darwesh et al., 2014; Hoballah et al., 2014), oxidation and reduction, organic matter transformation (Eida et al. 2012) …*etc*. Bacteria play its vital role in soil through production and release of several important enzymes (Bhat et al., 2015; Eida et al., 2011). Additionally, soil bacteria have been focused for biotechnological and industrial applications according to its vast diversity and important functions. Based on above, soil microbes have been utilized for initiating bioprocesses for industrial and commercial activates (Nikel, 2016).

Microbial enzymes are well recognized as essential metabolic catalysts which utilized to enhance different environmental, medical and industrial bioprocesses (Darwesh et al., 2018;

Hasanin et al., 2018). The applications of enzymes extended to biofuels, animal feed and personal care, among others. The demand on industrial and pharmaceutical enzymes is continuously raised motivated by a mounting demand for sustainable and economically competitive solutions for specific problems (Demain and Adrio, 2008).

L-asparaginase (L-asparaginase, EC 3.5.1.1) is a critical enzyme of medical importance as an anti-cancer agent, as anti-microbial agent, treatment of infectious diseases, autoimmune diseases and many other medical relevance applications. A wide interest recently focused on asparaginase to be used in food industry to prevent or reduce the formation of the human carcinogenic acrylamide during heat treatments of starchy foods (Zuo et al., 2015; Cachumba et al., 2016). Currently, L-asparaginase represents a 40% of the overall global enzymes demand (Vimal and Kumar, 2017).

Asparaginase could be divided into two main groups, Microbial-type and plant-type (Moreno-Enríquez et al., 2012). Asparaginase types (periplasmic asparaginase, extracellular asparaginase, intracellular asparaginase, and glutaminase-asparaginase) were produced significantly, cost-effectively and eco-friendly by various microorganisms (Cedar and Schwartz, 1967; Hüser et al., 1999; Krishnapura et al., 2016). Numerous kinds of microorganisms are known to produce asparaginase including bacteria, actinomycetes, yeast and fungi. The major asparaginase producing bacteria include *E. coli*, *S. marcescens*, *E. aerogenes*, *Pseudomonas aeruginosa, B. circulans*, *and Pectobacterium carotovorum.* When the most important actinomycetes are *Streptomyces albidoflavus* and *Streptomyces gulbargensis*, the key producing fungi are *Fusarium*, *Aspergillus terreus*, *Aspergillus tamarii*, *Aspergillus niger*, *Penicillium* and *Cladosporium* sp. (Krishnapura et al., 2016).

Respecting to the pharmaceutical, environmental and industrial importance of L-asparaginase, the current study aimed at isolation and identification of asparaginase producing bacteria from Egyptian agricultural soils. Asparaginase production, characterization and optimization by the most potent bacterial isolates were also evaluated.

MATERIALS AND METHODS

Screening and isolation of L-asparaginase producing bacteria

For bacterial isolation, two rhizosphere soil samples (10-30 cm depth) were collected from the garden of National Research Centre (Garden Soil, GS) and from potato's farm (Farm Soil, FS) located in Giza governorate. The samples were collected under aseptic conditions in sterile plastic bags and immediately transferred to lab for bacterial isolation.

The 10-fold dilution method was followed for isolation of L-asparaginase producing bacteria on Modified Brain heart infusion agar medium (mBHI) containing (g/l): Brain extract, 7.8; Dextrose, 2.0; Asparagine, 5.0; Na_2HPO_4, 2.5; Heart extract, 9.7; Protease peptone, 10.0; KH_2PO_4, 3.0; NaCl, 5.0; $MgSO_2$, 2.0; $CaCl_2$, 1.0; Agar, 15 was utilized (Atlas, 2010). A 0.1 ml from 10^{-4} – 10^{-8} dilutions was spread on mBHI plates containing 5 µg / ml of nystatin as antifungal agent. The inoculated plates were incubated at 37 ˚C for 48 h. Developed bacterial colonies were picked up in new plates, purified and maintained on nutrient agar slants at 4˚ C and re-cultured periodically for further studies.

Qualitative and Semi-quantitative screening of L-asparaginase producing bacteria

All bacterial isolates were screened for L-asparaginase production ability using plate assay method according to Gulati et al. (1997) with slight modifications. Briefly; cultures of isolated bacteria (24 h old on nutrient agar) were inoculated onto the surface of mBHI agar plates supplemented with 0.09% phenol red (prepared in ethanol) as pH indicator. The plates were incubated at 37 ˚C for 48 h. Colonies surrounded with pink zones were considered as L-asparaginase producer and selected for further Simi-quantitative screening.

The semi-quantitative screening for L-asparaginase producing bacteria was preformed according to Kaur and Saxena (2014) following the agar well diffusion method with some modifications. In brief; the selected isolates were cultivated in asparagine brain heart infusion broth medium for 24 h. A 50 µl from each culture was pipetted into a well (5 mm in diameter) that centralized in asparagine brain heart infusion agar plates. All isolates were examined in triplicates. A control treatment received a 50 µl of un-inoculated media was running in parallel with inoculated plates. All plates (including control) were incubated at 37 ˚C for 24 h, and then observed for the formation of pink zone around the wells. The diameter of formed pink zone was recorded as indication for L-asparaginase production level.

The isolates with higher pink zone were selected for identification, characterization and optimization.

Identification of the most potent L-asparaginase producing bacterial isolates

Based on the previous screening, the most potent isolates were subjected to primary identification according to Bergey's Manual of Systematic Bacteriology (Holt et al., 1994). These organisms were identified on the basis of morphological and biochemical characters. Further biochemical characterization of the selected isolates was carried out using Biolog System and GEN III MicroPlate (Biolog, Inc., Hayward, USA) according to manufacturer manual.

The results of morphological and biochemical identification of selected isolates was confirmed through molecular and phylogenetic methods. The genomic DNA was extracted from the most potent L-asparaginase producing isolates and the 16S rRNA gene from each was amplified by PCR using a Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, CA, USA) as previously described (Barakat et al., 2015; Barakat et al., 2017). The PCR products were purified using a QIAquick PCR purification Kit (Qiagen, USA). The purified 16S rRNA fragments were analyzed by agarose gel electrophoresis and visualized using UV-transilluminator (Kheiralla et al., 2016).

Sequencing of the amplified 16S rRNA fragments were perform using a BigDyeR Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA, USA) on an Applied Biosystems 3730xl DNA Analyzer. Similarities of the bacterial nucleotide sequences with other known sequences were examined by comparisons with the National Center for Biotechnology Information (NCBI) database for reference and type strains using the BLASTN program (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree based on partial 16S rRNA sequences was constructed using the neighbor-joining method contained within the Clustal X program (Thompson et al., 1997) and MEGA6 software (Tamura et al., 2007). The obtained sequences were submitted to GenBank (NCBI).

L-asparaginase production by the most efficient isolates in submerged fermentation system

The most potent L-asparaginase producing isolates were grown under submerged fermentation condition for L-asparaginase production. From each isolate, a loopful was inoculated into 250 ml conical flask containing 50 ml of sterilized BHI-Broth with 1% asparagine at pH 7.0. Un-inoculated control flask was incubated under the same conditions with inoculated ones on an orbital incubator shaker at 37 ◦C for 24 h at 120 rpm. Bacterial cells were removed by centrifugation at 10.000 rpm for 10 min at 4 ◦C. Cells-free cultural supernatants were utilized to determine the total protein content as well as enzyme activity and specific enzyme activity.

L-asparaginase and protein activity assay

L-asparaginase activity was determined in the cultural supernatants of selected bacterial isolates according to the method described by Ren et al., (2010). Protein concentration was measured using the Lowry method with Bovine Serum Albumin as the standard (Lowry et al., 1951). One unit of L-asparaginase activity is defined as that amount of enzyme which catalyzes the formation of 1µm of ammonia per min under the conditions of the assay while specific activity of L-asparaginase is defined as the units of enzyme per milligram protein (Bansal et al., 2010; Yano et al., 2008). All assays were carried out in triplicates.

Optimization of Enzyme production

The optimum growth conditions for maximizing L-asparaginase production by the most potent producing isolates were determined in liquid cultures. The tested strains were inoculated into 250 ml Erlenmeyer conical flasks containing 100 ml of the production medium and incubated for 24 h. Cultivation was performed on different carbon (glucose, sucrose, maltose and lactose at 1% w/v) and nitrogen sources (Yeast extract, malt extract and ammonium nitrate at 1 % w/v) with different initial pH values (ranged from 6.5 to 8.0) and different incubation temperatures (ranged between 25 and 40 ◦C). After incubation, the cultures were centrifuged, and the supernatant was analyzed for L-asparaginase activity as described above.

Partial purification and characterization of bacterial L- asparaginase enzyme

After optimization of growth conditions, the most potent L-asparaginase producing isolates were cultivated on mBHI medium for 24 h at 120 rpm under their individual optimum enzyme production conditions (from the last experiment).

The culture filtrates (containing crude enzyme) from the two chosen bacterial isolates

were centrifuged at 8000 rpm for 10 min at 4 ºC to remove cells. Appropriate volume of crude enzyme sources (cultural supernatant) were mixed with four volumes of ice-cold acetone and incubated with constant stirring at 4 ºC. The precipitated proteins were centrifuged and air dried then dissolved in minimal volume of 50 mM Tris-HCl (pH 8.6). L-asparaginase activity and protein concentration were determined as previously mentioned while the molecular weight of the protein was determined by SDS-PAGE as described by Darwesh et al., (2015).

RESULTS AND DISCUSSION

Agricultural soil-inhabiting bacteria are recognized as noticeable option for recent biotechnological purposes according to their enormous applications (Daniel, 2004). L-asparaginase that improves the hydrolysis of amino acid L-asparagine to L-aspartate and ammonia is fit to a cluster of homologous amidohydrolases. This enzyme produced by some microbial sources such as bacteria. As anti-cancer and food additive, the current demand on L-asparaginase is vastly increased. This enzymatic approach for cancer therapy seems to be very promising since numerous enzymes have confirmed to be active against various types of cancers. This approach is one of the most eco-friendly treatments for cancer cells, especially hydrolyses enzymes such as L-asparaginase and L-glutaminase. The main objective of this study was to obtain L-asparaginase producing bacterial strains from Egyptian environment. Optimization of growth conditions for enzyme production from selected isolates were also considered in this work.

Isolation and primary screening of L-asparaginase producing bacteria

Two soil samples were collected from different rhizosphere habitats *i.e.* garden (GS) and farm soils (FS) and were used to isolate bacteria capable of producing L-asparaginase enzyme. The total bacterial count were 6 x 10^6 cfu/ml and 11 x 10^6 cfu/ml for FS and GS samples, respectively after sub-culturing on modified BHI agar media. From both soil samples, 9 morphologically different isolates were found to produce pink color around bacterial colonies indicating the ability to produce L-asparaginase. The variation in color intensity surrounding the colonies was noticeable among different isolates. Several microbial groups generally inhabit soil ecosystem. However, the predominance of the L-asparaginase producing bacteria in different soils was previously reported (Shukla et al., 2014; El-Naggar et al., 2015; Devi et al., 2016).

After purification of preliminarily selected isolates, the capability of each isolate to produce L-asparaginase was assayed semi-quantitatively. Plate diffusion agar method was followed to detect the ability bacteria to change cultural pH from acidic to alkaline due to degradation of asparagine. In the presence of phenol red as pH indicator, producing pink zones around the wells and their diameters were correlated to L-asparaginase activity (Fig. 1). The results indicated a higher activity for garden soil isolates (GS-2, GS-4 and GS-7) compared to farm soil isolates (FS-4, FS-6 and FS-7). Among the 9 positive L-asparaginase producing isolates, FS-4 and GS-7 displayed the highest activity expressed as larger pink zones (5.2 and 4.5 cm, respectively). However, GS-2, GS-4, FS-6 and FS-7 isolates showed moderate activity with pink zones diameter ranged from 4.0 and 3.4 cm, while the lowest activities were recorded for GS-3, FS-2 and GS-5 isolates (3.0, 2.6 and 2.3 cm, respectively). Figure (2) shows the pink zones that formed around the wells containing the most potent L-asparaginase producing isolates from each isolation source (FS-4 and GS-7). All isolated bacteria displayed a high asparaginase activity. Gulati et al., (1997) indicated the 0.9 cm as a diameter of hydrolysis zone surrounding L-asparaginase producing bacterial isolates. In addition, the activity zone of L-asparaginase plate assay was measured to range from 0.8-1.3 cm for soil bacterial isolates (Devi and Ramanjaneyulu, 2016). In accordance with our results, Wakil and Adelegan (2015) found that L-asparaginase hydrolysis zone recorded for soil bacteria ranged from 3-5 cm.

Morphological and Biochemical characteristics of L-asparaginase producing bacterial isolates

All L-asparaginase producing isolates were preliminary identified depending on their morphological and biochemical characteristics. The preliminary identification classifies the isolates as following: *Escherichia* (FS-2), *Pectobacterium* (FS-4)*, Pseudomonas* (FS-6 and GS-2), *Bacillus* (FS-7 and GS-4), *Aeromonas* (GS-3), *Proteus* (GS-5) and *Serratia* (GS-7).

The L-asparaginase has been known to be produced by several bacterial genera including *Escherichia*, *Pectobacterium, Pseudomonas*, *Bacillus*, *Aeromonas*, *Proteus* and *Serratia*

(Izadpanah et al., 2018; Bhagat et al., 2016; Cachumba et al., 2016 Kuwabara et al., 2015).

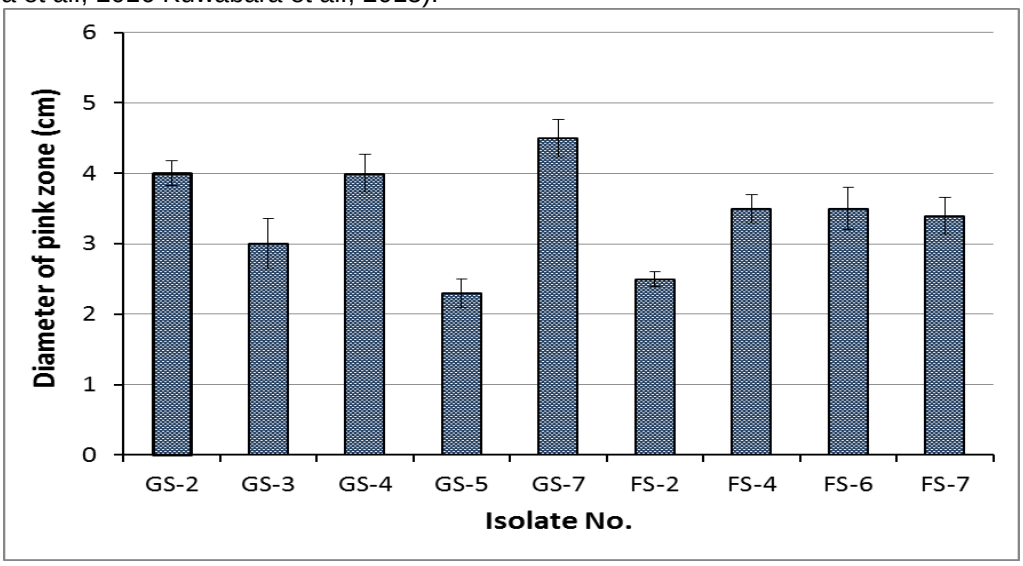

Figure (1). Screening the L-asparaginase production abilities of preliminary selected bacterial isolates. Column show the mean diameter of the pink color zone surrounding the wells containing cultural supernatant of selected isolates. The error bars represent ±sd.

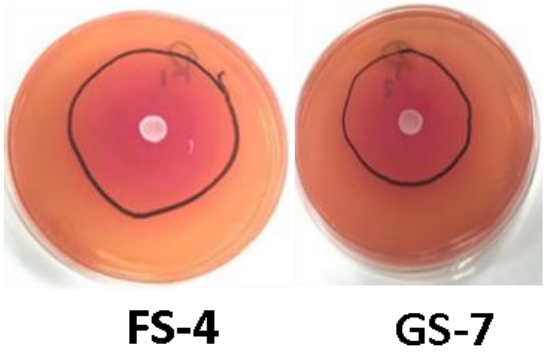

Figure (2). Zone of pink color performed around the wells containing cultural supernatant of selected isolates indicating their L-asparaginase production abilities.

Biochemical and molecular identification of the most potent L-asparaginase producing isolates

The most potent isolates that showed high L-asparaginase productivity (FS-4 and GS-7) were identified using the BIOLOG System. The MicroPlate "Metabolic finger print" displayed a high probability that FS-4 and GS-7 could be identified as *P. carotovorum* and *S. marcescens,* respectively, when compare the results with the Biolog MicroLog database. These results were confirmed using molecular characterization (16S rRNA gene analysis).

The results of NCBI GenBank BLASTn search as well as the phylogenetic tree (Fig. 3) created based on the similarity of partial sequences 16S rRNA genes indicated that the most effective L-asparaginase producing isolated could be identified same as formerly obtained via BIOLOG system. The nucleotide alignment and distance matrix showed high similarity value (99 %) with *P. carotovorum* and *S. marcescens* for FS-4 and GS-7, respectively. These sequences of the selected strains were deposited in the GeneBank under accession numbers MG808385 and MG808384.

As previously shown, the most potent L-asparaginase producing isolates were identified as *P. carotovorum* (FS-4) and *S. marcescens* (GS-7) by the mean of biochemical and molecular

based methods. These strains have been recognized as efficient L-asparaginase producers (Ghosh et al., 2013; Kumar et al., 2016).

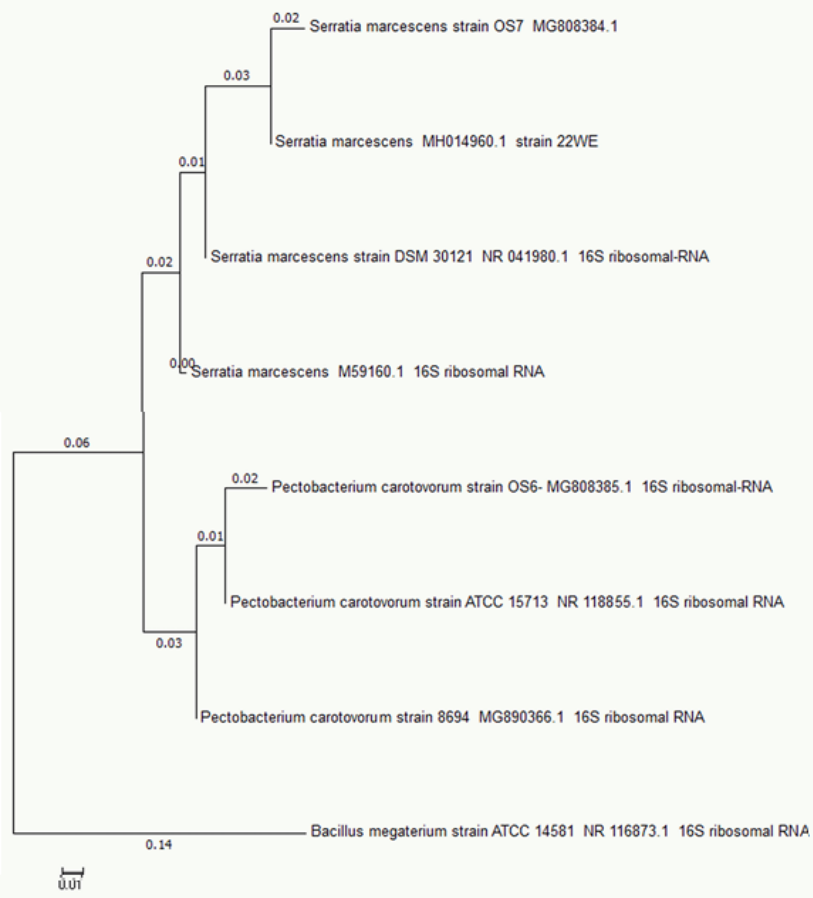

Figure (3). Phylogenetic tree based on the on the relationship among the 16S rRNA gene sequences of *P. carotovorum* OS6 and *S. marcescens* OS7 strains with sequences available in the GenBank. Scale bar represents the number of substitution per nucleotide position.

Optimization for L-asparaginase production by *P. carotovorum* and *S. marcescens* strains

Several cultivation conditions were adapted to enhance the microbial production of L-asparaginase by the selected bacterial isolates *i.e. P. carotovorum* and *S. marcescens*. These environmental conditions included; incubation temperatures, pH, carbon and nitrogen source.

Effects of incubation temperature and medium pH on L-asparaginase production

Temperature is one of the important process parameters for the enzyme production and plays an important role in microbial growth and production of microbial byproducts including enzyme. The effects of incubation temperatures on L-asparaginase production by *P. carotovorum* and *S. marcescens* strains were investigated. The bacterial strains were incubated at 4 different temperatures (25, 30, 35 and 40 ˚C). Results of L-asparaginase productivities were illustrated in Figure (4a). Although the finding of Kumar et al. (2009) that indicated that 40 ºC as optimal incubation temperature for producing L-asparaginase by *P. carotovorum*, 35 ˚C was recorded as the ideal temperature for recently isolated strain from Egyptian soil (4.494 Uml^{-1}). In accordance with our results, Ghosh et al., (2013) reported the 30º C as optimum temperature for L-asparaginase production by *S. marcescens* under current study condition (4.503 Uml^{-1}). Several reports recommended 30 to 37 ˚C as optimum temperature range for L-asparaginase production and it could be varied from one isolate to another (Erva et al., 2017; Ghosh et al., 2013; Kamble et al., 2006).

Consistent with Sanjay et al., (2017), pH value of culture medium plays an essential role for

improving L-asparaginase production efficacy. The influence of four initial medium pH values (6.5, 7.0, 7.5 and 8.0) on L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates were studied. According to our results both potent bacterial isolates produced higher L-asparaginase at neutral pH as indicated by the activity measured in culture media at different pH values. Even though the highest (2.95 Uml^{-1}) L-asparaginase production by *P. carotovorum* was displayed at pH 8-9 (Erva et al. 2017; Kumar et al., 2011), the local isolate produced a higher L-asparaginase (4.497 Uml^{-1}) at pH 7. On the other hand, L-asparaginase production by *S. marcescens* strain was peaked (4.443) at pH 7.5 in agreement with the results of Ghosh et al., (2013). The results exhibited that changing pH value from neutral zone (7-7.5) to acidity or alkalinity decreased L-asparaginase production by both selected isolates (Fig.4b).

Effect of carbon and nitrogen sources on L-asparaginase production

The nutritional requirements and culture conditions are critical for the biosynthesis of L-asparaginase and it varies from one microorganism to another. Four carbon sources namely; glucose, sucrose, maltose and lactose were applied to enhance L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates. In agreement with the results of Erva et al., (2017), lactose was the best carbon source for L-asparaginase production by *P. carotovorum* (4.802 Uml^{-1}) as shown in Figure (5a). Although Venil and Lakshmanaperumalsamy (2009) reported the higher production of L-asparaginase using galactose and lactose, the locally isolated *S. marcescens* strain sowed better performance (5.006 Uml^{-1}) utilizing sucrose as a carbon source (Fig.5a). Additionally, Venil et al., (2009) recommended sucrose as the most efficient carbon source for producing L-asparaginase by *S. marcescens*. They indicated that sucrose as an inexhaustible carbon source and its role in enzyme stabilization could explain its efficacy in L-asparaginase production.

Nitrogen is the second affecting nutritional factor on microbial growth, performance and enzyme production (Mc Tigue et al., 1994). Asparagine as the main nitrogen source was replaced by yeast extract, ammonium nitrate and malt extract to study the influence of nitrogen source on production of L-asparaginase by *P. carotovorum* and *S. marcescens* isolates. Results presented in Figure (5b) revealed that the best nitrogen source was ammonium nitrate for *P. carotovorum* (4.839 Uml^{-1}), while application of asparagine relatively enhanced L-asparaginase by *S. marcescens* (4.238 Uml^{-1}) under optimized conditions. The results indicated that influence of carbon and nitrogen source on L-asparaginase production is isolate-depended.

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

The most potent L-asparaginase producing bacterial isolates *P. carotovorum* and *S. marcescens*, were cultured on modified BHI liquid medium. Activities of produced L-asparaginase enzyme from normal and optimized cultivation conditions were evaluated. The Enzyme activities in pre-optimized cultivation conditions were 4.497 Uml^{-1} and 4.238 Uml^{-1} for *P. carotovorum* and *S. marcescens*, respectively. However, the specific activities were found to be 6.424 Uml^{-1} and 8.476 Uml^{-1} for *P. carotovorum* and *S. marcescens*, respectively (Fig. 6 a & b).

Optimization of culture conditions for L-asparaginase production both in batch and continuous fermentation is critical step to improve its productivity. Production of this enzyme depends on various parameters like concentration of carbon and nitrogen sources, pH of culture medium, temperature, fermentation time and oxygen transfer rate…*etc*. L-asparaginase enzyme was produced by *P. carotovorum* and *S. marcescens* isolates under optimum conditions for each bacterium.

The results showed variation in produced L-asparaginase at the optimum condition between the tested bacterial isolates compared to the original condition (Fig. 6 a). Results clearly showed increase in production of L-asparaginase by both *P. Carotovorum* (4.835 Uml^{-1}) and *S. marcescens* (5.221 Uml^{-1}) isolates and the specific activity was reached to 6.9 and 10.4 Uml^{-1} with *P. carotovorum* and *S. marcescens*, respectively. Optimization growth of growth parameters improved L–asparaginase activity by 7.51% and 23.19% for *P. carotovorum* and *S. marcescens*, respectively (Fig. 6 b).

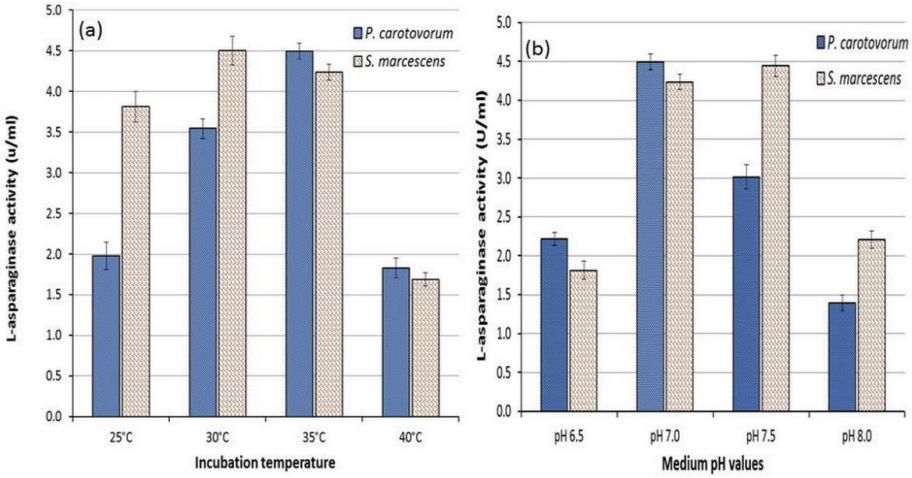

Figure (4). L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates under different incubation temperatures (a) and medium pH values (b). Error bars represent ±sd.

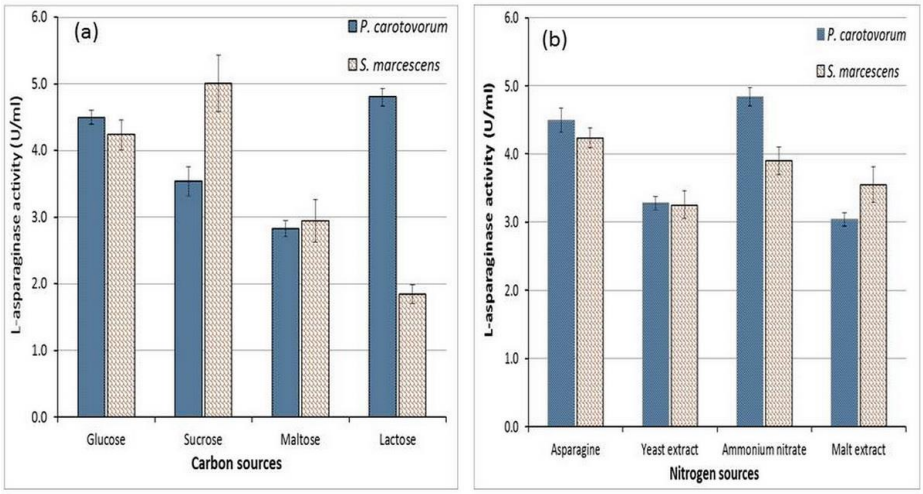

Figure (5). L-asparaginase production by *P. carotovorum* and *S. marcescens* isolates under different carbon (a) and nitrogen (b) sources. Error bars represent ±sd.

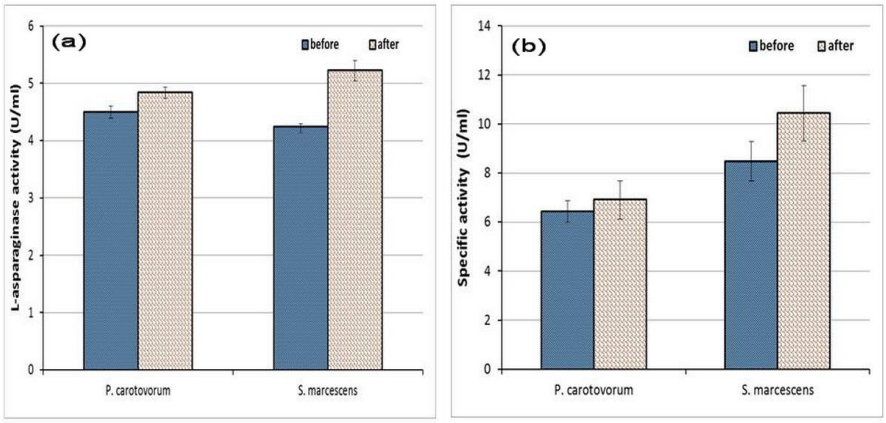

Figure (6). L-asparaginase activities (a) and specific activities (b) by *P. carotovorum* and *S. marcescens* before and after optimization. Error bars represent ±sd.

Characterization of L- asparaginase enzyme from *P. carotovorum* and *S. marcescens*

L-asparaginase purity and molecular weight was confirmed by the SDS–PAGE. The obtained results exhibited that the molecular weight of the partially purified L-asparaginase was approximately 34 and 40 kDa for *P. carotovorum* and *S. marcescens*, respectively. Purified L-asparaginase displayed molecular weight of 33.5 kDa for *Erwinia carotovora* (recently known as *P. carotovorum*) in agreement with the results of Kamble et al., (2006). Although the reports indicating higher molecular weight (171-180 kDa) for *S. marcescens* (Boyd and Phillips, 1971; Michalska et al., 2006), the present results reported that *P. carotovorum* and *S. marcescens* locally isolated strains from Egyptian soils produced L-asparaginase with relatively low molecular mass (~ 40 kDa).

CONCLUSION

The current study could conclude that L-asparaginase producing bacterial would be isolated from different agricultural rhizosphere soils. *P. carotovorum* and *S. marcescens* are considered as efficient L-asparaginase producing bacteria locally isolated from Egyptian agricultural soils. Optimization of cultural nutrients and growth condition exhibit that influence of pH, temperature, carbon and nitrogen source on L-asparaginase production were strain dependent. The L-asparaginase activity of both *P. Carotovorum* and *S. marcescens* strains reached 4.835 Uml^{-1} and 5.221 Uml^{-1} after optimization with average increase of 7.51% and 23.19% for *P. carotovorum* and *S. marcescens*, respectively. These isolates could be promising sources for microbial L-asparaginase production which needed in this regards.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors would like to express their thanks to the National Research Centre, Egypt for their support and funding this work.

AUTHOR CONTRIBUTIONS

All authors contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

REFERENCES

Abou-Shanab RAI, Khalafallah MA, Emam NF, Aly MA, Abou-Sdera SA, Matter IA, 2012. Characterisation and identification of carbofuran-utilising bacteria isolated from agricultural soil. Chem Ecol. 28(2): 193-203.

Atlas RM, 2010. Handbook of Microbiological Media. 4th ed. ISBN: 978-1-4398-0406-3, CRC press, Taylor & Francis group, 2035 p.

Bansal S, Gnaneswari D, Mishra P, Kundu B, (2010). Structural stability andfunctional analysis of L-asparaginase from *Pyrococcus furiosus*. Biochem. (Moscow). 75(3): 375-381.

Barakat KM, Hassan SW, Darwesh OM, 2017. Biosurfactant production by haloalkaliphilic *Bacillus* strains isolated from Red Sea, Egypt. Egypt J Aquat Res. 43: 205-211.

Barakat KM, Mattar MZ, Sabae SZ, Darwesh OM, Hassan SH, 2015. Production and Characterization of Bioactive Pyocyanin Pigment by Marine *Pseudomonas aeruginosa* OSh1. Res J Pharm Biolog Chem Sci. 6(5): 933-943.

Bhagat J, Kaur A, Chadha BS, 2016. Single step purification of asparaginase from endophytic bacteria *Pseudomonas oryzihabitans* exhibiting high potential to reduce acrylamide in processed potato chips. Food Bioprod Proc. 99: 222-230.

Bhat MR, Nair JS, Marar T, 2015. Isolation and identification of l-asparaginase producing *Salinicoccus* sp. M KJ997975 from soil microbial flora. Int J Pharm Sci Res. 6(8): 3599.

Boyd JW and Phillips AW, 1971. Purification and properties of L-asparaginase from Serratia marcescens. J Bacteriol. 106(2): 578-587.

Cachumba JJM, Antunes FAF, Peres GFD, Brumano LP, Santos JCD, Silva SSD, 2016. Current applications and different approaches for microbial l-asparaginase production. Braz J Microbiol. 47: 77-85.

Cedar H and Schwartz JH, 1967. Localization of the two L-asparaginases in anaerobically grown *Escherichia coli*. J Biol Chem. 242(16): 3753-3755.

Daniel, R. 2004. The soil metagenome–a rich resource for the discovery of novel natural products. Curr Opin Biotechnol. 15(3): 199-204.

Darwesh OM, Moawad H, Barakat OS, Abd El-Rahim WM, 2015. Bioremediation of textile reactive blue azo dye residues using nanobiotechnology approaches. Res J Pharm Biol Chem Sci. 6(1): 1202-1211.

Darwesh OM, Moawad H, Abd El-Rahim WM, Barakat OS, Sedik MZ, 2014. Bioremediation of textile Reactive Blue (RB) Azo Dye Residues in Wastewater using Experimental Prototype Bioreactor. Res J Pharm Biol Chem Sci. 5(4): 1203-1219.

Darwesh OM, Sultan YY, Seif MM, Marrez DA, 2018. Bio-evaluation of crustacean and fungal nano-chitosan for applying as food ingredient. Tox Rep. 5: 348–356.

Demain AL and Adrio JL, 2008. Contributions of microorganisms to industrial biology. Mol Biotechnol. 38(1): 41p.

Devi AL and 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.

Eida MF, Nagaoka T, Wasaki J, Kouno K, 2011. Evaluation of cellulolytic and hemicellulolytic abilities of fungi isolated from coffee residue and sawdust composts. Microb Environ. 26(3): 220-227.

Eida MF, Nagaoka T, Wasaki J, Kouno K, 2012. Isolation and characterization of cellulose-decomposing bacteria inhabiting sawdust and coffee residue composts. Microb Environ. 27(3): 226-233.

Eida MF, Nagaoka T, Wasaki J, Kouno K, 2013. Phytate degradation by fungi and bacteria that inhabit sawdust and coffee residue composts. Microb Environ. 28(1): 71-80.

El-Naggar NEA, Moawad H, El-Shweihy NM, El-Ewasy SM, 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 inter. 2015 (Article ID 627031): 17 pages. http://dx.doi.org/10.1155/2015/627031

Erva RR, Goswami AN, Suman P, Vedanabhatla R, Rajulapati SB, 2017. Optimization of L-asparaginase production from novel *Enterobacter* sp., by submerged fermentation using response surface methodology. Prep Biochem Biotechnol. 47(3): 219-228.

Ghosh S, Murthy S, Govindasamy S, Chandrasekaran M, 2013. Optimization of L-asparaginase production by *Serratia marcescens* (NCIM 2919) under solid state fermentation using coconut oil cake. Sustainable Chem Processes, 1(1): 9p.

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

Hasanin MS, Mostafa AM, Mwafy EA, Darwesh OM. 2018. Eco-friendly cellulose nano fibers via first reported Egyptian *Humicola fuscoatra* Egyptia X4: Isolation and characterization. Environ Nanotechnol Monit Manag. 10: 409-418.

Hoballah E, Saber M, Matter I, Zaghloul A, 2014. Bioremediation of aliphatic hydrocarbons in a sewaged soil by certain remediative amendments followed by phytoremediation. RJPBCS, 5(1):769-788.

Holt JG, Krieg NR, Sneath PA, Staley JT, Williams ST, 1994. Bergey's Manual of determinate bacteriology.

Hüser A, Klöppner U, Röhm KH, 1999. Cloning, sequence analysis, and expression of ansB from Pseudomonas fluorescens, encoding periplasmic lutaminase/asparaginase. FEMS Microbiol let. 178(2): 327-335.

Izadpanah F, Homaei A, Fernandes P, Javadpour S, 2018. Marine microbial L-asparaginase: Biochemistry, molecular approaches and applications in tumor therapy and in food industry. Microbiol Res. 208: 99-112.

Kamble VP, Rao RS, Borkar PS, 2006. Purification of L-asparaginase from a bacteria *Erwinia carotovora* and effect of a dihydropyrimidine derivative on some of its kinetic parameters. IJBB, 43(6): 391-394.

Kaur P and Saxena SG, 2014. Screening of Endophytic Fungi for Production of Asparaginase Enzyme, MSc thesis. Thapar University, Patiala, India.

Kheiralla ZH, Hewedy MA, Mohammed HR, Darwesh OM, 2016. Isolation of pigment producing actinomycetes from rhizosphere soil and application it in textiles dyeing. Res J Pharm Biol Chem Sci, 7(5): 2128- 2136.

Krishnapura PR, Belur PD, Subramanya S, 2016.

A critical review on properties and applications of microbial l-asparaginases. Crit Rev Microbiol. 42(5): 720-737.

Kumar S, Dasu VV, Pakshirajan K, 2011. Studies on pH and thermal stability of novel purified L-asparaginase from *Pectobacterium carotovorum* MTCC 1428. Microbiol. 80(3): 355-362.

Kuwabara, T., Prihanto, A.A., Wakayama, M. and Takagi, K., 2015. Purification and characterization of *Pseudomonas aeruginosa* PAO1 asparaginase. Proc Environ Sci. 28: 72-77.

Lowry OH, Rosebrough NJ, Farr AL, Randall R J, 1951. Protein measurement with the Folin phenol reagent. J Biol Chem. 193 (1): 265–75.

Mc Tigue MA, Kelly CT, Fogarty WM, Doyle EM, 1994. Production studies on the alkaline amylases of three alkalophilic *Bacillus* spp. Biotechnol lett. 16(6): 569-574.

Michalska K and Jaskolski M, 2006. Structural aspects of L-asparaginases, their friends and relations. ACTA Biochimica Polonica-English Edition, 53(4): 627.

Moreno-Enríquez A, Evangelista-Martínez Z, González-Mondragón EG, Calderón-Flores A, Arreguín R, Pérez-Rueda E, Huerta-Saquero A, 2012. Biochemical characterization of recombinant l-asparaginase (AnsA) from *Rhizobium etli*, a member of an increasing rhizobial-type family of l-asparaginases. J Microbiol Biotechnol. 22(3): 292-300.

Nikel PI, 2016. Systems and Synthetic Biology Approaches for Metabolic Engineering of *Pseudomonas putida.* In Microbial Models: From Environmental to Industrial Sustainability (pp. 3-22). Springer, Singapore.

Prashar P, Kapoor N, Sachdeva S, 2014. Rhizosphere: its structure, bacterial diversity and significance. Rev Environ Sci Bio/Technol. 13(1): 63-77.

Ren J, He F, Zhang L, 2010. The construction and application of a new PPY-MSPQC for l-asparaginase activity assay. Sensors and Actuators B: Chemical. 145(1): 272-277.

Sanjay K, Anand AP, Veeranki VD, Kannan P, 2017. Kinetics of growth on dual substrates, production of novel glutaminase-free L-asparaginase and substrates utilization by *Pectobacterium carotovorum* MTCC 1428 in a batch bioreactor. Korean J Chem Eng. 34(1): 118-126.

Shukla D, Shrivastav VK, Jana AM, Shrivastav A, 2014. Exploration of the potential L-asparaginase producing bacteria from the soil of Gwalior (India). Int J Curr Microbiol Appl Sci. 3: 665-672.

Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 24(8): 1596-1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25(24): 4876-4882.

Venil C and Lakshmanaperumalsamy P, 2009. Solid state fermentation for production of L-asparaginase in rice bran by Serratia marcescens SB08. Int J Microbiol. 7(1):1-6.

Venil CK, Nanthakumar K, Karthikeyan K, Lakshmanaperumalsamy P, 2009. Production of l-asparaginase by *Serratia marcescens* SB08: optimization by response surface methodology. Iran J Biotechnol. 7(1): 10-18.

Vimal A and Kumar A, 2017. Biotechnological production and practical application of L-asparaginase enzyme. Biotechnol Genet Eng Rev. 33(1): 40-61.

Wakil SS and Adelegan AA, 2015. Screening, production and optimization of L-asparaginase from soil bacteria isolated in Ibadan, South-western Nigeria. J Basic Appl Sci. 11: 39.

Yano S, Minato R, Thongsanit J, Tachiki T, Wakayama M, 2008. Overexpression of type I L-asparaginase of *Bacillus subtillis* in *Escherichia coli*, rapid purification and characterisation of recombinant type I L-asparaginase. Ann Microbiol. 58: 711-716.

Zuo S, Zhang T, Jiang B, Mu W, 2015. Recent research progress on microbial L-asparaginases. Appl Microbiol Biotechnol. 99(3): 1069-1079.