ISSN: 0975-833X

*Available online at http://www.journalcra.com*

*International Journal of Current Research*
*Vol. 11, Issue, 04, pp.3220-3226, April, 2019*

**DOI: https://doi.org/10.24941/ijcr.35148.04.2019**

**INTERNATIONAL JOURNAL OF CURRENT RESEARCH**

## RESEARCH ARTICLE

# PRODUCTION OF L- ASPARAGINASE AS ANTICANCER AGENT BY ALOMONASALKALIANTARCTICA ISOLATED FROM MARINE SAMPLES

**Al-Zahrani, S.H.M., Al-Zahrani, N.H., Alamodi, K.H. and Al-Refai, A.**

Department of Biology, Faculty of Science, University of Jeddah, KSA

**ARTICLE INFO**

***Article History:***
Received 20th January, 2019
Received in revised form
16th February, 2019
Accepted 24th March, 2019
Published online 30th April, 2019

***Key Words:***

Time Response, Health Service, Emergency Installation.

****Corresponding author:***

**ABSTRACT**

L-Asparaginase enzyme that proved clinically as a treatment of Acute Lymphoblastic Leukemia (ALL). This study focused on screening, optimization, and partialypurification of L-asparaginase. Six bacteriial strains isolated from six marine samples, screening was conducted on Glucose Asparagin agar m edium supplemented with L-asparagine and phenol red as an indicator dye pH7. Pink colour around the colony was a sign of L-asparaginase activity was screeened for L-asparaginase production isolates. Resulting from16S rDNA sequencing of highest L-asparaginase production isolate was identified as *Halomonasalkaliantarctica*. Then, it was optimized the effect of nutritionaland inoculum size parameters. *H. alkaliantarctica* recorded maximum L-asparaginase production (126.67U/min/ml) in medium supplemented with asparagine 0.1% and 1% tryptoone without carbon source at pH8 inoculated with 20% of (3.020×107CFU/ml) incubated for 48h,in shaking incubator (150rpm). The extractted enzyme of *H.alkaliantarctica* was partially purified using ammonium sulfate fractionation 80%.



***Citation: Al-Zahrani, S.H.M., Al-Zahrani, N.H., Alamodi, K.H. and Al-Refai, A.,* 2019.** "Production of l- asparaginase as anticancer agent by alomonasalkaliantarctica isolated from marine samples", *International Journal of Current Research*, 11, (04), 3220-3226.

## INTRODUCTION

Current cancer treatments causing side effects on the human in some instances. Extracted natural products from medicinal plants considered as a significance cancer treatment. The marine organisms able to produce novel chemicals against extreme variations which are unique in diversity, structural, and functional features (Kathiresan *et al*., 2008). L-asparaginase was known as L-asparagine amidohydrolase (EC. 3.5.1.1). L-asparaginase is useful antileukemia enzymme because of its ability in hydrolyzing L-asparagine into aspartic acid and ammonia (Dias and Sato, 2016). In the proliferation of leukemic cell L-asparagine is an important amino acid (Narta *et al*., 2007). Researchers mentioned that it can be isolated L-asparaginase producer from a wide diveersity of microbe especially bacteria which are most studied because of the high stability of isolation as claimed (Sanjeevir oyar *et al*., 2010). Halomonadaceae, *Halomonas* is one of the genera most frequently isolated from hypersaline waters and soils by conventional-culture techniques (Ventosa *et al.*, 2008; Kaye and Baross, 2000). The ecological role that *Halomonas* species play in these habitats and their relationships with other halophilic and non-halophilic microorganisms are still unknown. Most species of the genus *Halomonas* have been recognized as having potential applications in biotechnology due to their capacity to produce compatible solutes, enzymes or exopolysaccharides and for their role in the degradation of pollutants, such as several aromatic compounds (Ventosa, 2006; Rafael *et al*., 2011; Oren, 2010).

## MATERIALS AND METHHODS

**Samples:** Save marine samples were collected from the west coast of Saudi Arabia at red sea.At shore (*Nerita* sp.) and (*Atactodeaglabrate)*, from depth of 50 cm one species of brown algae (*Dictyota* sp.), one species of green algae (*Ulva lactuca*) and at the depth of 20 m, one species of solitary disc coral *(Ctenactiscrassa),* and two species of soft coral (*Sarcophton*sp, *Xenia umbellate)*.

**Isolation of L-asparaginase producing bacteria:** Suspensions were prepared by mixing 10 g of sediment samples into conical flasks containing 100 mL of steerile phosphate buffer, rotated at 50 rpm/min for 30 min, the suspending matter and the clear supernatant was decanted and serially diluted. Further, 100 UL of the different dilutions spread on glucose Asparagine solid medium (glucose 20g, asparagine 5g, KCl 0.5g, $K_2HPO_4$ 1.0g,$MgSO_4$. $7H_2O$ 0.5 and pH 7.0±0.2 in 1000ml sea water). The medium was supplemented with phenol red (2.5%): 0.04-0.36 mL indicator. The plates were then incubated at 35±2°C for 24-72h, to obtain colonies with pink zones around them (Gulati *et al.*, 1997).The more potent strains were selected for fermentation process.

**Preparation of inoculums:** The bacterial strain was inoculated in nutrient agar plates and incubated at 35±2°C for 24-48h. One or two colonies of bacterial growth were inoculated into 50 ml of nutrient broth in 250 ml flask and kept in a rotary shaker (150 rpm) at 35±2°C for 24h.

**Assay of L-asparaginase:** Assayed through Nesslerization method described by (Imada *et al.*, 1973). The measurements were by spectrophotometer at 450 nm. The reaction mixture was assayed in triplicates (Basha *et al.*, 2009).

**Molecular characterization of bacterial isolate:** Genomic DNA isolation using Gene JET Genomic DNA Purification Kit

1.Thermo Fisher Scientific.B acteria isolates identified by16S rDNA universal oligonucleotide primers designed by (Stravato and Cappelli, 2001). **PCR mixture**: 2µL DNA, 1µL of each primer, 12.5µL Master mix and 9.5µL sterile $dH_2O$. **PCR amplification conditions:** Initial: 5 min at 94 °C, Denaturation: 1 min at 94 °C, Annealing: 1 min at 55 °C and Extension: 2 min at 72 °C, Number of cycles: 35. Final extension: 10 min at 72 °C.

**Optimization of L-asparaginase production by**

***Halomonasalkaliantarctica* Effect of shaking incubation on L-asparaginaseproduction:** Two sets of cultures flasks; one was incubated in static incubation another on a rotary shaker 150rpm at 35±2˚C. After 48h of incubation cultures filtrates were collected and centrifuged at 4°C for 30 min at 10000 rpm. The supernatant used as a source of the crude enzyme.

**Carbon Sources.** To investigate the eject of carbon sources on L-asparaginase production by *Halomonasalkaliantarctica*, the carbon source(glucose) had been replaced by different carbon sources in AG broth medium such as lactose, sucrose, and glucose, each added at a concentration of 1% (w/v) (Amena*et al.* 2010; Narayana *et al*., 2008), keeping other components constant.

**The inoculum size was studied from the range of 10-40ml/100ml medium**

**Amino acids and other Nitrogen Sources**: Deferent amino acids, asparagine, arginine, glutamine, glycine and mithonine, and nitrogen source rather than amino acids, yeast extract, peptone, tryptone, were added at a concentration of 0.5 % (w/v) to the fermentation medium broth, keeping other ingredients constant (Amena *et al*. 2010). Inorganic nitrogen sources were not tried as it would interferein enzyme assay. In another study L-asparagine as the best amino acid, was added in different concentrations, 0.25, 0.5, 0.75 and 1.25% (w/v). Trypton was the best nitrogen source other than amino acids, was used in different concentrations varied from 0.25, 0.5, 0.75, 1.0 and 1.25% (w/v), and 0.1% (w/v) of L-asparagine was added. The effect of inoculum size on the production of L-asparaginase was studied, four different size (10 ml, 20ml, 30ml and 40ml/100ml medium) of inoculum was examined. After inoculation, the flasks were incubated at 35±2˚C on a shaking incubator (150rpm) and the Free-Cell Filtrate (FCF) assayed for ASNase accumulation after 72h.

## RESULTS

**Screening of L-asparaginaseproduction of isolated bacteria by plates:** The primary screening was carried out using phenol red as pH indicator. Colonies with pink zone were selected as asparagine degrading bacteria. After 72h of incubation at 35±2˚C. Six bacterial isolates of sex marine samples had a pink colour around its colonies, Isolates showed a great ability to change the color of the medium to pink in all incubation periods Table1 and Fig.1.

**Molecular identification of bacterial isolates based on 16S rDNA:** DNA sequences analyzed using Nucleotide BLAST alignment tools identified the highest L-asparaginase production isolate. It was identified as *Halomonasalkaliantarctica.* The sequence submitted into the Bacterial or Archaeal 16S ribosomal RNA sequences database under accession number MK072693.

**Optimization the production of L-asparagenase by *Halomonas alkali antarctica:*** The highest production of the enzyme 59.48 U/min/ ml, when the culture of *H. alkaliantarctica* was incubated in shaking incubator after 72h at 35±2ºC at 150rpm, while under static conditions it was 13.79 U/min/ ml. The isolate produced different amount of the enzyme in the presence of different carbon sources, but compared with control, the amount of the enzyme was higher in the presence of asparagine as sole organic source, 70.69 U/min/ml, Fig.3.Results in Fig.4 revealed that, presence of L-asparagine as the sole nitrogen and carbon source reveeled to the highest amount of the enzyme, 106.09 U/min/ml while glycine was the best second source, 102.16 U/min/ml compared with control. Further, it was equal in the presence of with or without methionine *by H.alkaliantarctic*. Increasing of L-asparagine as nitrogen source in the fermentation medium increased the production of ASNasein the fermentation medium, the highest amount of ASNase was 98.37 U/min/ml when 1.0% of L-asparagine was added after 48h of incubation Fig.5. Fermentation medium supplemented with different nitrogen sources rather than amino acids individually. Results in Fig.6 showed the highest L-asparaginase produced was 58.32 U/min/ml in the presence of tryptone as an organic nitrogen source. Results in Fig. 7showed that in the presence of 0.25% (w/v) tryptone mixed with 1.0% (w/v) asparagine maximized ASNase production to 120.66 U/min/ml compared with control. Incresing the inoculum size to 20/100ml medium (v/v) revealed to the highest ASNase production to126.67

U/min/ml. Fig. 8. Crude ASNase of *Halomonasalkaliantarctica* in cuture free cell filtrate was partially purified by ammonium sulfate. Partial ASNase was precipitated at80%by ammonium sulfate. Fig.9 showed that the maximum ASNase activity at pH 7 was148.28 U/min/ml. However, the lowest ASNase activity at pH 9 decreased to 55.77 U/min/ml. Fig.10showed the best activity of ASNase activity was 149.48 U/min/ml respectively at 35°C.

## DISCUSSION

About six bacterial isolates were obtained from six marine samples from the west coast in Jeddah city, Saudi Arabia, based on the formation of a pink zone around the bacteria as an indication of the ASNase production on the Glucose Asparagin agar (GA agar) plates, supplemented with phenol red (Asselin *et al*., 1993; Siddalingeshwara and Lingappa, 2010). The present study was to screen and evaluate ASNase activity of the selected bacteria that isolated from different marine


**Fig. 1. Screening of L-asparaginase production of 1SFC isolated bacterial by plates**

**Table 1. Screening of L-asparaginase production by isolated bacteria by plaates**

| Marine Samples | Sources of bacterial isolates | Bacterial isolates | Qualitative productionof L-asparaginaseafter | | |
|---|---|---|---|---|---|
| Soft coral | *Sarcophton* sp. | 1SFC | one day | two days | three days |
| | | | +++ | ++++ | ++++ |
| | Xenia umbellaate | 2SFC | _ | _ | _ |
| brown alga | *Dictyota* sp. | 1D | + | +++ | ++++ |
| green alga | *Ulva lactucaa* | 1U | ++ | +++ | ++++ |
| solitary disc corals | *Ctenactiscrassa* | 1CC | + | + | + |
| Nerite Snail | *Nerita* sp. | 1N | + | +++ | +++ |
| Bivalvia | *Atactodeaglabrrate* | 1A | + | ++++ | ++++ |

**Table 2. Identity percentage of 16S rDNA between the ASNase activity strains isolated from marine samples from different sites in the west coast of Jeddaah in Saudi Arabia and the relative strains in the gene bank.**

| Bacterial | Name and Accession of No. of the most related | | Identity | Coverage | Suggested Name ar |
|---|---|---|---|---|---|
| isolates | strain in NCBI GenBank. | | | | isolatess obta |
| Seq1 | MG456867.1 | *Halomonassalkaliantarcti ca* | 100% | 100% | MK072693 |

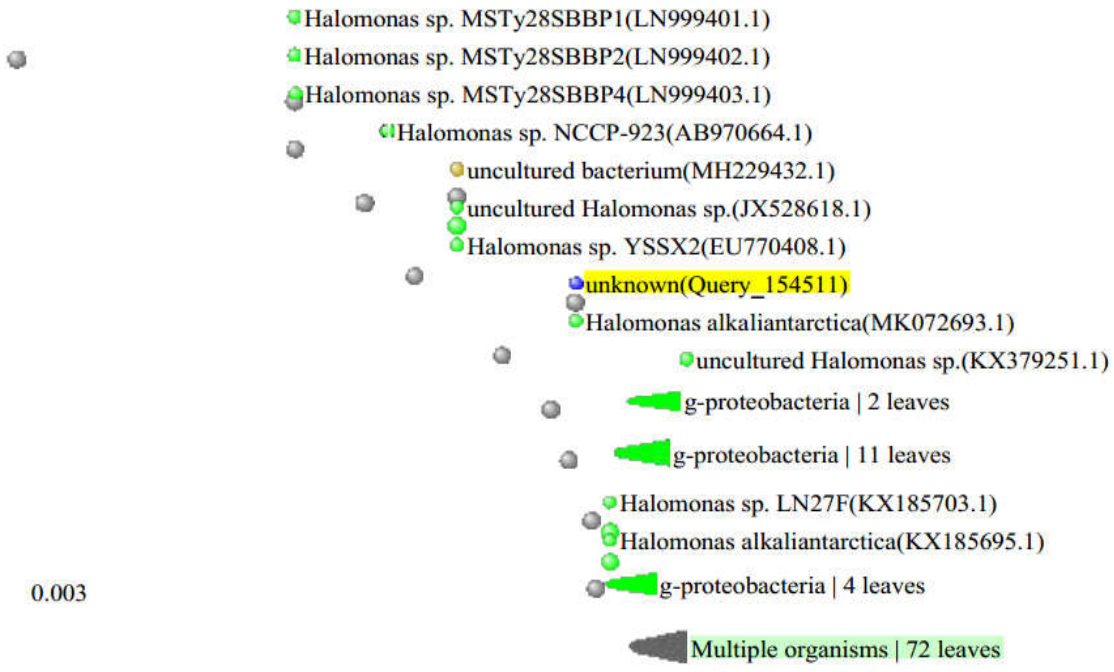


**Fig.2.Phylogenetic tree analysis of ASNase activity isolates based on 16S rDNA**

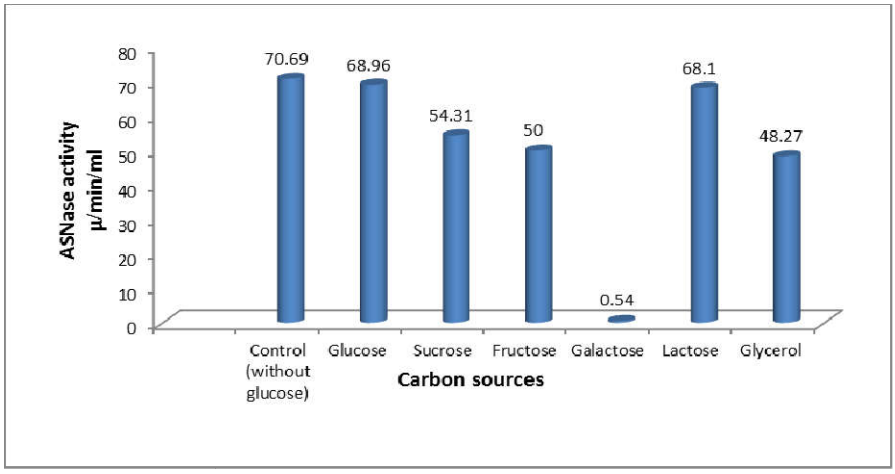


**Fig.3: Effect of different carbon sources onL-asparaginase by *Halomonasalkaliantarctica.***

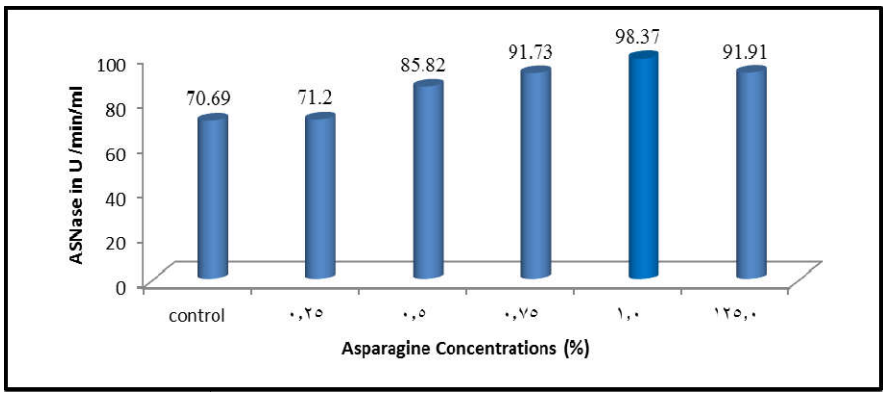


**Fig4: Effect of different amino acids on the production of L-asparagenase by *Halomonasalkaliantarctica***

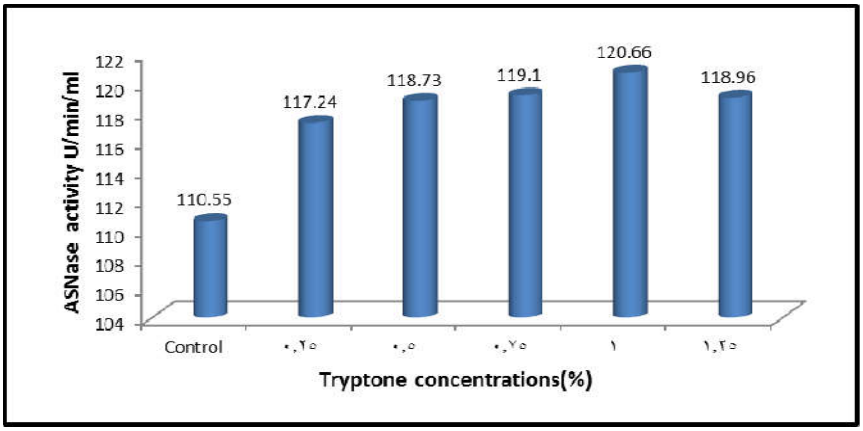


**Fig.5: Effect of different concentrations of L-asparagine on the production of L-asparagenase by *Halomonasalkaliantarctica*.**

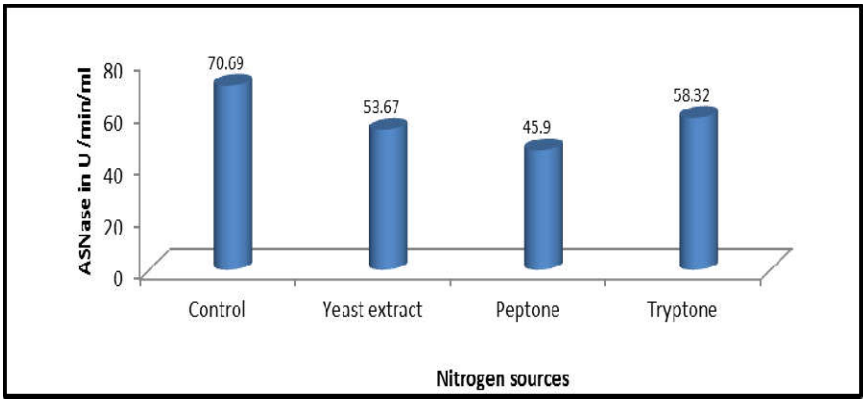


**Fig.6: Effect of different nitrogen sources on L-asparagenaseproduction by *Halomonasalkaliantarctica.***

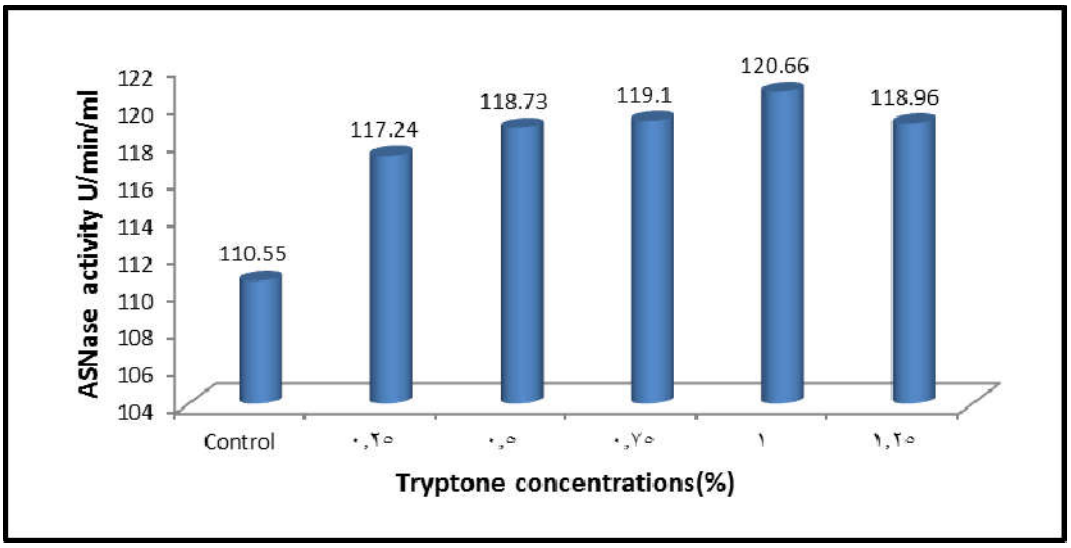


**Fig.7: Effect of different tryptone concentrations on L- asparaginaseproduction in presence of asparagine by *Halomonasalkaliantarctica.***

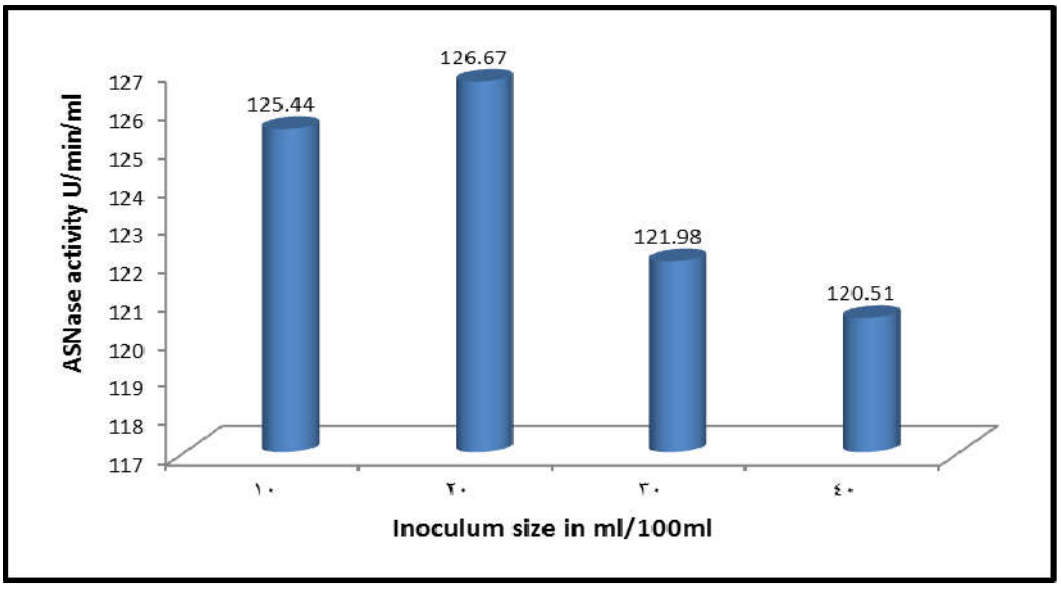


**Fig. 8. Effect of inoculum size on L-asparaginase activity by *Halomonasalkaliantarctica***

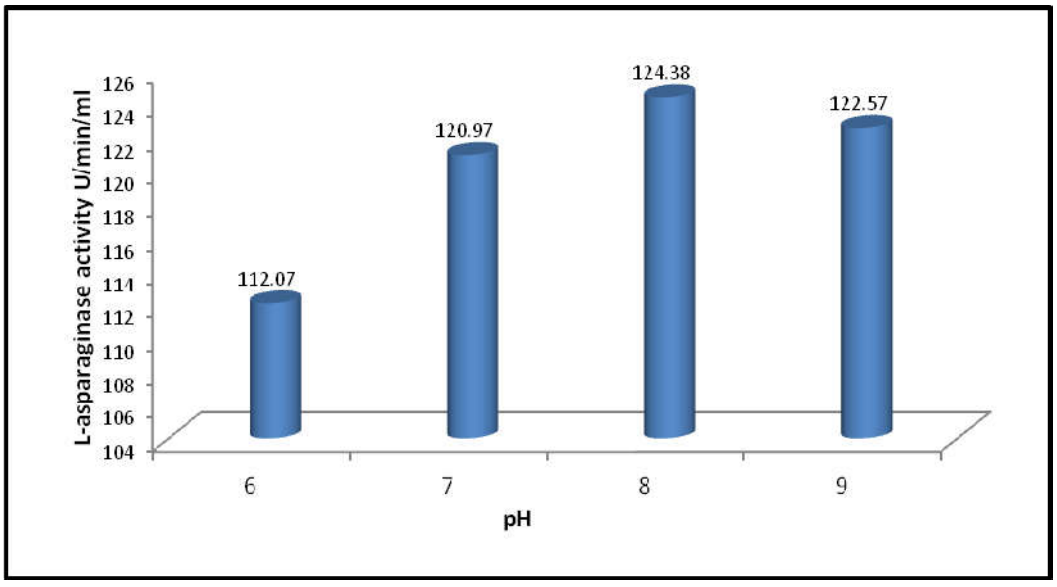


**Fig. 9: Effect of pH on enzyme activity by *Halomonasalkaliantarctica***

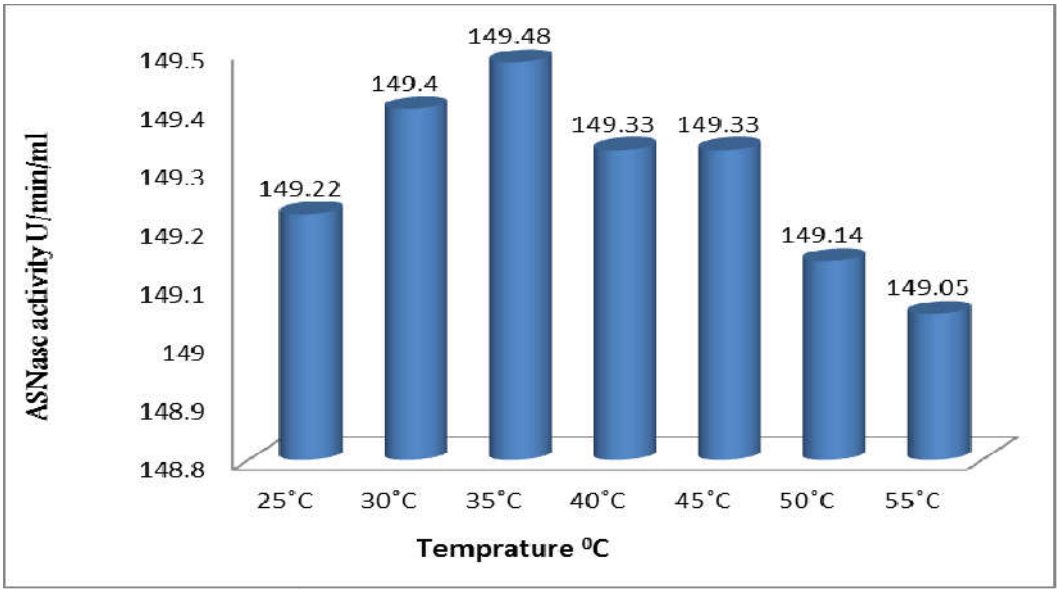


**Fig. 10: Effect of temperature on enzyme activity *Halomonasalkaliantarctica***

*Halomonasalkaliantarctica*. (Kalyanasundarram *et al*., 2015) reported, the presence of nitrogen sources along with nitrogenous compounds present in the substrate promotes enhanced growth and consequent enzyme production. Others reported yeast extract supported high yield of ASNase, while tryptone and yeast extract stimulated ASNase synthesis in *Erwiniacarotovora* (Maladkar *et al.,* 1993) and *Escherichia coli* (Kenari*et al*., 2011). This results showed a high level of ASNase production when tryptone at concentration (1%)followed by asparagine (1%). Similar, S harma and Husain (2015) found culture medium emended withh yeast extract has affirmative effect on enzyme production followed by tryptone by *Enterobacter cloacae*. The inoculum concentration results showed maximum production 126.67U/min//ml at20 ml/100ml inoculums *Halomonasalkaliantarctica*. Palllem *et al.* (2011) reported the maximum production of L-asaparaginase with inoculum volume of 1.5 ml of 7 days old *Fuusariumoxysporum* through SSF. L- asparaginase in culture freee-cell filtrate was partially purified by ammonium sulfate fr actionation. Crude enzyme was precipitated with 80% of ammmonium sulfate by *Halomonasalkaliantarctica*. Our result agre ed with that found by Ahmed *et al.* (2016). pH and temperature of enzyme incubation with substrate is one of the imporrtant factors which influences the enzymatic reaction rates.

The temperature optimum of ASNase from strains shown in at range of temperatures from 30–40°C. (Vidya *et al.,* 2011) reported the maximum activity of ASNase from *Saccharomyces cerevisiae* at 377°C.

## Conclusion

In conclusion, the marine isolate *Halomonasalkaliantarctica* was identified as a promising candidate for L-asparaginase production and it showed the enzyme activity maximized in nutrition medium supplementeed with 1.0% L-asparagine and 1.0 % tryptone without carbon source. It is highly recommended that prospects of this enzyme should be explored for its utility in pharrmaceutical as anticancer agent and food industry.

## REFERENCES

Ahmed, A. M. M., Abo-Dahab, N. F., Taha, T.M. and Hassan, F. S. M. 2016. Production, purification and characterization of L-Asparaginase from maarine endophytic *Aspergillus* sp. ALAA-2000 under submergged and solid state fermentation, *Journal of Microbial and Biochemical Technology*, 7(3): 165-172.

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

Asselin, B. L., Whitin, J. C., Coppola, D. J., Rupp, I. P., Sallan, S. E. and Cohen, H. J. 1993. Comparative pharmacokinetic studies of three asparaginase preparations, *Journal of Clinical Oncology*, 11(9): 1780-1786.

Basha, N. S., Rekha, R., Komala, M.and Ruby, S. 2009 Production of extracellular anti-leukaemic enzyme lasparaginase from marine actinomycetes by solid state and submerged fermentation: Purification and characterization, *Tropical Journal of Pharmaceutical Research*, 8(4): 353-360.

Baskar, G. and Renganathan, S. 2011. Optimization of media components and operating conditions for exogenous production of fungal L-asparaginase. *Chiang Mai Journal of Science,* 38(2): 270-279.

Deshpande, N., Choubey, P. and Agashe, M. 2014. Studies on optimization of growth parameters for L-asparaginase production by *Streptomyces ginsengisoli*, *The Scientific World Journal,* 2014:1-6.

Dias, F. F. and Sato, H. H. 2016. Sequential optimization strategy for maximum l-asparaginase production from Aspergillus oryzae CCT 3940. *Biocatalysis and Agricultural Biotechnology*, 6: 33-39.

Ellaiah, P., Adinarayana, K., Bhavani, Y., Padmaja, P. and Srinivasulu, B. 2002. Optimization of process parameters for glucoamylase production under solid state fermentation by a newly isolated *Aspergillus* species, *Process Biochemistry*, 38(4): 615-620.

Ghosh, S., Murthy, S., Govindasamy, S. and Chandrasekaran, M. 2013. Optimization of L-asparaginase production by *Serratia marcescens* (NCIM 2919) under solid state fermentation using coconut oil cake, *Sustainable Chemical Processes*, 1:1-8.

Gulati, R., Saxena, R. K. and Gupta, R. (1997) A rapid plate assay for screening l-asparaginase producing micro-organisms*, Letters in applied microbiology*, 24(1): 23-26.

Hosamani, R., & Kaliwal, B. 2011. L-asparaginase an anti-tumor agent production by Fusarium equiseti using solid state fermentation. *International Journal of Drug Discovery, 3*(2), 88-99.

Imada, A., Igarasi, S., Nakahama, K. and Isono, M. 1973. Asparaginase and glutaminase activities of micro-organisms, *Microbiology*, 76(1): 85-99.

Kalyanasundaram, I., Nagamuthu, J., Srinivasan, B., Pachayappan, A. and Muthukumarasamy, S. 2015. Production, purification and characterisation of extracellular L-asparaginase from salt marsh fungal endophytes, *World Journal Pharmacy and Pharmaceutical Sciences,* 4(3): 663-677.

Kathiresan, K., Nabeel, M. A. and Manivannan, S. (2008) Bioprospecting of marine organisms for novel bioactive compounds, *Scientific Transaction Environmental Technovation*, 1: 107-120.

Kaye, J. Z. and Baross, J. A. 2000. High incidence of halotolerant bacteria in Pacific hydrothermal-vent and pelagic environments, *FEMS Microbiology Ecology*, 32(3): 249-260.

Kenari, S. L. D., Alemzadeh, I. and Maghsodi, V. 2011. Production of l-asparaginase from *Escherichia coli* ATCC

*******