Extraction and purification of L-Asparaginase II from local isolate of *Proteus vulgaris*

Rawaa J. Toma *** ***Asmaa M. Suo'd * ***Shahlaa A. Hassan******
Methal A. Abd Aon ***Sarab K. Salman****

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Abstract:

Forty one isolates of genus *Proteus* were collected from 140 clinical specimens such as urine, stool, wound, burn, and ear swabs from patients of both sex. These isolates were identified to three *Proteus spp*. *P. mirabilis*, *P. vulgaris* and *P. penneri* .The ability of these bacteria to produce L-asparaginase II by using semi quantitative and quantitative methods was determined. *P. vulgaris* Pv.U.92 was distinguished for high level of L-asparaginase II production with specific activity 1.97 U/mg. Optimum conditions for enzyme production were determined; D medium with 0.3% of L-asparagine at pH 7.5 with temperature degree 35°C for incubation.

Ultrasonication was used to destroy the *P. vulgaris* Pv.U.92 cells then ASNase II was extracted and purified throughout several purification steps including precipitation with $(NH_4)_2SO_4$(60-80%), DEAE-cellulose ion exchanger chromatography followed by Sephacryl S-300 filtration. The specific activity was 155.6 U/ mg and the purification fold was 27.3 with 10.4% yield.

Key words: L-Asparaginase II; *P. vulgaris*; DEAE-cellulose; Sephacryl S-300.

Introduction:

L-Asparaginase (ASNase) is L-asparagine amido hydrolase enzyme, the systematic code is E.C.3.5.1.1, belongs to an amidase group that produces aspartic acid and ammonia by asparagine hydrolysis [1,2].

L-asparagine + H2O = L-aspartate + NH3

L-Asparaginase II (ASNase II) was one of the bacterial extracts that produced by many bacteria, by using the affinity chromatography to purification ASNase from the microorganism, found it's a tetramer with identical subunits[3]. ASNase II is high-affinity enzyme located in the periplasm, which is the space between the inner membrane (cytoplasmic) and outer membrane and only ASNase II[4].

Nearly all of the ASNases produced from the microorganism was the biologically active isozyme[5]. ASNase consider anti-neoplastic drug used in the treatment of cancer such as, lymphoblastic leukaemia chemotherapy [6], melanosarcoma cancers (skin cancers) that causes by *Epstein-Barr* virus [7], acute lymphocytic leukemia (mainly in children), and lymphosarcoma[8]. ASNase killed the leukemia cells by rapidly depleting L-asparagine, blocking protein synthesis and tumor cell proliferation, especially at G1 phase of the cell cycle [9].

Proteus especially *Proteus vulgaris* is another bacteria that produce ASNase aerobically with antitumor activity, with molecular weight of L-Asparaginase about 120.000 Dalton, the Km is 2.6 x 10-5M, the pI is 5.08 at optimum pH is 7-8[3].

* Ministry of Science and Technology.
** Baghdad University/ collage of Science/ department of Biotechnology.
*** Baghdad University/ collage of Science/ department of Biology

Since, there were no local studies on ASNase II production from *P.vulgaris*, thus this study was aimed to determined the qualified *Proteus* isolates for ASNase II production, study several environmental conditions that may affect production of ASNase II, and purification of ASNase II from selected isolate.

Materials and methods:

A- Enzyme activity: was measured by Indophenol methods [10].

B- Protein concentration: was measured according to Lowery method [11].

C- Media used for production ASNase II:

- **Medium A:** L-Asparagine broth (salt solution) and L-Asparagine agar[12].
- **Medium B (Luria broth)** [13]: it mixed with L-asparagine Composition per 100 mL from Peptone 1 gm, NaCl 0.1 gm, Yeast extract 0.5 gm, and L-asparagine 0.1 gm.
- **Media C** [13]: Composition per 100 mL from L-Asparagine 0.1 gm, Peptone 0.5 gm, Yeast extract 0.05 gm, and Glycerol 1 ml.
- **Media D** [14]: Composition per 100 ml (L-Asparagine 0.1 gm, Peptone 1 gm, and KH2PO4 0.34 gm).
- **Media E** [13]: it was prepared by dissolving 3 gm of nutrient broth, and 0.1 gm L-asparagine in 90 ml of DW, pH was adjusted to 7.0.

D- Purification of ASNase II: DEAE-cellulose was prepared according to Whitaker and Bernhard [15]. And Sephacryl S-300 column was prepared according to Pharmacia with dimensions 34.2 x 2.5 cm and the gel was equilibrated with 0.2 M PPB pH 8.

- **Determination of ASNase II-producing *Proteus* isolates:** after collected 140 clinical specimens and identified the Proteus spp. the ASNase II producing was determinate by two methods:

a- Semi-quantitative methods: activated isolates on brain heart infusion agar were transferred to the center of L-asparagine agar plate and incubated at 37ºC for 24-48 hr. The control plate was left without inoculation. Appearance of a purple zone around the colony was measured according to the equation below. Also activated isolates were transferred to L-asparagine broth, changing the color of medium to yellow represented a positive result and isolates were selected according to reduced pH [16,1].

Hydrolysis zone =Diameter of zone/diameter of bacterial colony

b- Quantity method (Indophenol Method): measurement of ASNase II activity [17] after activated isolates 0.2 ml of each broth culture growth isolate was transferred to salt L-asparagine medium without phenol red and incubated at 37ºC for 24hr. then participated by cold centrifuge at 4000 rpm for 30 min. the supernatant was discarded and the pellets were washed twice with 0.2 M PPB buffer pH 8. After that 0.2 ml of each suspension transferred to other new tube and added 0.05 ml of reaction solution of 0.1 M L-asparagine that currently preparation all tubes were incubated in a shaker water bath at 37ºC for 30 min, The reaction was stopped by adding 5 mL of **(A)** indicator and 5 mL of **(B)** indicator, with mixing and incubated in water bath at 37ºC for 20 min. and the O.D was measured at 625 nm, the activity of enzyme was calculated also protein concentration was estimation [11]

- **Determination of different conditions on ASNase II production**

1) Effect of different media: the activity of enzyme, and protein concentration were measured in each media.

2) Effect of different concentrations of L-asparagine: the production medium at different concentration of L-asparagine (0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 %) after that the activity of enzyme, and protein concentration were measured.

3) Effect of temperature: at different temperatures (20, 25, 30, 35, 40 and 45ºC) the crude enzyme was incubate after that the cells were precipitated and the activity of enzyme, protein concentration were measured.

4) Effect of pH: Selective medium was prepared at different pH values (6, 6.5, 7, 7.5, 8, 8.5, and 9), the activity of enzyme and protein concentration were measured.

- **Purification of ASNase II**

1) Extraction of enzyme from bacterial cells by Ultrasonication: at different times (2, 4, 8, and 16 min.), the activity of enzyme and protein concentration at each time were measured.

2) Precipitation of enzyme by ammonium sulfate: enzyme was precipitated with different concentration of (NH4)2SO4 (20, 30, 40, 50, 60, 70, 80, and 90%) saturation under cooled condition, at each concentration the activity of enzyme, protein concentration and specific activity were measured.

3) Ion exchange chromatography (DEAE-cellulose): The dialyzed enzyme was passed through DEAE-cellulose column, and washed with 0.005 M PPB buffer pH 8 after that the enzyme was eluted with 0.005 M PPB containing certain concentration of NaCl by stepwise at flow rate 0.05mL/8 sec. (22.2 mL/I hr)**.**

4) Gel filtration by Sephacryl S-300: The concentrated enzyme solution was applied to the column of Sephacryl S-300 at flow rate was 0.05ml/30 sec, enzyme activity was measured in the peak fractions and the active fractions were collected, volume, protein and enzyme activity were measured. Specific activity, yield %, and fold purification were calculated in each purification step.

Result and Discussion:

1- Screening for L-asparaginase II producing by Proteus spp.: by Semi-quantitative screening methods: it's simple and rapid assay method for the detection of ASNase from different strain of bacteria [16,1].

- **Screening on solid media:** Table (1) showed that *Proteus* species were able to produce ASNase II but with differences in their quantities, the ability of isolates to produce ASNase II was ranged between 4 to 27 mm. Notable *P. vulgaris* isolates were appeared to be the most efficient isolates to produce this enzyme (ASNase II).
- **Screening in broth media:** The ability of *Proteus* species to produce ASNase II in broth medium was determined according to the changing of the pH value (table 2). The average of final pH value was ranged from 6.9-8.6. Furthermore, it can be noticed that *P. vulgaris* isolates were the more efficient to produce ASNase II, followed by *P. mirabilis*. This result was corresponded to previous study that referred to the ability of Enterobacteriaceae for production ASNase II [18]. Because the *P. vulgaris* was more able to utilize the medium compound or they were had multiple *ansB* genes (that code for ASNase II production) in their genome to produce this enzyme in higher level [19] compared with other *Proteus* spp.. Therefore *Proteus spp.* isolates that recorded average pH value up to 8 were selected for quantitative detection of ASNase II production.

2- Quantitatively screening (Indophenol method) for ASNase II production: Table (3) indicated the quantitative activity of enzyme for 12 selected isolates of *Proteus spp*. It was revealed that isolate *P. vulgaris* Pv.U.92 had the highest activity of ASNase II. Thus *P. vulgaris* Pv.U.92 was selected for further study.

3- Effect of environmental factors on production of ASNase II by *P. vulgaris* Pv.U.92:

- **Effect of media compounds:** Figure (1) illustrated that D medium was the best one for production enzyme; this medium was enhanced enzyme production.
- Notable, El-Bessoumy and his team was recommended for using similar medium for enhances production of ASNase II by *Pseudomonas aeruginosa* 50071 bacteria [20]. Obviously, the type of carbon, nitrogen source as well as culture condition of media were affected the level of ASNase II production [21] by effected on microorganism growth or on enzyme united with substrate.

Table (1): ASNase II produced by *Proteus* species on solid medium

Proteus isolates	Diameter of asparagine hydrolysis zone (mm)	*Proteus* isolates	Diameter of asparagine hydrolysis zone (mm)
Pm.W.01	13	Pm.U.77	16
Pp.S.06	22.9	Pm.B.82	23
Pm.U.14	6	Pp.S.85	13
Pm.U.15	20	Pv.U.88	22
Pm.U.16	22	Pm.U.89	13
Pm.U.19	26.5	Pm.W.90	25
Pm.U.20	7	Pv.U.92	27
Pm.W.23	25	Pm.U.95	11
Pm.W.24	22.5	Pp.U.98	20
Pm.B.25	1.5	Pp.W.100	24
Pm.U.30	7	Pm.U.103	23
Pm.U.32	21	Pm.E.108	25
Pv.U.34	25	Pm.U.115	9
Pm.U.35	16	Pm.W.120	12
Pv.E.50	26	Pm.W.125	5
Pm.U.56	18	Pm.W.130	4
Pm.E.67	18	Pv.W.133	22
Pv.E.68	24	Pm.E.135	10
Pm.S.70	21	Pm.U.137	8
Pv.U.72	27	Pv.U.140	14
Pm.U.73	22	Control	0

- The result represent average of duplicate reading
- Pm= ***P. mirabilis***,
- Pv = ***P. vulgaris***
- Pp = ***P. penneri***

- E= ear swabs
- U = urine sample
- S = stool sample
- W =wound swab

Table (2) ASNase II produced by *Proteus* species in broth medium according to pH value

Proteus isolates	pH value	*Proteus* isolates	pH value
Pm.W.01	7.1	Pm.B.82	7.6
Pp.S.06	7.4	Pp.S.85	7.2
Pm.U.14	7.0	Pv.U.88	**8.2**
Pm.U.15	7.2	Pm.U.89	7.0
Pm.U.16	7.7	Pm.W.90	7.7
Pm.U.19	7.9	Pv.U.92	**8.5**
Pm.U.20	7.0	Pm.U.95	7.4
Pm.W.23	7.2	Pp.U.98	7.5
Pm.W.24	7.6	Pp.W.100	**8.0**
Pm.B.25	7.3	Pm.U.103	**8.6**
Pm.U.30	7.5	Pm.E.108	**8.4**
Pm.U.32	7.4	Pm.U.115	7.0
Pv.U.34	**8.3**	Pm.W.120	7.2
Pm.U.35	7.0	Pm.W.125	**8.4**
Pv.E.50	7.9	Pm.W.130	6.9
Pm.U.56	7.2	Pv.W.133	**8.0**
Pm.E.67	7.4	Pm.E.135	7.1
Pv.E.68	**8.5**	Pm.U.137	7.3
Pm.S.70	7.8	Pv.U.140	**8.6**
Pv.U.72	**8.3**	Control	6.9
Pm.U.73	**8.1**		
Pm.U.77	7.8		

- The result represent average of duplicate reading

Table (3) Activity of ASNase II production by *Proteus* species by using quantitative method

Proteus isolates	Activity of ASNase II (U/ml)	Concentration of protein (mg/ml)	Specific activity (U/mg)
Pv.U.34	4.4	3.9	1.13
Pv.E.68	7.1	4.63	1.50
Pv.U.72	3.1	2.5	1.24
Pm.U.73	5.8	3.65	1.59
Pv.U.88	6.4	4.3	1.49
Pv.U.92	**6.67**	**3.38**	**1.97**
Pp.W.100	5.8	3.5	1.66
Pm.U.103	3.3	2.5	1.32
Pm.E.108	5.6	3.1	1.81
Pm.W.125	6.6	4.7	1.40
Pv.W.133	1.5	2	0.75
Pv.U.140	7.3	4.5	1.62

- **The result represent average of duplicate reading**

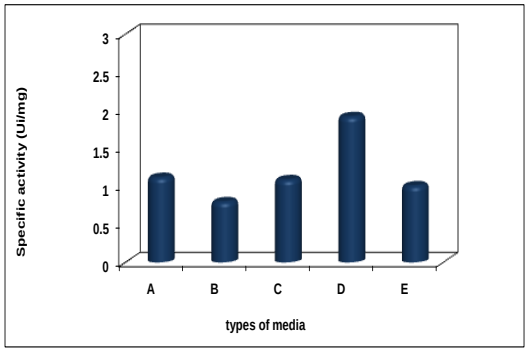

Fig.(1) effect of media on ASNase II production by *P. vulgaris* Pv.U.92 (at pH 7.0 in 37°C for 24 hr.)

- **Effect of L-asparagine concentration:** Figure (2) indicated that the specific activity of ASNase II produced by *P. vulgaris* Pv.U.92 was increased gradually by addition of L-asparagine to D medium until it reached 1.8 U/mg at 0.3gm of L-asparagine. This amino acid (L-asparagine) was enhanced the bacterial growth and produced ASNase II [22]. The substrate affinity of L-asparaginase II for L-asparagine is related to its degree of effectiveness against(1).

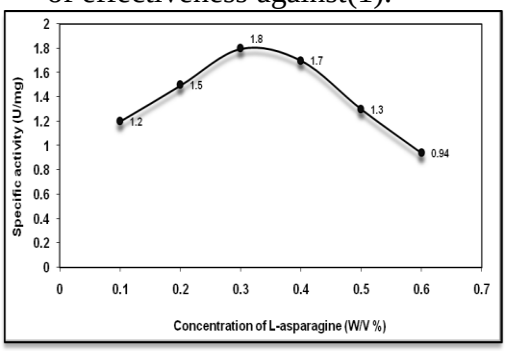

Fig.(2) Effect of L-asparagine concentrations on ASNase II production from *P. vulgaris* Pv.U.92 (at pH 7.0 in 37°C for 24 hr.)

- **Effect of pH:** The specific activity of ASNase II produced by *P. vulgaris* Pv.U.92 was increased at pH 7.5, then as shown in figure (3) this referred to produce the ASNase II approximately was in medium tend to equal and alkaline pH. It can conclude that *P. vulgaris* produced ammonia when growth in medium this increased pH of medium therefore it enhanced to produced this enzyme. The optimum pH was found to be 7.5 for ASNase II in Marine actinomycetes which is close to blood pH [1], compared to L-asparaginases from other bacterial sourcessuch as *Serratia marcescens*, *Mycobacterium* spp. and *Pseudomonas* spp showed optimum pH in the range of 8.0 to 8.5 [1], while Tosa and the others [23] referred to the optimum pH of *P. vulgaris* OUT8226 for production ASNase II was 7.0. pH value may change during the incubation time as a result to bacterial metabolism processes; this also differed according to bacterial strain, nature of enzyme and components of media. pH effect on ASNase II activity by ionization free enzyme or on enzyme-substrate complex [24].

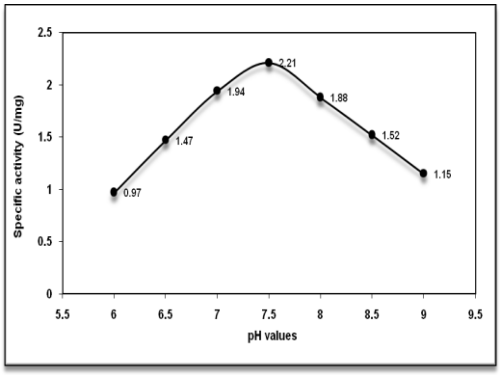

Fig.(3) Effect of pH on ASNase II production from *P. vulgaris* Pv.U.92 (at 37°C for 24 hr.)

- **Effect of temperature:** Figure (4) showed that the specific activity of ASNase II produced by *P. vulgaris* Pv.U.92 cultivated in D medium was increased gradually by increasing the temperature of incubation, thus it was noticed that incubation of culture at 35ºC enhancing the specific activity of ASNase II for production to 1.62 U/mg. The temperature it effect on stability of enzyme and on solubility the substrate also on ionization enzyme -substrate complex [24, 25].

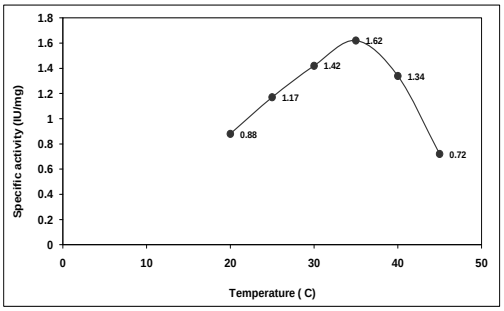

Fig.(4) Effect of temperature on ASNase II production from *P. vulgaris* Pv.U.92 (at pH 7.5 for 24 hr)

4- Extraction of ASNase II by Ultrasonication from *P. vulgaris* Pv.U.92 cells: Cells of *P. vulgaris* Pv.U.92 cultivated in D medium at pH 7.5 and temperature 35ºC were exposed to Ultrasonicator at different times. Figure (5) demonstrated that the specific activity of ASNase II was increased (2.13 U/mg) when cells of this isolate were disrupted for 4 min, and at this time about 90.3% of ASNase II was released from the cells, and the prolonged time was effect on the tetramer protein structure of this enzyme

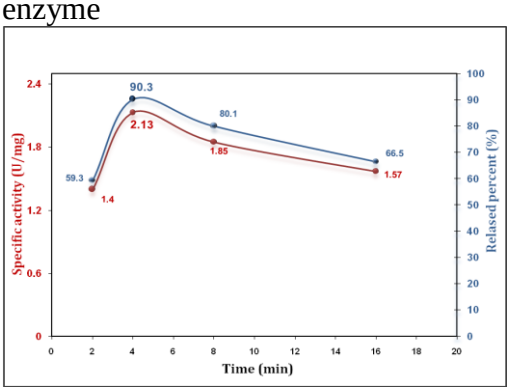

Fig. (5) Specific activity and released percent of ASNase II production from *P. vulgaris* Pv.U.92 at different times of Ultrasonication

5- Purification of ASNase II produced by *P. vulgaris* Pv.U.92: It was included three steps:

- **Precipitation by Ammonium sulfate $(NH_4)_2SO_4$:** It was observed that the specific activity of ASNase II was increased gradually with increasing $(NH_4)_2SO_4$ concentrations and it would be 2.2 U/mg at 70 % of $(NH_4)_2SO_4$ (figure 6). Ammonium sulfate was used because it has higher soluble ability, easy to use and its cheap [27], also $(NH_4)_2SO_4$ act to equal the charge on protein surface therefore the ASNase II became insoluble and precipitated [28]. Notable, Basha and the other observed similar result when worked on Marine actinomycetes [1].

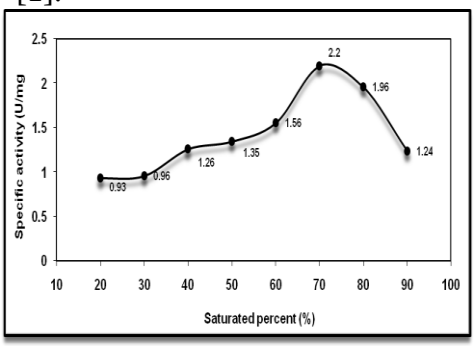

Fig.(6) Precipitation of ASNase II produced by *P. vulgaris* Pv.U.92 with different concentrations of $(NH_4)_2SO_4$

- **Purification ASNase II by DEAE-cellulose ion exchange chromatography:** The result in figure (7) revealed 3 peaks in washing fraction and 7 peaks in eluted fraction. The activity of enzyme was measured at each eluted peak. Only three peaks were displayed enzyme activity at eluted fraction. Peak number 2 showed the maximum specific activity (table 4), it was 20 U/mg in comparison with others. This result may suggest that ASNase II produced by *P. vulgaris* Pv.U.92 had negative charge. From this result, it can be considered that ASNase II has many forms (isoenzyme) because it revealed multiple activity peaks. However, almost bacteria belonged to Enterobacteriaceae family have isoforms for this enzyme [4] such as *E. coli* [29] and *pseudomonas aeroginosia* [20].
- Isoforms of any enzyme had approximately the same molecular

weight but they differ in isoelectric point (pI) which makes their separation impossible by gel filtration, because they have small differences in charging properties resulting from altered amino acid composition. Thus ion-exchange chromatography can use for separation isoenzymes [30].

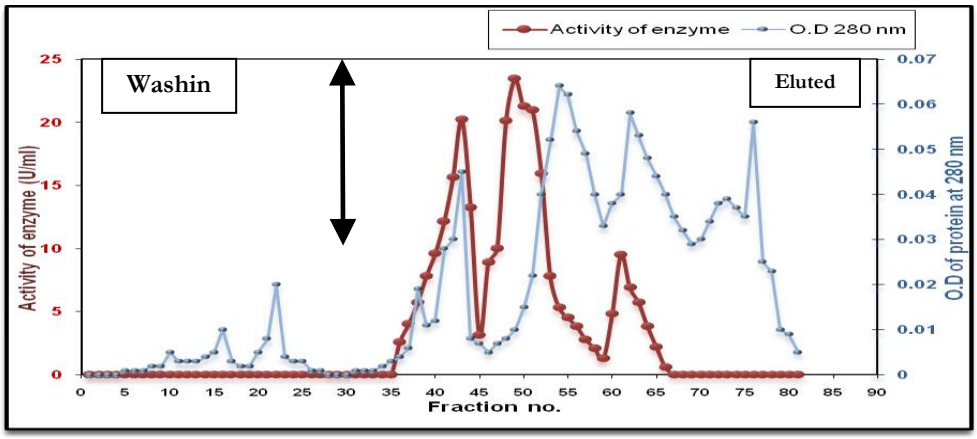

Fig. (7) DEAE-cellulose ion exchange to purified ASNase II from *P. vulgaris* Pv.U.92 with collection volume (3ml) at flow rate 22.2 mL/1 hr

Table (4) purification schedule of Ion exchange peaks

No. of peak	Volume (ml)	Activity of enzyme (U/ml)	Concentration of protein (mg/ml)	Specific activity (U/mg)	Total activity (U/ml)	Yield (%)	Fold purification
1	12	7.56	0.32	23.6	90.7	9.7	4.1
2	15	20	0.4	50	300	31.9	8.8
3	15	5.8	0.63	9.2	87	9.3	1.6

▪ **Purification of ASNase II by Sephacryl S- 300 gel chromatography:** Fraction tubes that represented peak 2 were selected for purification by gel filtration chromatography. Firstly, fractions were concentrated by sucrose, and then specific activity of ASNase II was measured and then gel filtration step was applied. Results in table (5) showed the increasing of specific activity of this enzyme. Subsequently, the concentrated enzyme was purified by Sephacryl S-300 column. Figure (8) demonstrated 3 peaks at optical density 280nm. The specific activity of each peak was estimated. Only one peak had enzyme activity. It was 155.6 U/mg with 10.4% yield and 27.3 fold of purification. In general, the differences in the specific activity of ASNase II may return to the concentration of proteins or kind of purification materials (gel) as well as type of methods that used for purification [20,30]. Sephacryl S 300 it's suitable for fast run (short run times), high recovery separations at laboratory and industrial scale [30].

Table (5) purification schedule for ASNase II purification

Purification steps	Vol. (ml)	enzyme activity (U/ml)	Concentrate of protein (mg/ml)	Specific activity (U/mg)	Total activity (U/ml)	Yield (%)	Fold purification
Crude enzyme	40	23.5	4.1	5.7	940	100	1
Precipitate with $(NH_4)_2SO_4$ (60-80%) (after dialysis)	18	35.8	2.3	15.6	644.4	68.6	2.7
DEAE-cellulose column	15	20	0.4	50	300	31.9	8.6
Concentrate with sucrose	8	26.4	0.4	66	211.2	22.5	11.6
Sephacryl S-300 column	**7**	**14**	**0.09**	**155.6**	**98**	**10.4**	**27.3**

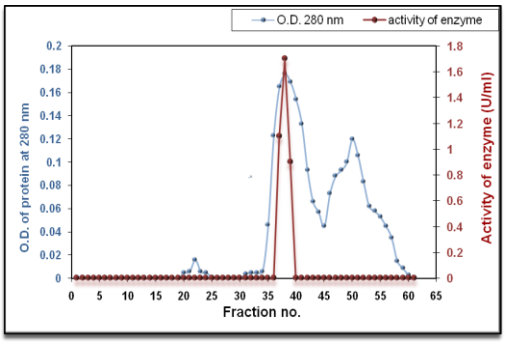

Fig.(8): Gel filtration of ASNase II produced by *P. vulgaris* Pv.U.92 by Sephacryl-S 300 column with volume of fraction was (3ml) at flow rate 0.7ml/min

References:

1. Basha, N. S.; Rekha, R.; Komala, M.; and Ruby, S. **2009**. Production of Extracellular Anti-leukaemic Enzyme Lasparaginase from Marine Actinomycetes by Solidstate and Submerged Fermentation: Purification and Characterisation. Tropical J. of Pharm.Res., vol.*8* (4): 353-360
2. Capizzi,R.L; Poole, M.; Cooper, M.P.; Richards, F.; Stuart, J.J.; Jakson, D.V.; White,D.R.; Spurr, C.L.; Hopkins, J.O.; and Muss, H.B. **1984**. Treatment of poor risk acute leukemia with sequential hig-done ARA-C and asparaginase. Blood. *63*:649-700**.**
3. Wriston, J.C.**1985**. L-Asparagine amidohydrolase. ***In:*** Meister, A.(ed.) Methods in enzymology, volume 113. Academic Press,Inc**.**
4. Campbell,H.A.; Mashburn,L.T.; Boyse,E.A.; and Old, L.J. .**1967**. Two L-asparaginase from *Escherichia coli* B. Their separation, purification, and antitumor activity. Bioche. *6*:721-730**.**
5. Roberts,J.; Burson,G.; and Hill,J.M. **1968**. New procedures for purification of L-Asparaginase with high yield from *E. coli.* J. Bacteriol.. *95*(6):2117-2123.
6. Keating, M.J.; Holmes,R.; Lerner,S.; and Ho,D.H. **1993**. L-asparaginase and PEG asparaginase – Past, present and future. Leuk. Lymphoma. *10*: 153-157**.**
7. Obama, K.; Tara,M.; and Nina,K. **1999**. L-asparaginase induced complete remession in Epstein-Barr virus, positive, multidrug resistant, cutaneous T-cell lymphoma. Int.J.Hematol. 69:260-262.
8. Soares,A.L.; Guimaraes, G.M.; Polakiewicz, B.; de Moraes Pitombo, R.N.; and Abrahao-Neto, J. **2002**. Effects of polyethylene glycol attachement on physicochemical and biological stability of *E. coli* L-asparaginase. Intern. J. of pharma.. vol. *237*:163-170.
9. Appel, I.M.; den Boer, M.L.; Meijerink, J.P.; Veerman,A.J.; Reniers, N.C.; Pieters,and R. **2006**. Up-regulation of asparagine synthetase expression is not linked to the clinical response L-asparaginase in pediatric acute lymphoblastic leukemia. Blood. *107*(11):4244-4249.

10. Weatherburn, M. W., **1967**. Phenol-hypochlorite reaction determination of ammonia. Anal. Chem. *39*(8):971-974.

11. Lowry, 0. H.; Rosebrough,N. J.; Farr,A. L.; and Randall, R. J. **1951**. Protein measurement with the Folin phenol reagent. J. Biol. Chem. Vol.*193*:265-275.

12. Beck, J. V. **1971**. Enrichment culture and isolation techniques particularly for aerobic bacteria. *In*: Jakoboy, W. B. (ed.) Methods in enzymology. vol. *22*:46-61. Academic press, New York.

13. Sukumaran,C.P.; Singh,D.V.; and Mahadevan,P.R. **1979**. Synthesis of L-asparaginase by *Serratia marcescens*. J.Biosci. *1*(3):263-269.

14. Bilimoria,M.H. **1969**. Conditions for the production of L.asparaginase II by coliform bacteria. Appl. Microbiol. *18*(6):1025-1030.

15. Whitaker, J. R.; and Bernard,R.A. **1972**. Experiments for an Introduction of Enzymology the Wibber press. Davis.

16. Gulati, R.; Saxena,R.K.; and Gupta,R. **1997**. A rapid plate assay for screening L-asparaginase producing microorganisms. Lett. Appl. Microbiol.vol. *24*: 23-26.

17. Novak,E.K.; and Phillips,A.W. **1974**. L-glutamine as a substructure for L-asparaginase from *Serratia marcescens*. J. Bact. Vol.*117*(5):593-600.

18. Imada, A.; Igarasi,S.; Nakahoma K.; and Isona,M. **1973**. Asparaginase and glutaminase activities of microorganism. J. Gen. Microbiol. vol. *76*:85-99.

19. Wade,H.E.; Robinson,H.K.; and Phillips,B.W. **1971**. The asparaginase activity of bacteria. J.Gen.Microbiol. vol.*65*.

20. El-Bessoumy, A. A.; Sarhan,M. and Mansour,J. **2004**. Production, Isolation, and Purification of L-Asparaginase from *Pseudomonas Aeruginosa* 50071 Using Solid-state Fermentation. J. Biochem. and Molecular Bio., 37(4): 387-393.

21. Saad, M.R. **1990**. Principles in Microbial physiology, higher education ministry, Mosel, Iraq (in Arabic).

22. Clark,P.H. **1980**. The utilization of amides by microorganism. ***In***: Payne J.W. (ed.) Microorganism and nitrogen sources. John Wiley and sons. New York. 563-576.

23. Tosa,T; Sano,R.; Yamamoto,K.; Nakamura, M.; Ando,K.; and Chibata,I. **1971**. L-Asparaginase from *Proteus vulgaris*. Amer. Soci. for Microbio. *22*(3): 387-392.

24. Mokrane,S.A. **2003**. Production, Purification, and Characterization of L-Asparaginase II from a local isolate of *E.coli*. College of science / Baghdad University. Iraq (thesis).

25. Pritsa, A.A.; Papazisis,K.T.; Kortsaris, A.H.; and Geromichalos, G.D. **2001** .Antitumor activity of L-asparaginase from *Thermus thermophilus*. Anti. Drugs. *12*(2):137-142.

26. Uhlig,H. **1998**. Industrial enzymes and their applications. John Wiley and sons. Inc. New York.

27. Voleskey,B.; and Luong,L. **1985**. Microbiol enzyme: production, purification and isolation. CRC. Biotecnology. Vol. *2*:119-146.

28. White,A.; Handler, P.; and Smith,E. **1972**. Principle of Biochemistry. M.C. Grow Hill Book Company. A blakiston publishing. New York.

29. Jennings, M. P.; and Beacham,I. R. **1990**. Analysis of the *Escherichia coli* gene encoding L-asparaginase II, ansB, and its regulation by cyclic AMP receptor

and FNR proteins. J. Bacteriol. 172(3): 1491–1498.

30. AL-Azawi, M.A.H. **2005**. Production and Purification of L-Asparaginase II from a local isolate of *Serratia marcescens*. College of science / Baghdad University. Iraq (Thesis).

استخلاص وتنقية أنزيم L-Asparaginase II المنتج من بكتريا *Proteus vulgaris* المعزولة محلياً

رواء جميل توما* ***اسماء محمد سعود**** ***شهلاء علي حسن*****

مثال عبد الكريم عبد عون* ***سراب سلمان كاظم****

*وزارة العلوم والتكنولوجيا.

**جامعة بغداد/كلية العلوم/قسم التقنيات الاحيائية.

*** جامعة بغداد/كلية العلوم/قسم علوم حياة

الخلاصــة:

تم الحصول على 41 عزلة تعود لجنس *Proteus* من مجموع 140 عينة سريريه من عينات مختلفة شملت عينات الإدرار، الخروج، الجروح، الحروق ومسحات الإذن لكلا الجنسين. هذه العزلات تم تشخيصها وفقاً لصفاتها المظهرية والاختبارات الكيموحياتية إلى ثلاثة أنواع هي *P. mirabilis*، *P. vulgari* و *P. penneri*. أختبرت قابلية هذه العزلات لإنتاج إنزيم L-asparaginase II باستخدام طرائق كمية وشبه كمية. وقد أختيرت بكتريا *P. vulgaris* Pv.U.92 كأفضل عزلة منتجة للأنزيم حيث بلغت الفعالية النوعية للإنزيم 1.97 وحدة/مليغرام. حددت الظروف المثلى للإنتاج وكان الوسط D المحتوى على 0.3% من المادة الأساس (L-asparagine) عند الأس الهيدروجيني 7.5 الأمثل لإنتاج الإنزيم عند حضنه بدرجة حرارة 35 مº.

أستخلص ASNase II من خلايا *P. vulgaris* Pv.U.92 بعد تكسيرها بطريقة الأمواج الصوتية الفائقة (ulrasonication) ثم رسب المستخلص الناتج بأملاح الامونيوم (60-80 %) ونقي الإنزيم باستعمال المبادل الأيوني DEAE-cellulose ثم مرر بعمود Sephacryl S-300 وقد بلغت الفعالية النوعية 155.6 وحدة/مل غرام وبمحصلة إنزيمية 10.4 % وبعدد مرات التنقية 27.3 .