# EFFECT OF THE ANTIBIOTICS ON GLUTAMINASE PURIFIED FROM *Serratia plymuthica* ISOLATED FROM DIYALA RIVE.

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ABSTRACT

*Serratia plymuthica* isolates were obtained at 20 isolates out of 40 water samples from Diyala river, Baquba city ,these isolates were tested for sensitivity against 10 different antibiotics .The results showed that the isolates have littlie resistance to Nitrofurantion(N),Chloramphenicol(C) and Streptomycin(S ), but they were giving whole activity (100%) toward Amoxicillin (AMX), Ampiclocx,(AM ) and Cephalothin(CF) antibiotics. Active of these isolates was selected to purify glutaminase through several stages of purification including (ammonium sulfate precipitation ,ion exchange by DEAE- cellulose, and gel filtration by sephadex G-100). The stock solution of these antibiotics was mixed with purified enzyme respectively The results showed that the activity was reduced to zero by using the ,(AM ) , Metronidazole, (CF) ,Erythromycin, and Rifampicin . On the other hand the Tetracycline and (C) were raised the specific activity of glutaminase to 10.8 I.U./mg and 15.31 U.I./mg respectively in the comparison with the control ( 3.1 I.U./mg ).

**Key words , *Serratia plymuthica* ,Antibiotics, Glutaminase.**

## INTRODUCTION

*Serratia* genus includes different species, *Serratia marcescens* , which is frequently involved in human and animals infection , and *Serratia plymuthica* a saprophytic fermentative, non –motile gram negative rod and produces red pigment (prodigiosin) ,The bacterium has been classified as an uncommon cause of human and animal infection (Ramos,1995).

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Most of the *Serratia plymuthica* strains have been isolated from fresh water and fish.Although *Serratia plymuthica* strains infections are relatively rare in human and animals ,their clinical and veterinarical importance is well documented .This is suggesting that may be potential opportunistic pathogen for animals and human (Rodriguez,1990;Vivas,2000;Raschle,2005) Thus , *Serratia plymuthica* was isolated from human clinical specimens , soil, water, plant surfaces and other environmental sites ,digestive tract of rodents, and insects.(Matthew,1979) .

Glutaminase is relatively wide spread enzyme, found in many animal tissues ,bacteria , plants,and in serum of certain rodents but not of man(Cornea,2002).Interest in glutaminase began with the discovery that agent responsible for the anti lymphoma effects of guinea pig serum was due to glutaminase , since glutaminase has a dual specificity for glutaminase and L-asparaginase (Novak,1974) .For this reason ,the aim of this experiment was to purify glutaminase and to study the effect of some antibiotics on glutaminase activity .

MARERIALS AND METHODS

A- Samples collection:

Fourty water samples were collected in sterile containers from the Diyala river in Baquba city . The water samples were filtered through 0.45 µm pore size nitrocellulose filters sterilization , the filters are then vortexed in peptone broth medium for recovering the bacteria .After removing the filters , the bacteria were cultivated at 37Cº for 20 hours and then centrifuged(Delabre,1998).

Isolation and characterization of *Serratia plymuthica* :

Samples were plated on blood agar and MacConkey agar plates .Plates were incubated at 30 Cº for 18- 24 h.(Cruickshank,1975). Several biochemical tests were done to differentiate *Serratia plymuthica* from other species . These include the following tests : methyl red , lysine decarboxylase production ,

ornithine decarboxylase production , acid production from lactose, melibiose and xylose fermentation (Garrity,2009).Beside API 20 E identification to differentiate *Serratia plymuthica* from the other types(John,2001) .

B- Purification of glutaminase from *Serratia plymuthica* :

*Serratia plymuthica* glutaminase was purified by modification method described by (Kazuaki,2005; Singer,2008) .Cells harvested after 24 hr. of incubation on tryptic Soya broth and washed twice with 0.05 M tris – hydrochloride buffer (pH 8.6) and suspended in two volume of cold buffer .The sonically disrupted suspension was centrifuged by cooling centrifuge at 10000xg for 20 min. To the supernatant fluid 0.05 ml of 1.0 M $MnCl_2$ was added for removal of nucleic acid .**The mixture** was then stirred for 2 hours and re centrifuged .The supernatant glutaminase activity was assayed by treating the fluid by solid ammonium sulfate ratio of saturation 50% .The mixture was centrifuged , then the supernatant was dialyzed against distilled water and the glutaminase activity was assayed. One unit of glutaminase activity was defined as amount of enzyme that released 1µ mol of ammonia from L- glutamine Per minute (Kazuaki,2005) .

The supernatant was loaded on chromatographic column (2.5 by 20 cm) containing 100 ml of diethyl ammonium (DEAE)- cellulose which had been equilibrated with 0.01M tris ( pH8.6) containing 0.01M KCl .The column was washed with 5 to 10 volumes of 0.01M tris (pH8.6) containing 0.05 M KCl . A gradual elution was then raining from 0.1 M KCl to 0.4M KCl Fractions (5ml) were collected and assayed for glutaminase enzyme activity. The fractions that shown glutaminase activity were loaded on Sephadex G-100 column (1.5 by 70 cm ) containing 100 ml of Sephadex G-100 which had been equilibrated and washed with 0.2 M phosphate buffer and the elution done by the same buffer .

The fractions (5ml) were collected and assayed for glutaminase activity (Kazuaki,2005) .

GLUTAMINASE ASSAY:

Routine glutaminase assay was conducted by (Katikala,2009) 0.2ml solution of enzyme was added to 0.2ml of 0.05 M phosphate buffer (pH 8.2) as blank . The reaction was initiated by the addition of 0.2ml of enzyme solution to 0.2 ml of 0.04 M glutaminase in the same buffer and incubated at 37 Cº for 40 min. The reaction was stopped by addition of 0.25 ml of 1.5 M trichloroacetic acid for 10 min. Ammonia released in the reaction was determined by the addition of Nessler ´s reagent to the diluted supernatant fluid and after 15 min and observing the absorbency at 500 nm by visible spectrophotometer (Kazuaki,2005 ).

PROTEIN ASSAY :

Analysis for protein were carried out by the spectrophptometric assay at 600 nm by visible spectrophotometer, in each stage of glutaminase purification.(Prasanth,2009).

**C-** Determination of the level of antimicrobial activity **:**

The antibiogram test using Kirby- Bauer method was carried out using Mueller-Hinton agar plates (John,2001) .The antibiotic discs used were : Amoxicillin (AMX) 30 µg; Ampiclox (AX) 30µg; Metronidazole(MET) 30µg;Cephalothin (CF) 30µg;Erythromycin (E) 15µg;Streptomycin (S)10 µg; Chloramphnicol (C) 30µg ; Rifampicin (RA ) 5 µg; Nitrofurantion (FT) 300µg;Tetracycline(TE) 30µg .

EFFECT OF THE ANTIBIOTICS ON GLUTAMINASE ACTIVITY:

Stock solution from the antibiotics were prepared by using the two fold concentrations that were found in the antibiotics discs .The stock solutions used were Amoxicillin (AMX) 50 µg/ml; Ampiclox (AX) 60µg/ml; Metronidazole(MET) 60µg/ml;Cephalothin (CF) 60µg/ml;Erythromycin (E) 30µg/ml;Streptomycin (S)20 µg/ml; Chloramphnicol (C) 60µg/ml ; Rifampicin (RA ) 10 µg/ml; Nitrofurantion (FT) 600µg/ml;Tetracycline(TE) 60µg/ml.

0.2 ml of purified glutaminase solution was added to 0.2 ml of each antibiotic stock solution was used and, after 30 min .in 37Cº , The glutaminase activity and amount of protein were measured to find the specific activity(Brown,2008 ,Greenup,1997).

RESULTE AND DISSCTION

Isolation and characterization of *Serratia plymuthica*:

The results revealed that 20(50%) isolates of *Serratia plymuthica* were obtained out of 40 water samples .Since most of the *Serratia plymuthica* strains have been isolated from fresh water and fish suggesting that may be a potential opportunistic pathogen for animal and human (Rodriguez,1990 ; Greenup,1997 ) In a study by (Vivas,2000) found that *Serratia plymuthica* 68.5% and *Serratia liquefaciens* 0.8%.

SENSTIVITY TEST :

Ten different antibiotic discs were used to per form this test fig.1

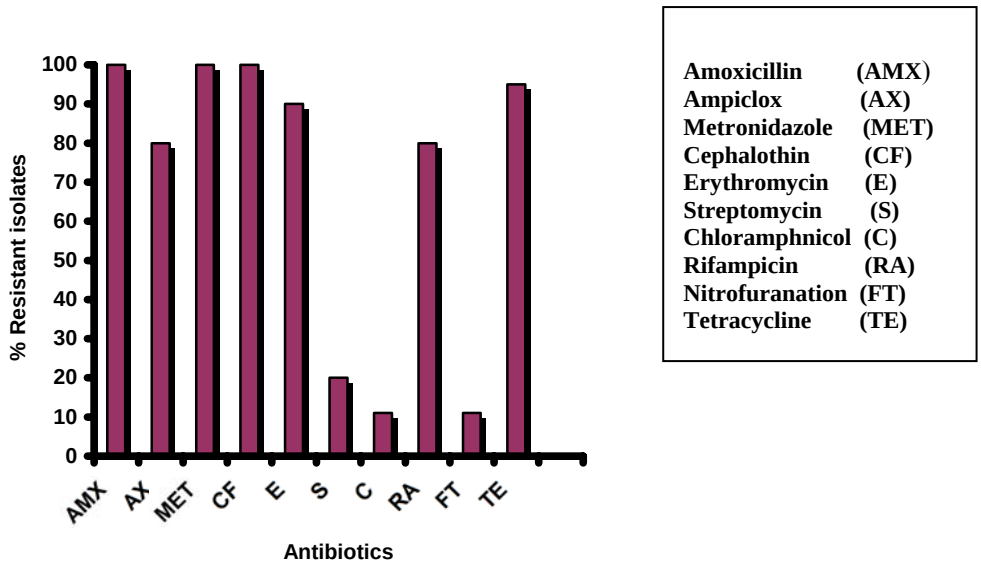


**Figure 1.** *Percentage of Resistant Isolates to Antibiotics* .

The results showed that most of *Serratia plymuthica* isolates were found to be Choroamphenicol (C),Nitrofurantion (FT) and Streptomycin (S) susceptible

and they show resistance 10% to (C) and (FT), 20% to (S) .Similar result was obtained according to a study of therapy for infection caused by *Serratia plymuthica.*

All isolates of *Serratia plymuthica* were showed multi antibiotics resistant toward Amoxicillin, Ampiclocx, and Cephalothin (100%). First the resistance to ß- lactam antibiotic ampiciline were due to ß- lactamase enzyme which hydrolyze ß- lactam rings and render such antibiotics incapable of reacting with bacterial transpeptidase enzymes another example is the aminoglycoside an antibiotic Kanamysin (bacterial protein synthesis inhibitor), enzymes conferring the kanamycin resistant phenotype inactivate the drug by covalent attachment of phosphate or other group. Second bacteria can develop mutation that render the target molecule unable to interact with the antibiotic . Finely , change bacteria cell permeability to an antibiotic can confer antibiotic resistance. ( Brown,2008;Wilk,2005;Fisher,2005 ) . While the strains with low antibiotics susceptibility( resistance) : with the following antibiotics order :MET(80%), E(90%), RA(80%) and TE(95%).

PURIFICATION:

The purification of glutaminase from *Serratia plymuthica* is summarized in (Table 1) .The producer described yield a 369 fold purification and 2.6% recovery of the enzyme .Solid ammonium sulfate at 50% saturation to the crude extract lead to rise in the enzyme activity .Approximately 80 to 90 % of the enzyme activity was salted out 55 and 65% saturation of ammonium sulfate as mentioned by (Singer,2008) .The precipitate was dialyzed against distilled water to remove the ammonium sulfate and loaded on DEAE – cellulose column . The typical elution profile from DEAE- column is shown in (fig. 2). In a study done by (Singer,2008;Dura,2002 ) were found that enzyme preparation were found to be more stable if they were purified on DEAE-column before ammonium sulfate precipitation .Additional purification was done on Sephadex G-100 column (1.5 by 70 cm) , the column was loaded with about 0.11 mg of protein after equilibration with 0.2 M phosphate buffer(pH = 7.5) , washed with several column volumes of the same buffer , and finally eluted with phosphate buffer . Fractions containing high specific activity were pooled and used for further studies as shown in (fig. 3). On the other hand

(Novak,1974) have shown that the fractions containing high specific activity were pooled and further purified on a hydroxylapatite column (1by 25 cm).

**Table 1 :** *Purification of Serratia plymuthica Glutaminase*

| Purification step | Size (ml) | Protein Conc. (mg/ml) | Activity U/ml | Specific Activity U/mg | Total activity | Purification Fold | Yield (%) |
|---|---|---|---|---|---|---|---|
| Crude extract | 50 | 25.0 | 21.0 | 0.84 | 1050 | 1 | 100 |
| (NH4)2SO4 | 25 | 10.2 | 15.2 | 1.49 | 380 | 1.77 | 37.2 |
| DEAE-cellulose | 9 | 0.11 | 7.20 | 65.45 | 64.8 | 77.91 | 6.3 |
| Sephadex G-100 | 9 | 0.01 | 3.10 | 310 | 27.9 | 369.04 | 2.6 |

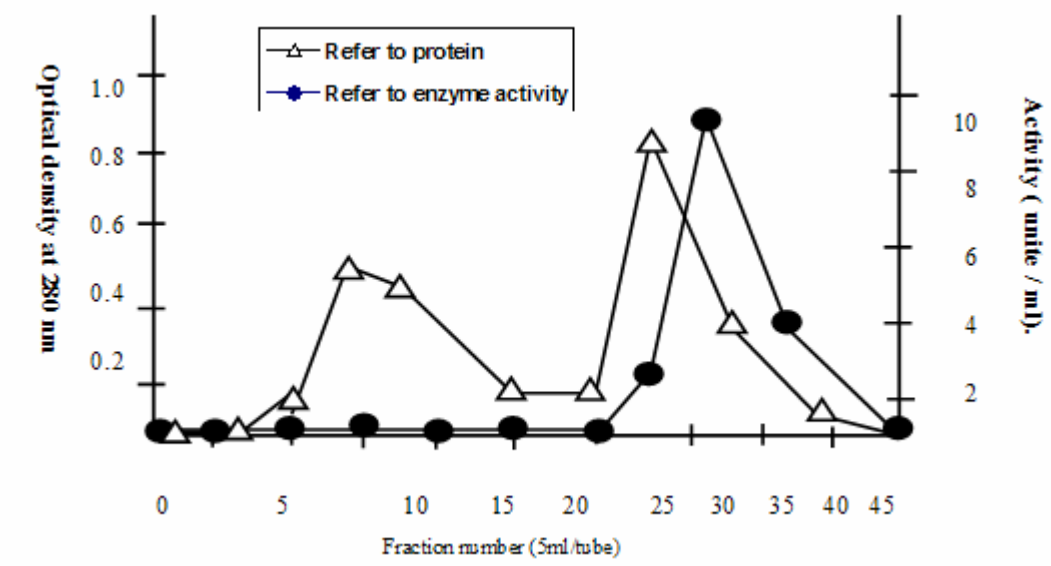


**Figure 2.** *Glutaminase purification from Serratia plymuthica by DEAE – Cellulose chromatography.*

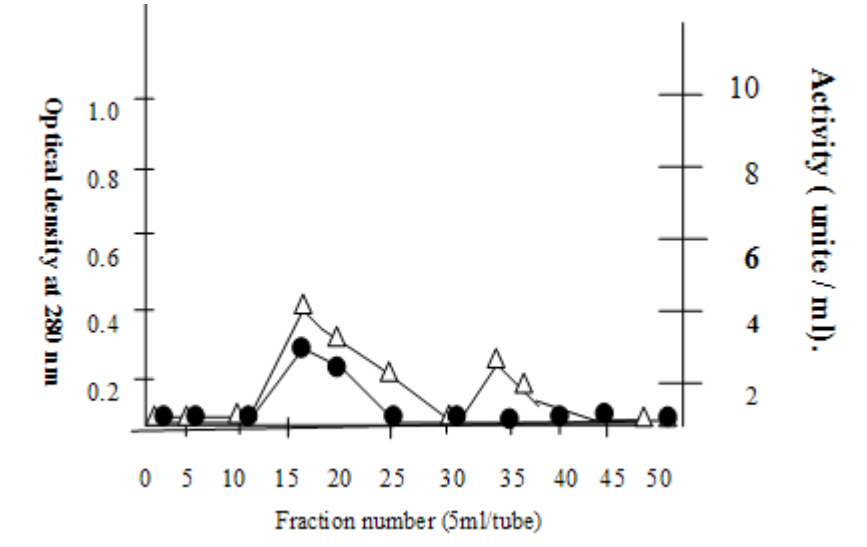


**Figure 3.** *Glutaminase purification from Serratia plymuthica by Sephadex G-100 chromatography.*

EFFECT OF THE ANTIBIOTICS ON GLUTAMINASE ACTIVITY:

The isolates that was chosen for glutaminase purification showed resistance to AMX,AX, MET, CF,E, RA, and TE while showed susceptibility to Streptomycine ,Nitrofuration ,and Chloramphenicol .

In this study when stock solution of these antibiotics were mixed with purified enzyme respectively , we found that the isolates were resistant to antibiotics but the antibiotics have effect on glutaminase specific activity , since this activity was reduced to zero by using the Ampiclox ,Metronidazole,Cephalothin,Erythromycin, and Rifampicin , but in using Amoxicillin has no effect on glutaminase specific activity . On the other hand the Tetracycline was raised the specific activity to 10.8 I.U./mg in the comparison with the control 3.1 I.U./mg as shown in the table- 2. Also these isolates were sensitive to Nitrofuration ;Chloramphnicol and Streptomycin, Thus ,the specific activity of glutaminase became zero in case of using Nitrofuration and Streptomycin, while the Chloramphnicol raised the specific activity to 15.31 U.I./mg .This lead to final conclusion that some antibiotics have effect on the glutaminase even these strains were resistant to antibiotics

and another of antibiotics led to arise of glutaminase activity .This refer to increase interest in using the glutaminase as the antilymphoma factor .

The enzyme glutaminase belong to a large group of serine beta- lactaminase and pencillin – binding proteins. Beta- lactamases catalyzed the hydrolysis of an amide bond (N-CO) in beta- lactam ring of pencillin and cephalosporin family constituting the most common mechanism of bacterial resistance (Brown,2008;Fisher,2005;Haddix,2009;Kliebe,1985).

**Table 2 :** *Effect of the antibiotics on glutaminase specific activity.*

| **Antibiotics** | **Antibiogram test** | **Spesific activity of glutaminase purified I.U/mg + Antibiotic** |
|---|---|---|
| control | - | 3.1 |
| Amoxicillin | R | 3.2 |
| Metronidazole | R | 0 |
| Cephalothin | R | 0 |
| Erythromycin | R | 0 |
| Streptomycin | S | 0 |
| Chloramphenicol | S | 15.3 |
| Rifammpicin | R | 0 |
| Nitrofuranation | S | 0 |
| Tetracycline | R | 10.8 |
| Ampiclox | R | 0 |

**C: control**

**S: sensitive**

**R: resistance**

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## تاثير المضادات الحياتية على فعالية انزيم الكلوتامينيز المنقى من العزلة *Serratia plymuthica* المعزولة من نهر ديالى في مدينة بعقوبة .

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## الخلاصة

تم الحصول على 20 عزلة تعود لبكتريا *Serratia plymuthica* من مجموع 40 عينة ماء ماخوذة من نهر ديالى في مدينة بعقوبة. أخضعت جميع العزلات لاختبار الحساسية باستخدام 10 مضادات حياتية .

أظهرت النتائج ان العزلات البكتيرية التابعة لجنس *Serratia plymuthica* أعطت مقاومة قليلة للمضادات الحياتية(S)Streptomycin, Nitrofurantion(N),Chloramphenicol(C) بينما أعطت مقاومة كاملة (100%) للمضادات ( Amoxicillin (AMX), Ampiclocx,(AM . and Cephalothin(CF) اختبرت أكفا العزلات لاستخلاص وتنقية إنزيم الكلوتامينيز بعدة مراحل تضمنت (الترسيب بكبريتات الامونيوم, التبادل الايوني باستخدام المبادل الايوني DEAE-cellulose والترشيح الهلامي باستخدام هلام Sephadex G-100) , ثم اختبر تأثير نفس المضادات الحياتية المذكورة اعلاه على فعالية الإنزيم المنقى و اظهرت النتائج أن استخدام بعض المضادات الحياتية ( Chloramphnicol (C و Tetracycline(TE) أدت إلى زيادة فعالية أنزيم الكلوتامينيز المنقى الى 10.8 I.U./mg and 15.31 U.I./mg على التوالي مقارنة بعينة السيطرة حيث بلغت الفعالية النوعية للإنزيم المنقى من العزلة *Serratia plymuthica* ( 3.1 I.U./mg