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Screening, Identification and Production of L-glutaminase by *Lactobacillus reutri* Isolated from feces of healthy adults and effect it on *P.aerogenosa* wound infection

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ABSTRACT

Lactobacillus reutri may be employed as a functional antibacterial agent. Optimizing the antibacterial activity of L-glutaminase extracted from L. reurri led to an increase in the enzyme's synthesis in low-medium production media. By employing L. reutri as antibiotics and antibacterial biofilms, this work aims to increase the production of high-activity L-glutaminase that has been isolated from the bacterium. In ion exchange chromatography, the specific activity was 4.7 U/mg of protein, with a yield of 65% and 6.6 times purification. In gel filtration, the specific activity was 10 U/mg of protein, with a yield of 60% and 16.6 times purification and the molecular weight of glutaminase was found to be 34-40 KD determined by sodium dodecyl sulfate polyacrylamide. The results indicated that L-glutaminase that was isolated from L.reutri had strong antibacterial and antibiofilm properties. The antibacterial activity of isolated L-glutaminase was shown to be substantial ($P<0.05$) when applied to P. aeruginosa isolates. Furthermore, the antibiofilm activity of purified L-glutaminase was found to be more potent than that of pipracillin tazobactam, the reference standard drug ($P<0.05$). This finding may prove promising for the development of natural probiotics or other treatments for bacterial illnesses in the future.

Key word: Lactic acid bacteria (LAB), Biofilms, Antibiotic, Antibiotic Susceptibility. Probiotics, L- Glutaminase.

INTRODUCTION

According to Charteris *et al*. (1998), probiotics are living microorganisms with nutritional and physiological benefits. One hundred years ago, Elie Metchnikoff was the first person to do research on probiotics (Muhammad and Ahmad, 2018). Many probiotic microorganisms are composed of lactic acid bacteria (LAB), including Bifidobacterium sp., Lactobacillus sp., Streptococcus sp., Enterococcus sp., and Pediococcus acidilactici (Sharma *et al*., 2014).L-glutaminase (EC3.5.1.2; L-glutaminase amid hydrolase). L-glutaminase breaks down L-glutamine into an ammonia group and glutamic acid (Sarkar *et al*., 2014; Ayodeji *et al*., 2019). Glutamine amino transferases, often known as glutamines, catalyze the bacterial conversion of glutamine to glutamate. Acid resistance and the production of the bioactive metabolites glutamate, -amino butyrate (GABA), and glutamyl γ gammapeptides are both influenced by glutamine deamination. Both prokaryotes and eukaryotes rely heavily on L-glutaminase for their nitrogen metabolism (Katikala *et al*., 2009).Potential sources of L glutaminase include bacteria, fungi, and yeast. It is preferable to use these microorganisms for L-glutaminase synthesis due to their easy processing, low production costs, and straightforward growth requirements (Sarkar *et al*., 2014). L-glutaminase is a very significant medicinal substance that is employed to treat illnesses like cancer and to enhance other organ ailments like gastrointestinal issues and antibacterial effects. Numerous earlier investigations used bacteria from various species and environments, including Lactobacillus ferment and Aspergillus niger, to screen, isolate, and purify L glutaminase. (Woraharn *et al*., 2014; Sushma *et al*., 2017; Dutt *et al*., 2014).The genus Lactobacillus is a member of the big LAB group and is frequently utilized in food as probiotics and as starters for fermented foods (Arena *et al*., 2016). Among the lactobacilli, Lactobacillus salivarius is a type of Gram-positive, anaerobic rods that do not form spores. It is commonly found in human saliva and other oral secretions, as well as in the digestive tracts of both humans and animals (Caralampopoulos *et al*., 2009). It's thought that many LAB have probiotic properties."Live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" is the definition of probiotics (Guarner *et al*., 1998). Numerous LAB-produced antimicrobial substances, such as bacteriocins, organic acids, diacetyl, and hydrogen peroxide, are capable of inhibiting a wide range of harmful microbes (Bamidele *et al*., 2013). A commensal bacteria called L. gasseri is present in the female lower urogenital tract and the human GI tract (Walter, 2008; Matsuda *et al*., 2018). The effect on women's health has been studied recently (Matsuda *et al*., 2018; Phukan et al., 2018). Mahdi *et al*. (2024) conducted a study on the effectiveness of l-glutaminase derived from clinical isolates of L. gasseri BRLHM as an antibacterial agent against P.aeruginosa that is resistant to many drugs.

MATERIAL AND METHODS

Isolation and identification of *L. reuteri* from feces

Fifty six isolates of *Lactobacillus spp* obtained from 111 samples of feces of healthy adults volunteer (22 to 30 years) The biochemical reactions of the *L. reuteri* isolates were, as displayed in table 3-3. All the bacterial isolates were oxidase, catalase, gelatinase and citrate negative; *Lactobacillus* forms a clear zone on MRS medium that is supported with $CaCO_3$, because of their production of lactic acid that would combine with calcium then precipitate. All

Lactobacillus isolates were capable to grow in the existence of 2.5 and 5 % NaCl while at 6.5 % NaCl they were not capable to grow. According to the morphological and biochemical tests, the isolates were identified as Lactobacillus spp. The slides underwent heat fixation, air drying, and Gram staining. The slides were next examined in accordance with , which studies the size, shape, color, and edge of colonies grown on MRS agar (Atlas *et al* .,1995; Goldman and Green ,2015).

Preparation of crude L-glutaminase BRLG:

L.gasseri in its raw form With a few modifications, L-glutaminase BRLG was produced in accordance with the methodology described by Padma *et al*. (2009). Subsequently, the culture was grown in L-glutamine broth (made by dissolving 1 g yeast extract, 5 g glucose, 5 g glutamine, 6 g Na2HPO4.2H2O, 3 g KH2PO4, 0.49 g MgSO4, 0.05 g NaCl, 0.002 g CaCl2, 20 gm of agar, and 2.5% w/v ethanolic phenol red solution 0.06 ml in 1 liter of deionized distilled water. The pH was then adjusted to 6.2 and the broth was autoclaved at 121 c for 10 minutes.) at 37°C for 24 hours under anaerobic conditions. The cells were separated by centrifugation at 8000 rpm for 15 minutes at 4°C. Aliquots of the supernatant were then pH-corrected to 6.2 and kept at 4°C until analysis. Semi-quantitative screening was conducted using the Gulati *et al*. (1997) approach. The procedure outlined by Mannan *et al*. (1995) was followed in the quantitative assay to ascertain the enzyme activity in every isolate. Imada *et al*.'s (1973) assay of enzyme activity was determined utilizing these procedures.

Determination of optimum condition for L-glutaminase production:

The optimum of carbon source, nitrogensource, pH, and temperature and incubation period was estimated according to the method described by Savitha *et al* (2016).

RESULTS

Isolation and identification of *L. reuteri* from feces

Fifty six isolates of *Lactobacillus spp* obtained from 111 samples of feces of healthy adults volunteer (22 to 30 years) The biochemical reactions of the *L. reuteri* isolates were, as displayed in table 1. According to the morphological and biochemical tests, the isolates were identified as Lactobacillus spp. Dependent on the results of API 50 KIT six species were identified as the following L. plantarum 22 isolates (39%) L. fermentum 13isolates (23%), L. reuteri 12isolates (21%), L. rhamnosus 6 isolates (10%) L. paracasei 2 isolates (3%) and L gasseri 1 (1%) as shown in fig 1.

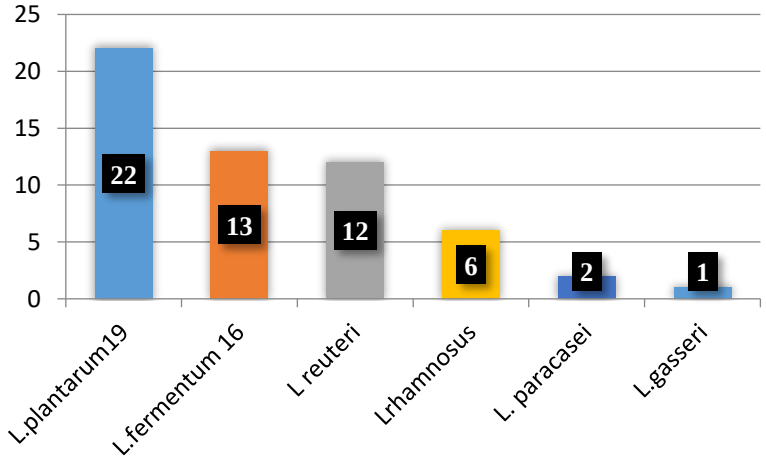

Fig (1): Percentage of Lactobacillus spp. isolated from feces of healthy adults volunteer

Semi Quantitative Screening of L- glutaminase produced from *L. reuteri isolates isolated from feces*

By using the L-Glutamine agar media containing phenol red were used for assay Semi Quantitative Screening of L- glutaminase produced from L. reuteri isolates isolated from feces . Results indicated a correlation between the pink color and the amount of generated enzyme. *L. reuteri* isolate number 3 could be regarded as the highest L-Glutaminase producer because it had the largest a visible pink zone when compared to the other isolates. Result showed that the isolates were L-glutaminase producers, as shown in (Table 1).

(Table 1).with different levels of productivity.

Isolates	Pink zone (Mean ±SD) (mm)
***L. reuteri* no.1**	10.22±1.93
***L. reuteri* no. 2**	7.49 ±1.64
***L. reuteri* no. 3**	13.57±3.8
***L. reuteri* no.4**	0±0
***L. reuteri* no. 5**	11.42±2.07
***L. reuteri* no. 6**	8.87± 0.56
***L. reuteri* no.7**	0±0
***L. reuteri* no. 8**	9.89±3.11
***L. reuteri* no. 9**	12.39 ±2.08
***L. reuteri* no.10**	11.52±2.23
***L. reuteri* no. 11**	6.71±1.22
***L. reuteri* no. 12**	13.03 ±1.66
***L. reuteri* no. 12**	13.03 ±1.66

Quantitative Screening of L- glutaminase produced from *L. reuteri isolates*

After Semi Quantitative screening, the specific L-glutaminase activity for L. reuteri isolates in liquid media were identified. The isolate No.3 provided the highest (89.06 U/mg protein) Specific activity. Moreover, isolate No. 8 showed the lowest specific activity 33.98 U/mg proteins. According to these results, the isolate No. 3 was selected to be used for improving its ability in L-glutaminase production .

Isolates	Enzyme activity	Protein mg/ml	Specific activity (U/mg)
no.1	**9.97**±o.58b	**0.122**±0.02	**78.66±3.42c**
no. 2	**5.32**±2.42d	**0.136**±0.03	**47.42±2.69d**
no. 3	**13.88**±1.71a	**0.161**±0.01	**89.06±1.99a**
no.5	**10.29**±1.06b	**0.143**±0.01	**81.05±3.06b**
8	**7.01**±1.45c	**0.122**±0.01	**33.98±2.14d**

no.9	10.09±2.16ab	**0.108**±0.04	**81.22±1.92b**
no. 10	**10.33**±1.62b	**0.139**±0.01	**79.55±0.93b**
no.12	**11.93**±2.51ab	**0.142**±0.02	**83.69±2.34a**
LSD	5.439**	**NS**	**6.38****

(Table **2**).LSD test was used to calculate the significant differences between tested mean, the letters (A, B,C and D) LSD for rows represented the levels of significant, highly significant start from the letter (A) and decreasing with the last one. Similar letters mean there are no significant differences between tested mean

Extraction of L- glutaminase produced from L. reuteri isolate no.3

The L. reuteri isolate no. 3 was selected to be used for improving its ability in L-Glutaminase production by optimization. This approach is used to screen P. fluorescens for L-glutaminase synthesis. Variations in the genes responsible for producing this enzyme may account for specimen discrepancies in their ability to make it (Kumari *et al*., 2015).A. faecalis is used to screen for L-glutaminase (Pandian *et al*., 2014).Ion exchange chromatography (DEAE-Cellulose)

The results in figure 3-6 showed that their Ionic exchange chromatography patterns showed one protein peak in wash elution and one peak in stepwise elution. Only one peak among the stepwise elution peaks represented glutaminase activity appeared by measuring the absorbance at 280 nm of each fraction, at elution fractions tube glutaminase s (56, 1.3) with 0.6 M of NaCl.The fractions were pooled and tested for glutaminase activity with 0.65 U/ml, specific activity 4 U/mg a fold purification of 6.6 and protein yield of 65 % as shown in table 3-5.LGlutaminase from Bacillus cereus were purified using a DEAE-cellulose exchanger.

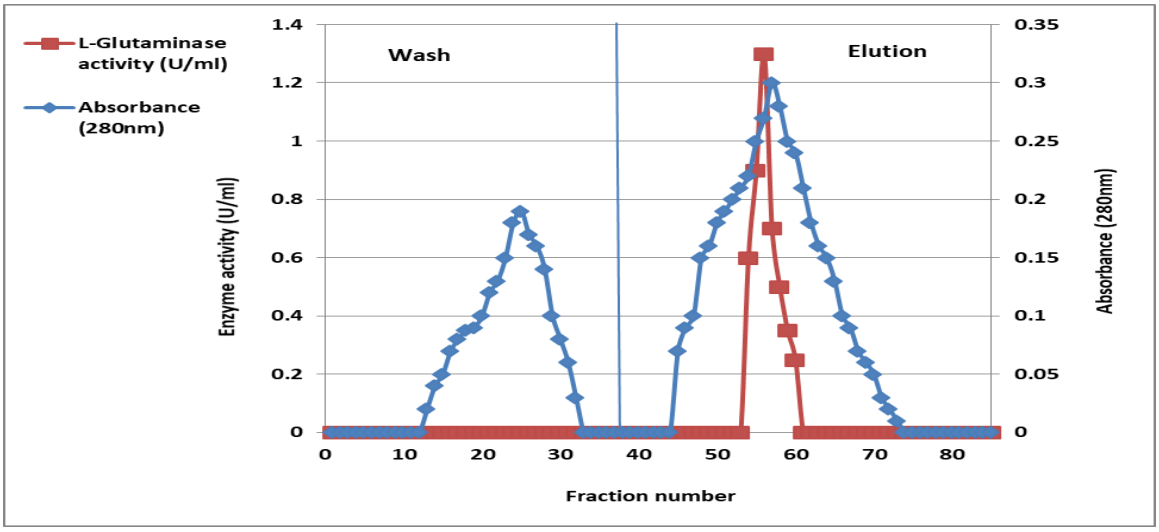

Fig (2): Ionic exchange chromatography for Rhamnocin LHM through DEAE cellulose column (2x 25) cm. The column was calibrated with 0.5 M phosphate buffer, pH7, flow rate 60ml/hrs and 3 ml fraction, eluted with stepwise (0.1-1) M NaCl.

Gel filtration chromatography

Further purification carried out by a gel filtration using Sephadex G-100 column L- glutaminase active fractions from DEAE-cellulose were pooled and passed through gel filtration column. The fractionation yielded one protein peak as absorbance reading at 280nm, this peak series (19,1) contained glutaminase activity **0.7** U/ml, specific activity **10** U/mg and the purification fold was **16.6** with yield of protein **60** % as mentioned in figure 3-7 and table 3-5. Gel filtration is unusual in that fractionation is based on the relative sizes of protein molecules and is carried out utilizing porous beads as the chromatographic support.

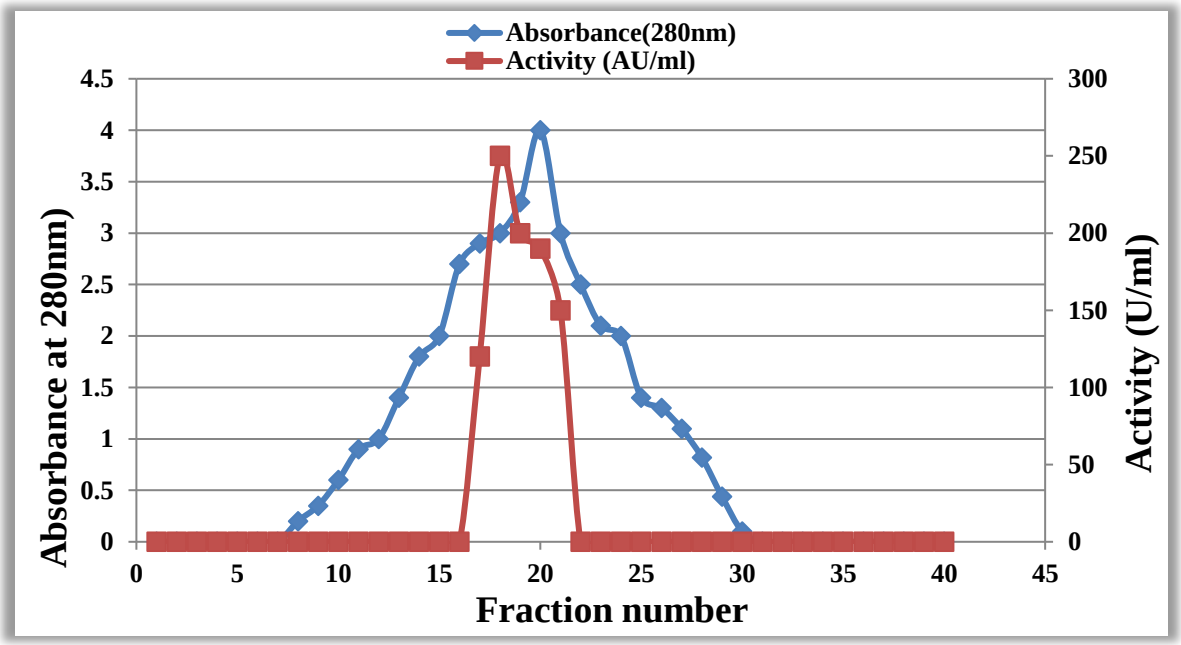

Fig (3): Gel filtration chromatography for purified l-glutaminase by using Sephadex G- 100 column (2x35) cm. The column was calibrated with 0.5 M phosphate buffer, pH7; flow rate 30 ml/hrs and 5 ml/fraction.

Steps of purification	Volume (ml)	Enzyme activity (U/ml)	Protein concentration (mg/ml)	Specific activity (U/mg)	Total activity (U)	Purification (folds)	Yield (%)
Crude enzyme	70	0.3	0.5	0.6	21	1	100
Ammonium sulphate precipitation 70%	14	1	0.45	2	14	3.3	66
DEAE-cellulose	21	0.65	0.15	4	13.6	6.6	65
Sephadex- G100	18	0.7	0.1	10	12.6	16.6	60

(Table 3**).Equations for purification table**

Determination of Molecular Weight of glutaminase

Sodium Dodecyl Sulfate-Polyacrylamide was used to determine the molecular weight of glutaminase. Gel electrophoresis by using SDS-PAGE, the molecular weight of glutaminase was calculated using the standard curve that depicts the relationship between log of the molecular weight and relative mobility (Rm) for standard proteins. The molecular weight of glutaminase was found to 34-40 KD, as illustrated in fig (4 ,5)

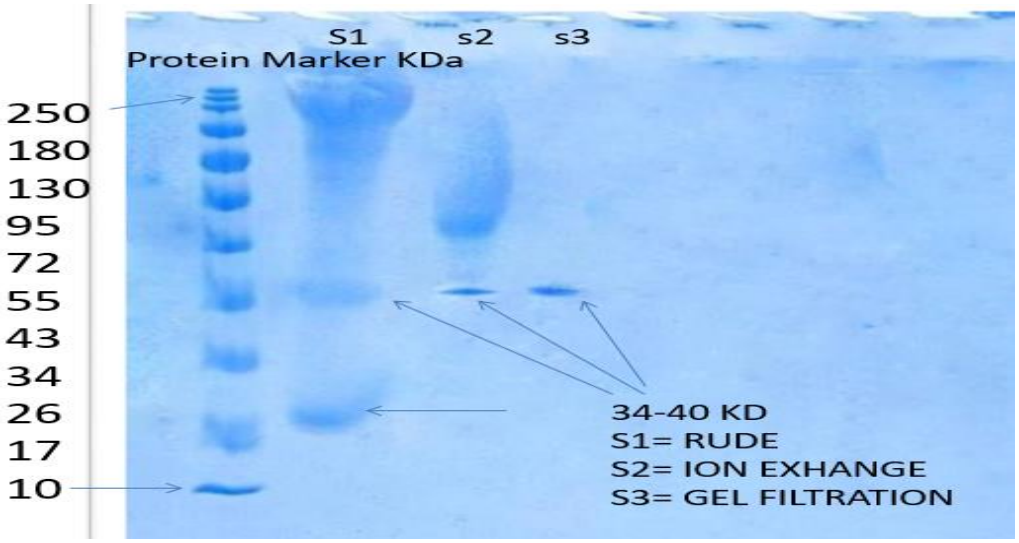

Figure (4**): Sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE) of the purified Rhamnocin LHM.**

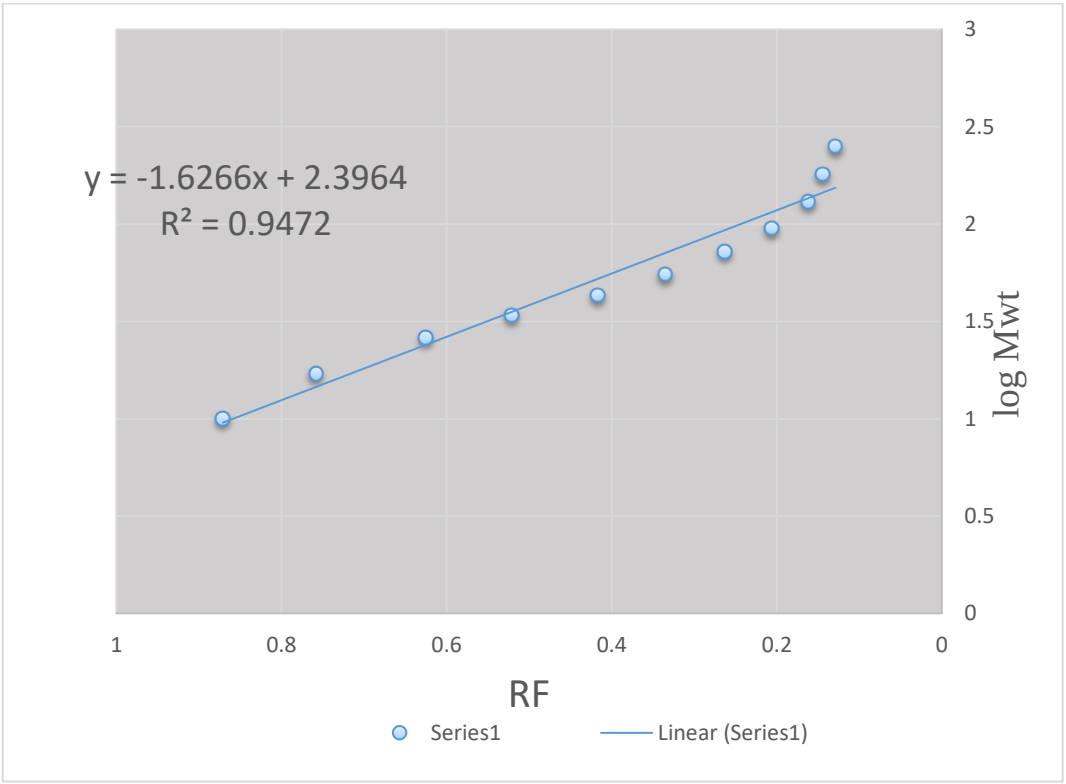

Fig (5**): Determination of molecular weight of purified glutaminase.**

Log Mwt: logarithm of molecular weight, Da: Dalton.

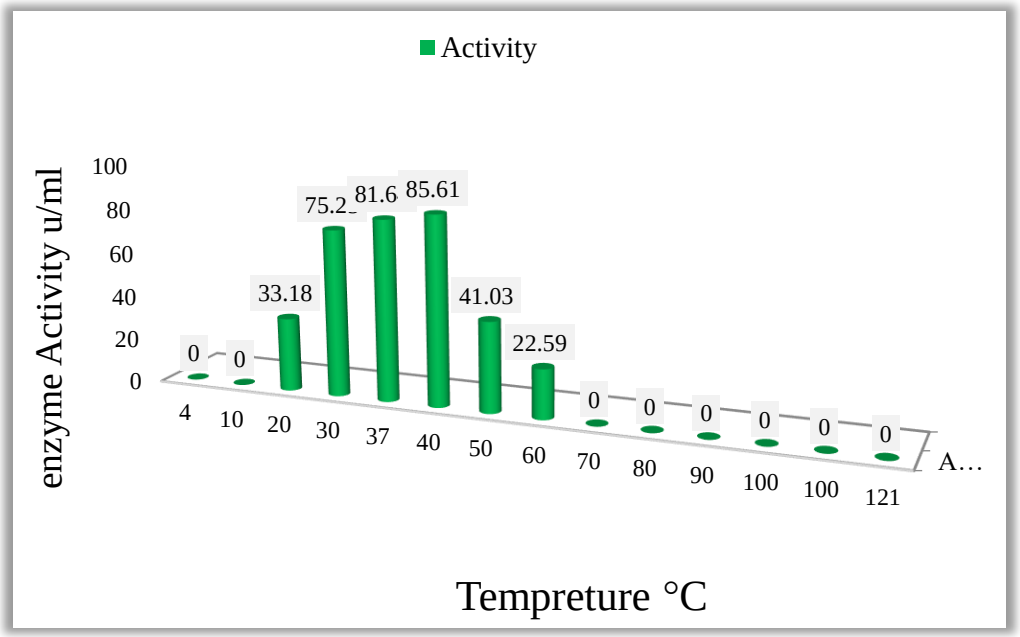

Fig (6): activity of purified L-glutaminase purified from L. reuteri isolate no.3 at different temperatures

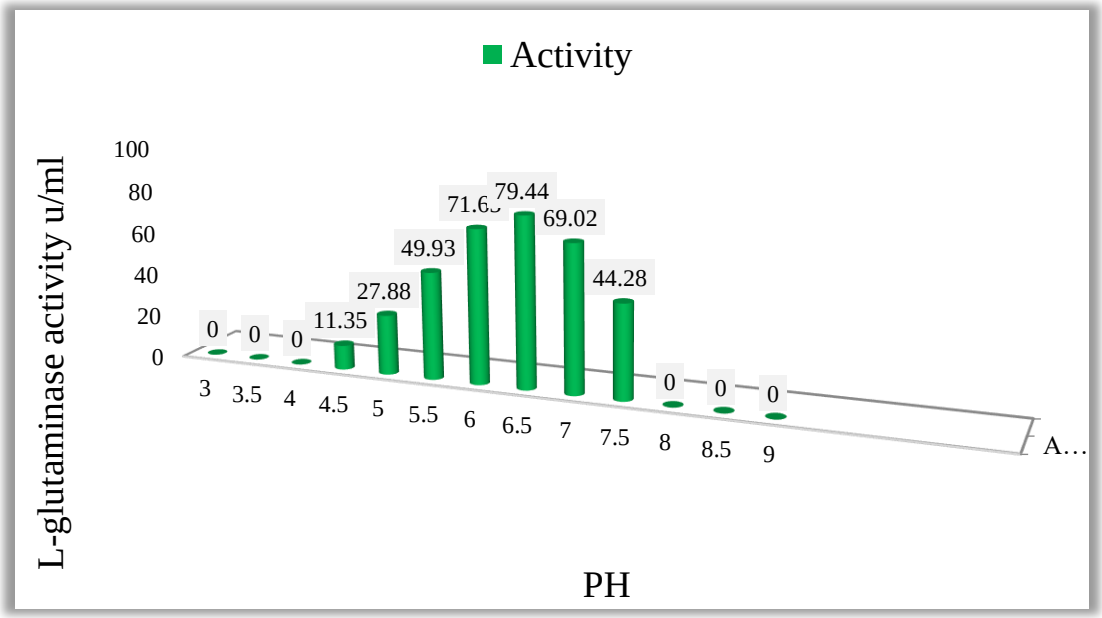

Fig (7) -: activity of L-glutaminase purified from L. reuteri isolate no.3 at different pH values.

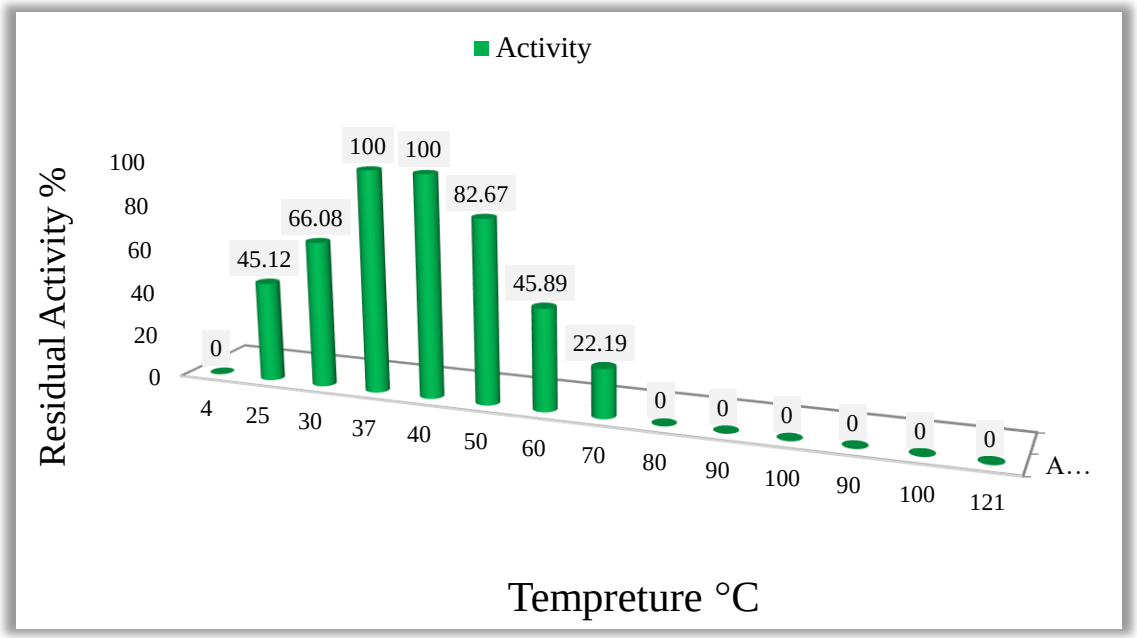

Figure (8): thermal stability of L-glutaminase purified from L. reuteri isolate no.3 at different temperatures

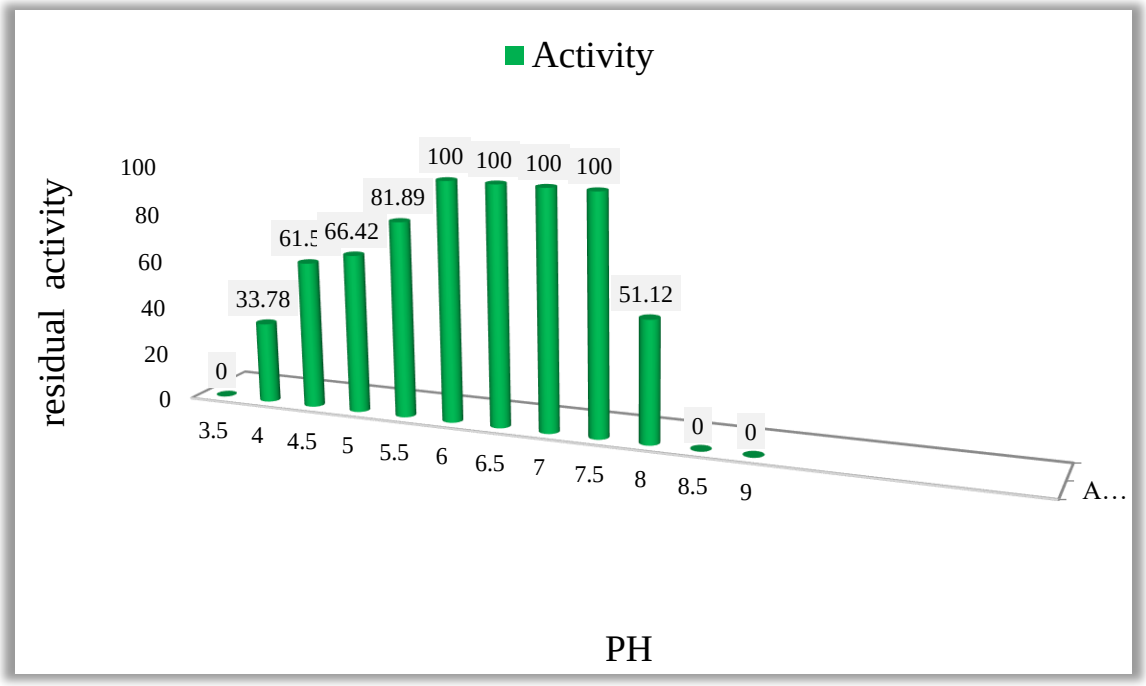

Fig (9): pH stability of L-glutaminase purified from L. reuteri isolate no.3 at different pH values.

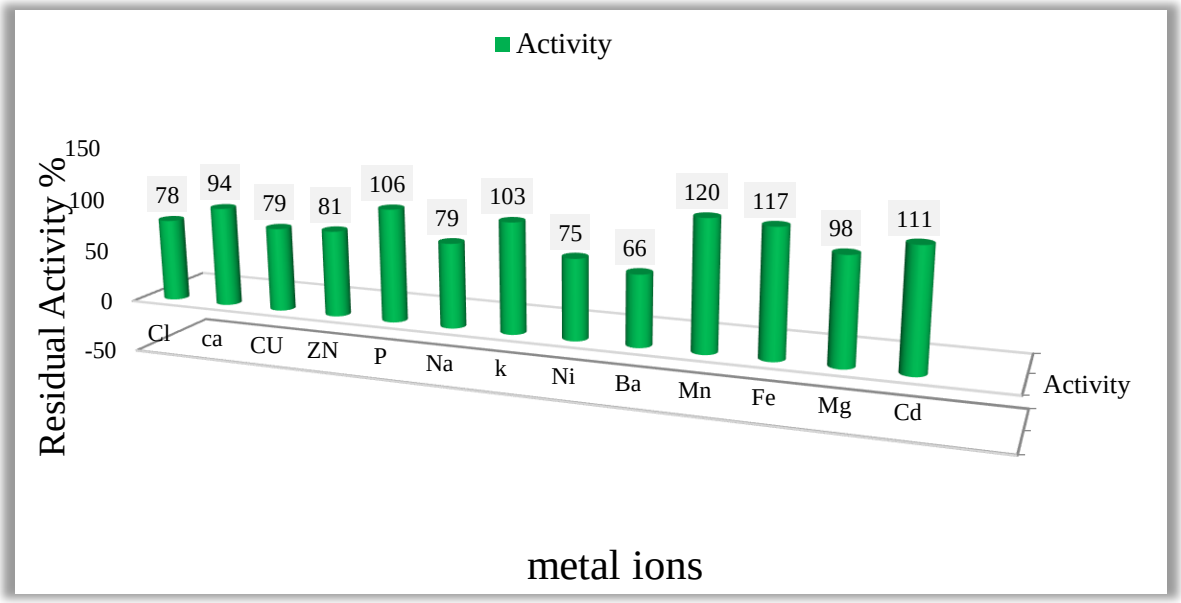

Fig (10**): effect of metal ions on L-glutaminase purified from L. reuteri isolate no.3activity**

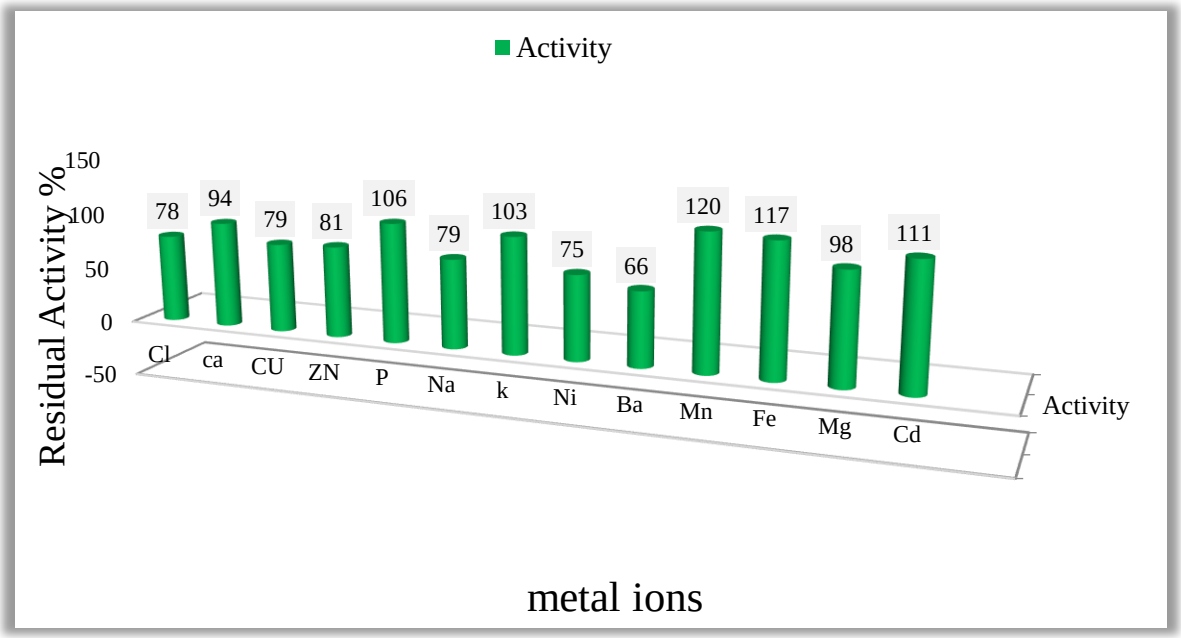

Fig (11): effect of metal ions on L-glutaminase purified from L. reuteri isolate no.3activity

Table (4): Antibacterial activity of L-glutaminase purified from L reuteri on *P. aeruginosa* isolates in-*vitro*

Isolates number		Concentration µg/ml			P value
		Crude	Purified L-glutaminase		
	Crude L-glutaminase 64	L-glutaminase 32	64	32	
P. aeruginosa 5	C 12.o7±2.11	C 14 .23±1.06	A 34.7±2.98	B 22.55±3.04	**0.01 ***
P. aeruginosa9	C 9.66±1.71	C 13.59±2.44	A 29.09±3.07	B 24.3±1.09	**0.01 ***
P. aeruginosa24	C 11.69±1.02	C 14.08±0.08	A 31.22±1.33	B 20.15 ±2.03	**0.01 ***
P. aeruginosa31	C 8.11±0.9	C 10.22.6±0.89	A 25.71±2.09	B 17.49±0.03	**0.01 ***
P. aeruginosa33	C 13.01±1.92	C 15.02±0.08	A 30.51±0.7	B 19.12±1.76	**0.01 ***
P. aeruginosa58	C 7.05±0.9	C 10.38±0.6	A 24.06±1.05	B 20.11±0.9	**0.01 ***
P value	**NS**	**NS**	**NS**	**NS**	

test was used to calculate the significant differences between tested mean,(*: significant erences

letters (A, B and C) LSD for rows represented the levels of significant, highly significant start m the letter (A) and decreasing with the last one. Similar letters mean there are no significant erences between tested mean.)

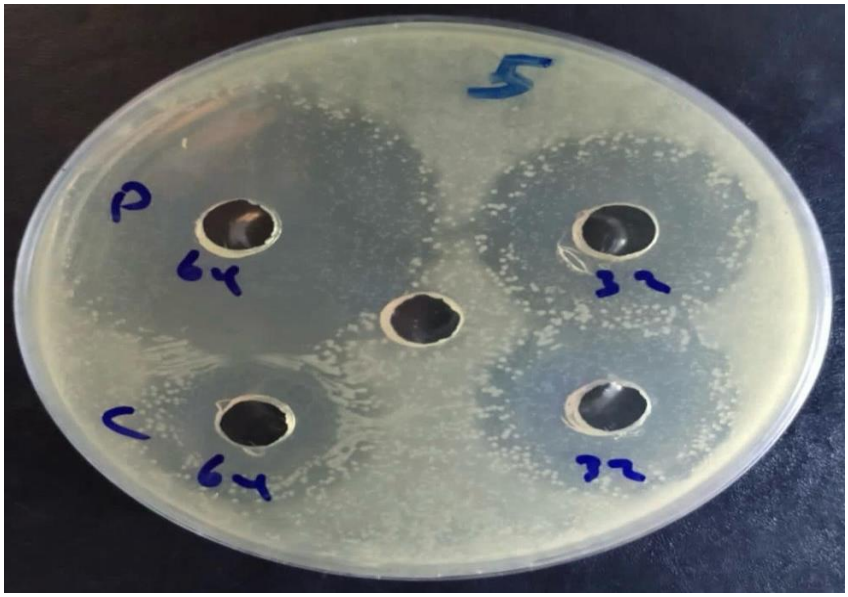

Figure (12): Antibacterial activity of crude and purified L-glutaminase against *P. aeruginosa.* A: at concentration 64 µg/ml, B: at concentration 32 µg/ml.

Determination of MIC and Sub-MIC of Pipracillin tazobactam and purified L-glutaminase

The six *P. aeruginosa* isolates were selected according to it is the ability to greater biofilm production and it is the capability of greater antibiotics resistance. (*P. aeruginosa* no. (5,9,24,31,33,58)isolates. Pipracillin tazobactam and purified L-glutaminase were tested by determining the MIC utilizing a microtiter plate with Resazurin stain.

Table *(* 5): MIC and sub MIC of Pipracillin tazobactam and purified L-glutaminase against *P. aeruginosa* isolates.

Treatment µg/ml	Ps. 5	Ps. 9	Ps. 24	Ps. 31	Ps. 33	Ps. 58
MIC of Pipracillin tazobactam	32	256	64	64	16	8
Sub-MIC of Pipracillin tazobactam	16	128	32	32	8	4
MIC ofpurified L-glutaminase	8	64	32	32	128	256
Sub-MIC of purified **L-glutaminase**	4	32	16	16	64	128

Inhibitory effect of purified L-glutaminase & **Pipracillin tazobactam on biofilm formation by P. aeruginosa isolates.**

In this study, the high resistance to antibiotics and high producing biofilm clinical isolates were chosen (**5,9,24,31,33, and 58**).To evaluate the antibiofilm activity of purified L-glutaminase from purified and Pipracillin tazobactam,sub MIC were used .Optical density exhibited significant differences before and after treatment. ($P < 0.05$) as displayed in table 5 purified **L-glutaminase** activity as antibiofilm agent was statisticaly significant higher than the activity of the Pipracillin tazobactam ($P < 0.05$) in vitro. On the other hand, after comparing among isolates, the results revealed that the isolates *P. aeruginosa* no.5, no.9 and no.58 were the most biofilm producing isolates after comparing with other clinical isolates.

Table (6**): Effect sub-MIC of purified L-glutaminase and Pipracillin tazobactam on biofilm formation from *P. aeruginosa***

No. of Isolates	O. D. Before treatmen	O. D. after Treatment with sub MIC		P value
		Pipracillin tazobactam	purified **L-glutaminase**	
Ps. 5	1.622±0.01 A	0.71±0.08 C	0.47±0.17 D	**SIG 0.05**
Ps. 9	1.738 ±0.22 A	0.53±0.05 C	0.34±0.05 E	**SIG 0.05**
Ps. 24	1.691 ±0.19A	0.59±0.11C	0.29±0.08E	**SIG 0.05**

Ps.31	1.42±0.46A	0.61±0.25 C	0.48±0.09D	**SIG 0.05**
Ps.33	1.29±0.81 B	0.39±0.09 D	0.29±0.07 E	**SIG 0.05**
Ps.58	1.622±0.92A	0.55±0.12 C	0.41±0.12 D	**SIG 0.05**
P value	0.05	0.05	0.05	----------

) test was used to calculate the significant differences between tested mean, the letters (A, B,C,D ar
) for rows represented the levels of significant, highly significant start from the letter (A) and decrea
h the last one. Similar letters mean there are no significant differences between tested mean.
nificantly)

CONCLUSION

Lactobacillus ***reutri*** are good probiotics and a good antibiotic for pathogenic bacteria, especially P. aeruginosa , as well as an anti-biofilm,and It is a good producer of the L-glutaminase enzyme , which allows the possibility of using them as a treatment in the future.

DISCUSION:

According to the measurement of L-Glutaminase activity in cell-free extracts, 5 Unit/mg of L. gasseri-specific activity and L-Glutaminase were produced. In applied and industrial microbiology, lactobacillus strains remain the most common bacteria that produce enzymes; these organisms represent a significant source of extracellular industrial enzymes (Ziadé *et al*., 2003).Since glutamine amino acid was the only carbon and nitrogen source in glutamine agar, organisms that could produce glutaminase could thrive on this medium. The PH indicator changed from yellow to pink in color. Around colonies, a pink zone was observed due to the activity of the L glutaminase enzyme, which broke the L glutamine amino acid and released ammonia, raising the pH level (Sarkar *et al*., 2014).Using this method to screen for L-glutaminase synthesis from P. fluorescens, the differences in specimens' abilities to make this enzyme could be attributable to genetic variants in the genes responsible for producing this enzyme (Kumari *et al*., 2015).Screened L-glutaminase from *A.faecalis* .Many probiotic microorganisms are composed of lactic acid bacteria (LAB), including Bifidobacterium sp., Lactobacillus sp., Strep. thermophilus, Enterococcus sp., and Pediococcus acidilactici (Pandian *et al*., 2014;Sharma *et al*.,2014).high-level pure L. gasseri BRLHM glutaminase was shown to have antioxidant properties (Hasson *et al*., 2021). Genetic variations in the genes that create this enzyme may be the cause of the variances in each specimen's capacity to manufacture it (Kumari *et al.*, 2015). This technique is employed to screen P. fluorescens for the synthesis of L-glutaminase.A. faecalis is used to screen for L-glutaminase (Pandian *et al*., 2014).According to Singh *et al*. (2016), purified L-glutaminase exhibited a specific activity of 7.44 U/mg and a purification fold of 14. 8.Using ion-exchange chromatography, L-glutaminase was also extracted with a purification fold of 46 from Streptomyces sp. (Desai *et al*.,

2016). The enzymes produced by Lactobacillus gasseri were further purified and characterized using a chromatography method (Hasson *et al*., 2021). Using gel filtration, L-glutaminase from marine Brevundimonas diminuta was isolated, achieving an activity of 60.15 U/mg and an enzyme recovery of 48.12% (Jayabalan *et al*,2010).The molecular weights of microbial L-glutaminase ranged between 35 ± 1 and 71 KDa, and electrophoresis is thought of as an additional purification step (Desai *et al*., 2016; Savitha *et al*.,2016). A few larger molecular mass exceptions have been found, including 50 KDa from Streptomyces sp. isolated from soil (Desai *et al*.,2016; Savitha *et al.,* 2016); 64 KDa L-glutaminase from Bacillus subtilis was also reported (Jambulingam and Sudhakar,2019). Whether it was crude or purified, the data indicate that a concentration of 64 µg/ml was more effective against P. aeruginosa than 32 µg/ml. This is consistent with a study by Mahdi *et al*. (2024), which found that the purified l-glutaminase had significant antimicrobial activity against isolates of P. aeruginosa ($p < 0.05$) and that it was more effective than the referral standard drug at preventing the formation of biofilms. According to Zhang *et al*. (2005), 28% of the Lactobacillus sp. isolates that exhibited enzyme activity also exhibited antibacterial activity. Elborai *et al*. (2023) reported that L-glutaminase shown efficacy against harmful microorganisms.According to Mahdi et al. (2019), Amikacin at sub-MIC dramatically reduced the production of P. aeruginosa biofilms. Mahdi et al. (2021) reported using sub-MICs of gentamicin to minimize the growth of biofilms over plastic sheets.

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فحص وتشـــخيص وإنتاج إنزيم L-glutaminase بواســـطة بكتريا *Lactobacillus reutri* المعزولة من براز البالغين الأصحاء وتأثيره على إصابة الجروح ببكتيريا *P.aerogenosa*

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المستخلص

ان Lactobacillus reutri لديها القدرة على ان تستخدم كتأثير دوائي ضد البكتريا المرضية في المستقبل. وتم تحسين التأثير المضاد للميكروبات لـ L-Glutaminase المعزول من L. reurri الذي عزز إنتاج الإنزيم في ظل الحد الأدنى من وسائل وطرق الإنتاج. و تكمن أهمية هذه الدراســة في تعزيز إنتاج L-Glutaminase المعزول من L. reutri بنشــاط عالي باســتخدام L-Glutaminase كمضاد حيوي ومضاد للاغشية الحيوية للبكتيريا. في كروماتوغرافيا التبادل الأيوني، كان النشاط النوعي هو 4.7 وحدة / ملغ من البروتين، مع إنتاجية 65٪ و6.6 لـــ purification folds. اما في الترشيح الهلامي، كان النشاط النوعي 10 وحدة/ملغ بروتين، مع إنتاجية 60% و16.6 لـ purification folds ، ووجد أن الوزن الجزيئي لانزيم L-Glutaminase يتراوح بين 34-40 كيلو دالتون تم تقديره بطريقة Sodium Dodecyl Sulfate-Polyacrylamide. وفقا للنتائج، أظهر L-Glutaminase المعزول من L.reutri المنقى نشــاطًا مضــادًا للميكروبات بشــكل كبير ضــد عزلات P. aeruginosa (P <0.05)، وكان نشــاط تكوين الغشــاء الحيوي للـــ L-Glutaminase المنقى أقوى من نشــاط المضــاد الحيوي للدواء القياسـي المرجعي، Pipracillin tazobactam (P <0.05). قد

يكون هذا اكتشافًا واعدًا لعلاجات الأمراض البكتيرية المستقبلية مثل البروبيوتيك الطبيعي.