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Evidence of Antioxidant Activity of Novel L-Glutaminase Purified from *L. Gasseri* BRLHM

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Abstract

Probiotic strains have the potential to be used as bio-preservatives and functional radical scavenging treatments in the future. Antioxidant tests, including DPPH radical scavenging, were used to evaluate the antioxidant effects of extracellular L- Glutaminase isolated from *L. gasseri* BRLHM. Parameters for the promoted production of the enzyme under minimal production media were optimized. The importance of this study lies in enhancing the production of the L- Glutaminase isolated from *L. gasseri* BRLHM with a high activity using these *L. gasseri* bacterial as antioxidant activity. In ion-exchange chromatography, the specific activity was 14.7U/mg protein, with 58.8% yield and 4.6 purification folds. In the gel filtration, the specific activity was 23.4 U/mg protein, with a yield of 54 % and 4.6 purification folds. According to the findings, L-Glutaminase isolated from *L. gasseri* BRLHM exhibited good antioxidant properties. As the concentration rose, there was a remarkable proportionate increase in scavenging activity. The IC_{50} values for control and L- Glutaminase were 36.09 1.12 and 619.8 gm/ml, respectively. The IC_{50} values were discovered to be 100 and 200 µg/ml, respectively. Conclusion: For the first time, the high of L- Glutaminase isolated L. gasseri BRLHM was shown to exhibit antioxidant. This could be a promising discovery for future radical scavenging treatments and antioxidant prophylaxis with natural probiotics.

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1.Introduction

Probiotics are live microbes that have nutritional and health benefits [1]. Elie Metchnikoff was the first to conduct probiotic research 100 years ago [2]. Lactic acid bacteria (LAB) such as *Bifidobacterium* sp., *Lactobacillus* sp., *Strep. thermophilus*, *Enterococcus* sp., and *Pediococcus acidilactici* make up many probiotic microorganisms [3]. Probiotics have several functional qualities that are beneficial to one's health. The study of probiotics' antioxidative capabilities than free radicals is an intriguing research topic. Several human diseases (e.g., inflammatory diseases, cancer, atherosclerosis, and cirrhosis) have been linked to oxidative damage [4].

Damage in DNA was induced by oxidative stress. Various malignancies have been found to have higher levels of oxidative DNA damage. Due to the increase of antimicrobial resistance, the inclusion of probiotics in food is a fascinating alternative to antibiotics that have piqued public interest [5]. Antioxidant activity of artificial antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and n-propyl gallate (PG) is powerful against a variety of oxidation processes. However, due to potential health hazards, the use of these antioxidants in food has been restricted in several countries. Because of the documented negative effects of some artificial antioxidants [6], natural antioxidants are likely to be more tolerable than those created chemically. the result, the hunt for natural, stable, safe antioxidant agents as alternatives to manufactured molecules have gained more attention. The aim of the study is to isolate L-glutaminases from *Lactobacillus gasseri* and their study antioxidant activity.

2.Experimental Procedure

Collection of samples78 vaginal swabs were isolated from healthy women. *Lactobacillus* spp. is diagnosed based on the findings of culture, microscopic, biochemical, and API 50 CHL testing [7]. Collected of *Lactobacillus* spp. from aged (15-45) years old from the Hospital AL-elweya of Obstetrics in Baghdad, Iraq, from December 2019 to March 2020. Detection, screening, and purification of L- Glutaminase purified from *L. gasseri* BRLHM Crude *L. gasseri* prepared by [8], and L-glutaminases was prepared according to [9] with a modification, local isolate *L. gasseri* purified by four steps: ammonium -sulfate precipitation at 80%, dialysis, ion-exchange using Sephadex-G100 and gel filtration chromatography [10], concentrated of protein by the Bradford method [11] at each step was measured. Study the effectiveness of L-Glutaminase purified from *L. gasseri* BRLHM as antioxidant.

- DPPH preparation: DPPH is 2,2-Diphenyl-2-picryl-hydroxyl, dissolve (3.3)ml ethanol with (2.3)mg of DPPH. The solution was shielded from light by using aluminum foil to cover the test tubes. Control positive use of ascorbic acid (10g/ml) concentration [12].
- Determination of antioxidant activity of L-Glutaminase by DPPH: Using the free radical scavenging action of L-Glutaminase was investigated [13]. The L-Glutaminase solution (1.0 mg/ml) was diluted to five different concentrations: 12.5 , 25 , 50 , 100, and 200 mg/ml. Each concentration of L-Glutaminase (10) was mixed with ethanol (490) being and added with a (500) DPPH solution [12]. A residual amount of DPPH was calculated based on a reduction in absorbance at 517 nm after 30 minutes of incubation at room temperature. The % inhibition of DPPH was estimated using the equation formula[14], which is shown below.

$$\text{Scavenging action \%} = \frac{OD^{A} - OD^{S}}{OD^{A}} \times 100 \quad \text{.....(1)}$$

OD = optical density

A=Control

S= Sample

3. Results and Discussion

3.1. Isolation of *Lactobacillus gasseri*

78 isolated go to the *Lactobacillus* genus were dependent on the microscopic examination, cultural examination, biochemical tests, and API 50 CHL system. *L. gasseri* and *L. crispatus* were isolated from vaginal of healthy women's (vaginal swabs') in high percentages (30.769)% (n=24) out of 78 *Lactobacillus* isolates, while *L. acidophilus* constituted (14.102)% (n=11), *L. plantarum* with (10.256)% (n=8), *L. casei* with (6.410)% (n=5), *L. vaginalis* with (5.128)% (n=4), and *L. jensenii* and *L. brevis* with (1.282)% (n=1) out of *Lactobacillus* isolates.as shown in figure 1.

The vaginal flora was found to be dominated by eight different lactobacilli species, the most common of which were *L .crispatus* and *L. gasseri*.

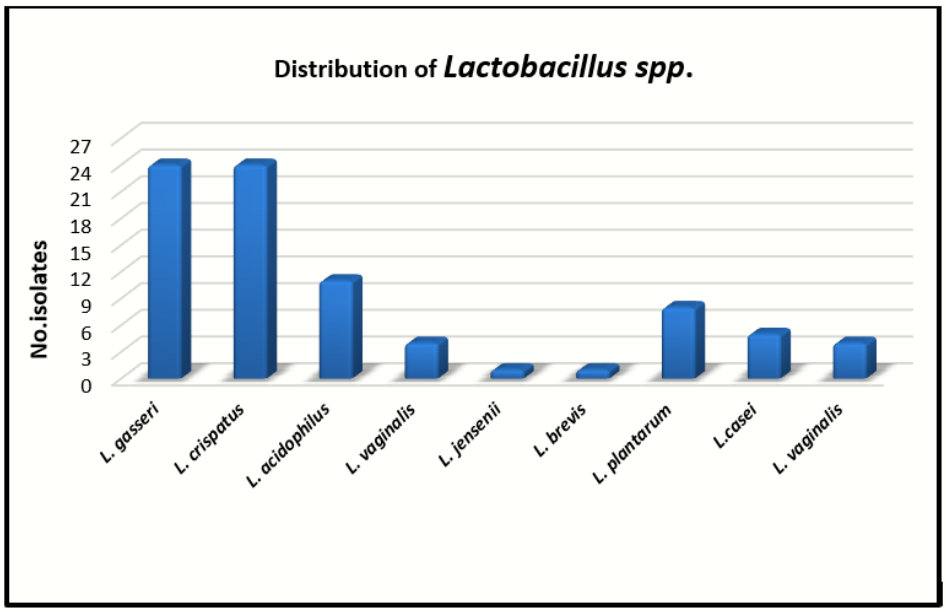

Figure 1: Distribution of Lactobacillus spp. from vaginal of healthy women's

3.2. Detection of L- Glutaminase purified from *L. gasseri* BRLHM.

L. gasseri isolate no.24 was more active in L-Glutaminase production according to the steps of the primary screening for L-Glutaminase production, thus was selected for the following steps of this current study. The selected isolates were characterized by their ability to produce heavy growth L-Glutamine-containing broth medium and had a red or pink color on L-Glutamine broth medium during semi-quantitative screening steps under optimum conditions for L-Glutaminase production, the initial pH (7), and incubation period (48) hr. at 37^{o}C. The L-Glutaminase activity indicated that the *L. gasseri* and specific activity reached 5 Unit/mg proteins.

3.3 Semi-Quantitative Screening

The *L. gasseri* BRLHM isolate no.24 isolate was cultured on an L-Glutamine broth medium containing L-Glutamine and monitored during 48 hr. of incubation, resulted from the reaction of ethanolic phenol red due to the variation in ammonia production between bacterial isolates since the active L- Glutaminase producing isolates increased NH3 and intensity of color production which indicated the activity of this L-Glutaminase screening method Figure 2. The 12 *L. gasseri* BRLHM isolates that grow on L-Glutamine broth during 24 hr. revealed pink color, while the other isolates formed pale yellowish color. The color intensity indicated bacterial activity to hydrolyze L-Glutamine and liberate glutamic acid and ammonia; this can be used as an indicator to produce L-glutamine. *L .gasseri* BRLHM isolate no.24 isolate was selected for further steps of this current study. This color change is due to changes in the pH of the medium, as L-Glutaminase causes the breakdown of the amide bond in L-Glutamine and liberates ammonia the L- Glutaminase activity present in Lactobacillus. Lactobacillus screening is based on the qualitative approach that Gulati *et al*., [20] identified. The change of color of the medium from yellow to pink is an indicator of the extracellular production of L- Glutaminase. This color shift is attributable to a change in the pH of the medium. Phenol red is yellow at acidic pH and turns pink at alkaline pH, thereby forming a pink zone around the bacterial colonies. The findings were those in agreement with (Savitha *et al*., [21].

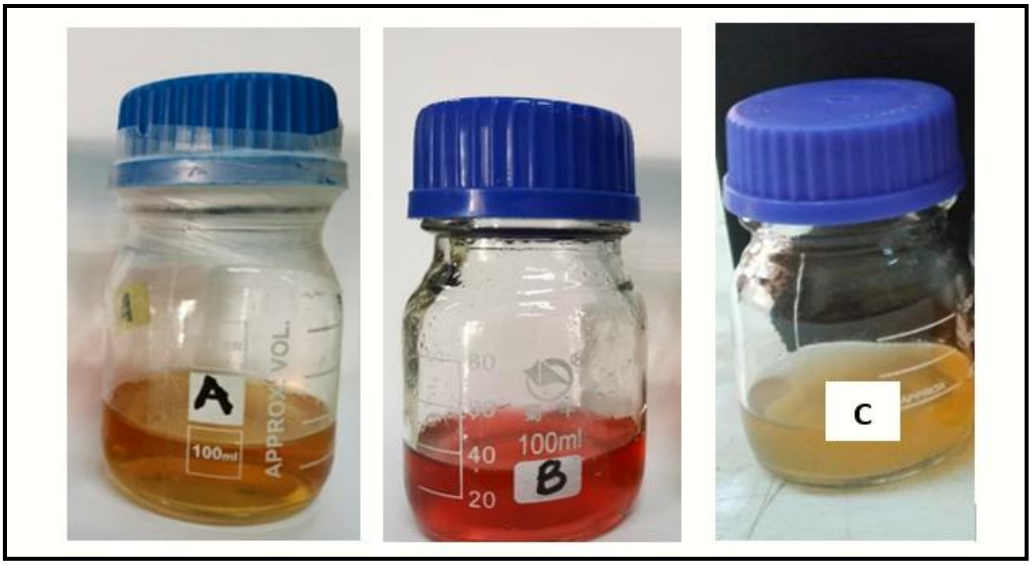

Figure 2: L- Glutaminase production on L-Glutamine broth medium during 48 hr. A= control, B= strong pink, C= pale pink weakly.

3.4. L-glutaminases Purification

Dialysis, Ion exchange technique using Sephadex-G100 column, with 80% ammonium sulfate precipitation saturation have been used to L-glutaminases purification was eluted using a linear gradient of 0.05–0.1 M NaCl. The first peak was found between 0.05 and 0.1 M, while the second peak was L-glutaminases, which eluted in 0.4–0.5 M NaCl. L-glutaminases that eluted in 5–10% of 1 M NaCl, and L-glutaminases that eluted in 40–50% of 1 M NaCl, are the first and second peaks, respectively. In ion-exchange chromatography, the specific activity of L-glutaminases was 14.7U/mg protein, with 58.8% yield and 4.6 purification folds. figure. 3. In the gel

filtration the specific activity of L-glutaminase was 23.4 U/mg protein, with a yield of 54 percent and 4.6 purification folds. figure 4. This study introduced a new L-glutaminase which belonged to the l-glutaminases group and was termed L-glutaminase BRLHM because it differed from other L-glutaminase identified by lactobacillus. Purification of L- Glutaminase was critical for gaining a better knowledge of the enzyme's function [15].

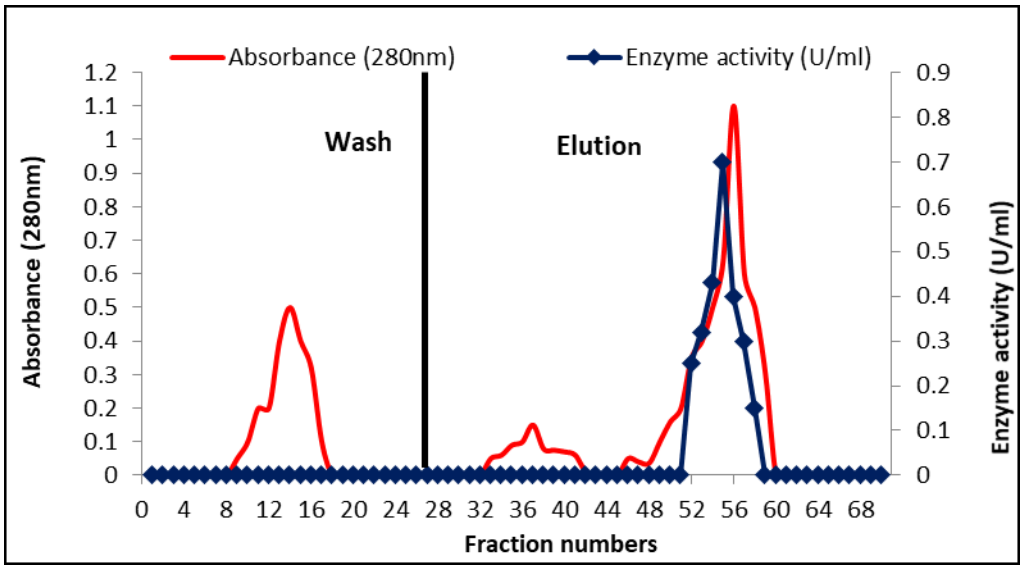

Figure 3: Ion exchange chromatography for L- Glutaminase.

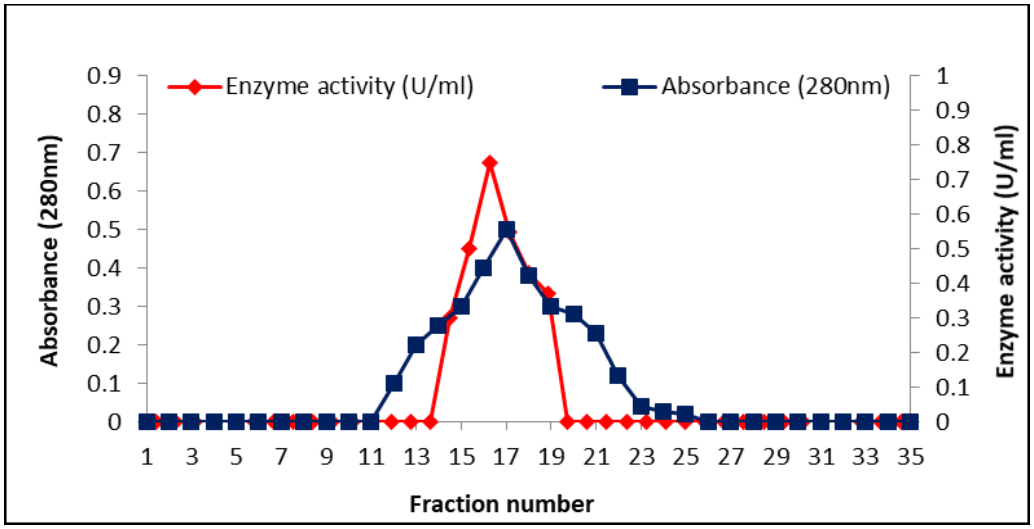

Figure 4: Gel filtration chromatography for L- Glutaminase.

3.4. Antioxidant activity of L- Glutaminase by using DPPH

The results of my study of L- Glutaminase purified from *L. gasseri* BRLHM isolate showed the DPPH root was displaced immediately proportional to the increase in concentration. The scavenged effect is assigned a concentration of (12.5,25,50,100, and 200) g/ml, as shown in figure 5. P-value test ascorbic acid was < 0.0012 at concentration 200 µg/ml. while the P-value test for purified L-Glutaminase was < 0.0001 at a concentration of

200 µg/ml. The IC_{50} value of control and L- Glutaminase purified from *L. gasseri* BRLHM isolate are (36.09± 1.12 and 619.8 µg/ml respectively. For DPPH radicals, the IC_{50} values were determined to be 100 and 200 g/ml, respectively. as indicated in table (1). The intact cells of L- Glutaminase purified *L. gasseri* isolate displayed powerful antioxidant activity *in vitro*, which was like the antioxidant activity of *L .plantarum* by [16]. These findings backed with confirmed of [17], who found that proteins isolated from *Bifidobacterium animalis* cells have antioxidant activity *in vitro*.

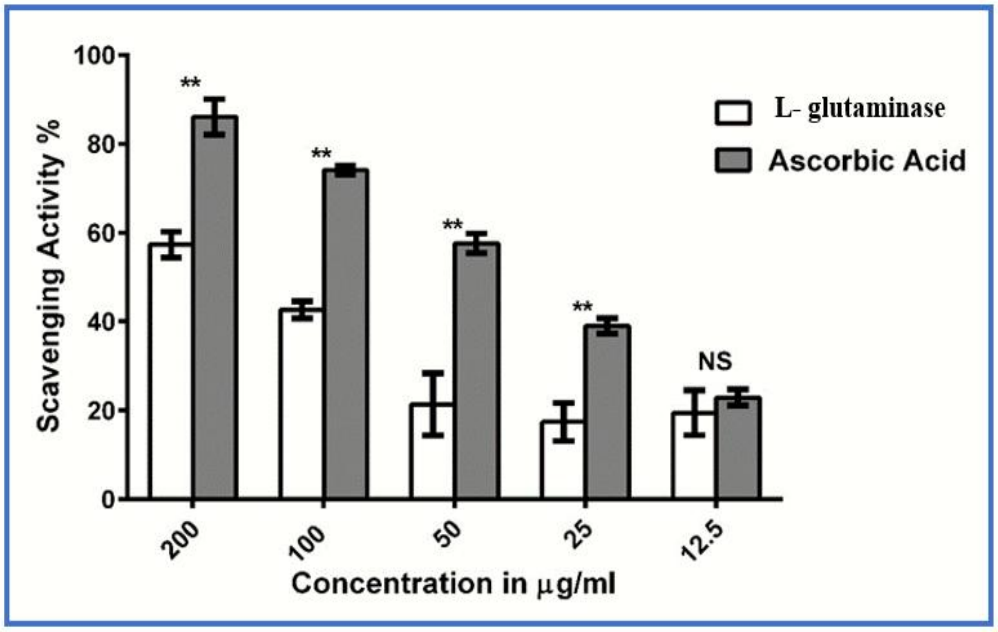

Figure 5: Antioxidant activity of L- Glutaminase purified from *L. gasseri* BRLHM isolate by DPPH.

One of the most often utilized tests is the scavenged effect test. Pure L-Glutaminases and a standardized antioxidant molecule are used to demonstrate the DPPH radical scavenging capability of purified L-Glutaminases. When antioxidant compounds returned an electron and a proton, the hue shifted to yellow [14].

Table 1. IC_{50} value for antioxidant activity of L- Glutaminase

Samples	IC_{50} value in µg/ml (DPPH free radical)	P value
L-Glutaminase	619.8	< 0.0001
Control	36.09± 1.12	< 0.0012

Glutamine can influence redox homeostasis, bioenergetics, nitrogen balance, and other processes, being a precursor to glutathione, the most important non-enzymatic cellular antioxidant, and it is convert glutamine to glutamate, which is then converted to alpha-ketoglutarate for further metabolism [18]. L-Glutaminase utilized scavenge free radicals generated *in vitro* by donating their H, which is the most widely used technique for

evaluating the antioxidant capacity of pure substances. As a result, discovering antioxidants from natural sources is becoming increasingly popular [19].

4. Conclusion

My study demonstrated for the first time that the high-level of L- Glutaminase was purified *L. gasseri* BRLHM to have antioxidant effects. This could be a promising discovery for future radical scavenging therapies and antioxidant prophylaxis with natural probiotics.

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Conflicts of interest: There are no conflicts of interest.

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