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# تأثير بكتريا *Lactobacillus gasseri* ضد الأصابة ببكتريا *Pseudomonas aeruginosa* في الفئران

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## الخلاصة

درس تأثير راشح بكتريا *Lactobacillus gasseri* ضد الأصابة ببكتريا *Pseudomonas aeruginosa* في الفئران ، اذ حقنت مجموعة من الفئران داخل الغشاء البريتوني ب 0.25 مل من راشح بكتريا *Lb. gasseri* مدة خمسة أيام ، بعدها حقنت ب50.2 مل خلايا حية لبكتريا *P. aeruginosa* ( 01 $^{8}$ خلية / مل ) داخل الغشاء البريتوني ، بينما حقنت مجموعة السيطرة ب 0.2 مل من دارئ الفوسفات.

مل دارئ الفوسفات داخل الغشاء البريتوني وأخذت محتوياته . تم عمل تخافيف من محتويات البريتون ، وزرع 0.1 مل على الأوساط الزرعية لحساب عدد المستعمرات البكتيرية

تم قتلت الفئران بعد 12 ساعة من حقنها ببكتريا *P . aeruginosa* ، اذ تم حقن 5 النامية . حسبت أعداد المستعمرات النامية وقورنت الأعداد بمعاملة السيطرة ، كذلك تم حساب نسبة الخلايا البلعمية ( Macrophage ) في محتويات البريتون .

أظهرت النتائج عدم تأثر الفئران المعاملة براشح بكتريا *Lb. gasseri* عند تعريضها لبكتريا *P. aeruginosa* خلافا للمجموعة التي عرضت لبكتريا *P. aeruginos* فقط من دون حقنها بالبكتريا الواقية وكانت نسبة الخلايا البلعمية هي 18 % في مجموعة السيطرة، بينما كانت نسبتها 27 % في مجموعة الأختبار ، مما يدل على التأثير الوقائي لبكتريا *Lb. gasseri* في الحماية من الأصابة ببكتريا *P. aeruginosa* .

# The Effect of *Lactobacillus gasseri* Against *Pseudomonas aeruginosa* Infection in Mice.

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## Abstract

The effect of local *Lactobacillus gasseri* filtrate against *Pseudomonas aeruginosa* infection in mice was studied . 0.25 ml of concentrated filtrate *Lactobacillus gasseri* was injected in intraperitoneally ( I.P.) 5 days before challenge with 0.2 ml viable *P. aeruginosa* ( $10^8$ cell/ ml).

Animals were sacrificed after 12 h. from challenge by cutting the femoral artery . To follow bacterial growth in the peritoneal cavity , its contents were washed out with 5 ml of PBS .The fluid was diluted, 0.1 ml from each dilution and was spread on culture media. The number of colonies in 5 ml of harvested fluid was expressed as Log 10 CFU ,and the percentage of Macrophage in the peritoneal cavity was counted.

Growth of *P. aeruginosa* in the peritoneal cavity was markedly inhibited in *Lb. gasseri* pretreated mice, wherease such inhibition of bacterial growth was not observed in another group ( mice were not treated with *Lb. gasseri* )

The percentage of macrophage detectable in the peritoneal cavity was 18% in control and 27% in test . It was suggested that macrophages activated with *Lb. gasseri* were involved in the protective to *P. aeruginosa*.

## Introduction

Lactic Acid Bacteria ( LAB) are gram-positive bacteria with cell wall components such as peptidoglycan, polysaccharide and teichoic acid, all of which have showed to own immunostimulatory properties. In addition to cell wall components, immunostimulatory effects were observed with antigens originated from the cytoplasms of some strains of LAB [ 1 ] .

Certain specific probiotic strains (for example, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus johnsonii* have well defined and proved the clinical effects for the treatment and/or prevention of diseases of intestinal and extraintestinal origin [ 1 ] . And have immunostimulatory properties, including modulation of cytokine production, increased phagocytic activity of polymorphs, adjuvant effects on specific humoral responses, T- lymphocytic function, and NK activity [2][3].

Probiotic bacteria are showed that they would promote the endogenous host defense mechanisms. In addition to the effects of probiotics on nonimmunologic gut defense, which is characterized by stabilization of the gut microflora , probiotic bacteria have showed their ability to enhance humoral immune responses and thereby promote the intestine's immunologic barrier. Moreover, probiotic bacteria have showed their ability to stimulate nonspecific host resistance to microbial pathogens .Thereby they would have the ability to aid in immune elimination, and to modulate the host's immune responses to potentially harmful antigens with a potential to down-regulate hypersensitivity reactions [4] [5].

Oral introduction of *Lactobacillus casei* and *Lactobacillus bulgaricus* activated the production of macrophages and administration of *L. casei* and *Lactobacillus acidophilus* activated phagocytosis in mice , enhanced phagocytosis was also reported in humans by *L. acidophilus*[2]. De Simone *et al* [6 ] studied the influence of a yogurt-supplemented diet on

the immunocompetence and survival of animals subsequently infected with *Salmonella typhimurium*. De Simone *et al.* reported that mice fed live LAB (*L. bulgaricus* and *Streptococcus thermophilus*)-containing yogurt for 7 and 14 days had a higher percentage of B lymphocytes than did mice fed a control diet supplemented with a cow milk. In a similar experiment, Puri *et al* [7]showed that intestinal lymphocytes from mice fed live LAB-containing yogurt had a higher proliferative response to LPS than did mice fed milk after a challenge with *S. typhimurium*.

Although the mechanism(s) of action involved in the inhibition of *P. aeruginosa* was not investigated in the present study , it is therefore believed that some factors may include bacteriocins,hydrogen peroxide, diacetyl and CO2 , or enzymes by the selected Lactic Acid Bacteria [ 8]. Finally , *L. casei* strain Shirota administration before or after an initial challenge dramatically inhibited *E. coli* growth in a murine model of urinary tract infection [9].

Most studies investigated the effects of *Lactobacillus* on pathogenic bacteria *in vitro*, whereas very few studies have investigated the effects of *Lactobacillus in vivo*. The aim of this work was to study the effect of *Lactobacillus gasseri* filtrate on prevention of *P. aeruginosa* infection in mice.

## Material and Methods

**Animals :**

Balblc- Male mice were obtained from Department of Biology – College of Science - AL-Mustansiriya University . Mice were used in experimentals at 7 to 9 weeks of age , 25-30 gram weight ( Five mice for each group).

**Bacterial isolates :**

1) *Lb. gasseri* **(** maintained from Department of Biology – College of Science - AL-Mustansiriya University ) was cultured on De Man Regosa Sharpe medium ( MRS) at 37 C for 48 hrs ,The *Lb. gasseri* filtrate was prepare according to [ 9].

2) A clinical isolate of *P. aeruginosa* was isolated from wound infection and identified according to [10].

**Bacterial infection:**

In the experiment to test the protective effect of *Lb. gasseri* filtrate against *P. aeruginosa* in mice , 0.25 ml of *Lb. gasseri* filtrate was injected in intraperitoneally ( I.P.) 5 days before challenged with 0.2 ml viable *P. aeruginosa* ( $10^8$ cell/ ml). [11].

**Determination of bacterial growth:**

The challenge dose of bacteria was injected I.P. to control mice and mice that had been treated with *Lb. gasseri* filtrate 5 days earlier. Twelve hour. after the challenge , animals were sacrificed by cutting the femoral artery . To follow bacterial growth in the peritoneal cavity , its contents were washed out with 5 ml of PBS . The fluid was diluted 10- fold with PBS , 0.1 ml from each dilution was spread on nutrient agar plates ( containing 0.4% glucose ) . The number of colonies in 5 ml of harvested fluid was expressed as Log 10 CFU [ 11] .

**Counting of WBCs in peritoneal cavity :**

Smear specimens for differential counts were prepared for Giemsa staining and examined **[**12].

## Results and Disscussion

In our study the protective effect of *Lb. gasseri* filtrate against *P. aeruginosa* infection in mice was studied . PBS treated control mice and those pretreated with 0.25 ml of *Lb. gasseri* filtrate 5 days earlier were inoculated I.P. with 0.2 ml viable *P. aeruginosa* and the growth of the bacteria in the peritoneal cavity was followed . The number of the *P. aeruginosa* in the peritoneal cavity decreased gradually to about $10^6$ CFU by 12 h. after challenge in control mice Fig. (1). In mice pretreated with *Lb. gasseri* filtrate , however,the bacteria were reduced rapidly to $10^3$ CFU in 12 h. . Although the mechanism(s) of action involved in the inhibition of *P. aeruginosa* was not investigated in the present study , it is therefore believed

that some factors may include bacteriocins,hydrogen peroxide, diacetyl and CO2 , or enzymes by the selected Lactic Acid Bacteria [ 8].

Differential cell counts of peritoneal leukocytes were studied consecutively after treatment with *Lb. gasseri* filtrate . The percentage of macrophage detectable in the peritoneal cavity was in control and 27% in test. Macrophages were characteristically increased in *Lb. gasseri* filtrate treated mice. These findings are in agreement with the previously reported result which showed that the administration of *Lactobacillus* or yogurt to young mice enhanced lung clearance of *P. aeruginosa* and phagocytic activity of alveolar macrophages [13**].**

Villena *et al* [14] found that pneumococcal colonization of lung and bacteremia were significantly greater in control group mice compared with the *Lb. casei* pretreated group. Although the number of bacteria in lungs and blood stream tended to decrease ($P < 0.05$) during infection in *Lb. casei* pretreated group mice, they suggested that the addition of *L. casei* to the repletion diet has a beneficial effect because it accelerates the recovery of the innate immune response and improves the specific immune mechanisms against *Streptococcus pneumoniae* respiratory infection in malnourished mice.

A limited number of animal studies were conducted on the effect of LAB on macrophages. Goulet *et al* [ 15 ] found that phagocytic activity of alveolar macrophages was significantly ($P < 0.05$) higher in mice fed milk fermented with *L. acidophilus* and *L. casei* than in control mice fed ultrahigh-temperature-treated milk. Perdigon *et al* [2] showed that feeding milk (100 µg protein/d) fermented with *L. casei* and *L. acidophilus,* or both for 8 d. increased the *in vitro* and *in vivo* phagocytic activity of peritoneal macrophages . Other studies in which reconstituted lyophilized LAB were administered orally or intraperitoneally showed enhancement of macrophage activation by LAB [ 16 ].

These observations reviewed together suggested that specific immunomodulatory properties of probiotic bacteria should be characterized during the development of clinical applications for extended target populations. Further experiments are required to establish the mechanism by which *Lb. gasseri* filtrate affects *P. aeruginosa* pathogenicity . In the future , the immunological aspects of the protective role of *Lb. gasseri* filtrate should be studied .

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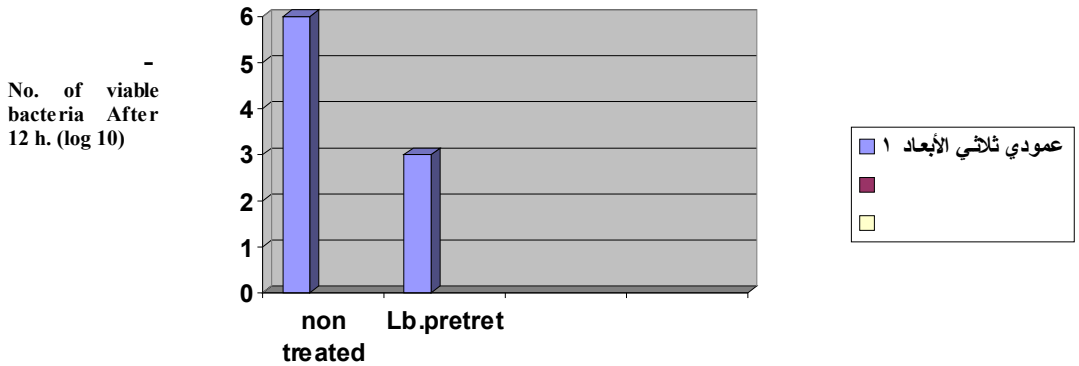


**Fig.(1): The growth of the bacteria in the peritoneal cavity after 12 h.**