

## Inhibitory effect of Probiotics on some Gram positive and negative Bacteria

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### Abstract

The present study's objective was to evaluate the inhibitory activity of the Probiotics *Lactobacillus acidophilus* and *Bifidobacterium* (obtained from the Agriculture Research Directorate, Ministry of Science and Technology, Iraq) and a suspension of a mixture between the two mentioned probiotics with two types of Gram-negative bacteria (*Pseudomonas spp* and *Proteus spp*) and one type of Gram-positive bacteria (*Streptococcus spp*) in vitro. The required tests were completed to verify the probiotics' purity, and the bacterial isolates used in the current investigation were assessed using biochemical assays and selected culture medium (culture and microscopic features). In addition, the inhibitory efficacy of the investigated Probiotics in different Gram positive and negative bacteria was evaluated by drug susceptibility testing (disc diffusion test as well as agar well diffusion test). Our data of the current study confirmed an excellent inhibitory activity of each *Bifidobacterium* (B) and the mixture of the two probiotics (MLB) via measuring the inhibition area, they had 25, 22mm, 28,-30 mm inhibition zone for *Pseudomonas spp*, 23, 25 mm, 26-27mm inhibition zone for *Proteus species spp*, and 22,20 mm, 33,29 mm inhibition quarter for *Streptococcus species*, by way of the usage of disc and agar well diffusion methods respectively. Where it was once weak inhibition activity of *Lactobacillus acidophilus* (L) on *Pseudomonas spp*, 0-3 mm and *Streptococcus species* 1-7 mm by the usage of the disc and agar well diffusion respectively. On the other, hand, Probiotic (*Lactobacillus acidophilus*) had available zone of inhibition on the *Proteus spp*

bacteria, which were 24, 24 mm through the disc and agar well diffusion respectively. In conclusion: the Probiotics were found to have good and active inhibitory action on Gram-positive microorganism (*Streptococcus*) and gram-negative microorganism (*Pseudomonas* and *Proteus*) in vitro by way of using disc and agar well diffusion test, and the combination of the two probiotics MBL of present study, had more potent inhibitory action than each one of the studied separate probiotics.

**Key words:** *Probiotics, Lactobacillus, Bifidobacterium, Inhibitory effect, Antibiotics Sensitivity test.*

## Introduction

Many years ago, there was a revolution in the food industry in the field of functional foods production as a result of increasing awareness of the consumers on the food positive role in wellbeing and health. (1). Development of probiotic foods received more focus due to its potential to prevent disease. Probiotics are numerous types of live microorganisms that have been shown to have positive impacts on human health. (2). According to reports from the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), probiotics are live microorganisms that, when given to a host in sufficient amounts, have a positive effect on their health in ways like improving the balance of their intestinal microbiota, modulating their immune system, lowering their serum cholesterol levels, inhibiting the growth of harmful bacteria, and reducing or preventing intestinal disorders. (3, 4). Present indicator suggests that some probiotics like *Lactobacillus* and *Bifidobacterium* can modulate gut microflora homeostasis, and may have protective

effects against diarrhea (5). The development of multiple antibiotic-resistant Gram-positive and Gram-negative bacteria in many areas such as new ways for quick healing in hospital and community settings and accurate diagnosis and treatment (6).

*Pseudomonas aeruginosa* can be defined as a common Gram-negative opportunistic multidrug-resistant pathogen which causes acute and chronic infections particularly in immunocompromised and diseased animals. (7).

*Proteus* is considered as one of the common pathogens among gram-negative bloodstream isolates causing the secondary infections of the urinary tract (UTIs) and usually associated with urinary catheters community-acquired. It may result in infections of the biliary tract, surgical wounds, and nosocomial infections. Due to immune evasion and the protective reservoir that urinary stones provide, *Proteus* has a remarkable ability to remain in the urinary tract despite the use of antibiotics and catheter exchange. It can

cause opportunistic infections and is a natural component of the gut flora (8).

*Streptococcus* is a group of gram-positive bacteria that has the ability to infect many animal species, leading to suppurative conditions like mastitis, metritis, polyarthritis and meningitis. It is distributed worldwide, most species live in the form of commensals on the mucosae of the upper respiratory tract and lower urogenital tract. *Streptococcus* is catalase-negative, facultative anaerobe, which is non-motile, fastidious and requires the addition of blood or serum to be cultured. (9).

The current study aimed to evaluate the inhibitory effect of probiotics against certain gram-positive and negative bacteria via using disc diffusion and agar well diffusion methods in vitro and to compare between the antimicrobial activities of these probiotics.

## Material and Methods

### Bacterial Isolates:

In the current study, *Pseudomonas spp* and *Proteus spp* as gram-negative and one species of gram-positive *Streptococcus spp* bacteria were used . All isolates have been taken from laboratory of Microbiology, College of Veterinary Medicine, University of Mosul, Iraq , during the period December 2021 to March 2022.

The isolates were similarly purified via subculture on brain heart infusion broth, cultured for two days at 37°C, and then transported to selective

medium that included *Pseudomonas* agar, Edward agar, and regular media such Nutrient broth, Nutrient agar, and. To verify the purity of all isolates, specific biochemical tests were carried out (10).

### Probiotics:-

The two isolates of *Lactobacillus acidophilus* and *Bifidobacterium spp.* were obtained from the agriculture Research Directorate, Ministry of Science and Technology, Iraq.

The probiotic isolates were further purified using a subculture of brain-heart infusion broth, after which they were incubated for one day at 37°C to achieve further differentiation. Finally, the isolates were cultured on specific media (De-Man, Rogosa, and Sharpe broth (M.R.S. broth and Agar) with 5% Co2 for 48 hours at 37°C. In addition, we examined the combination of these two probiotics (*Lactobacillus* and *Bifidobacterium spp*) (MLB) for the estimation of the antagonism effects of these Probiotics on the bacterial study.

### Preparations of Bacterial Suspension:

These procedures involved transferring 4-5 colonies of each isolate of bacterial species from the selective media into nutrient broth-filled tubes, which were then incubated for 14–16 hours at 37°C (11). Physiological salts solution was used for dilution, and comparisons were made with the standard control tubes (McFarland tubes), which were used in this learning experience (10).

### Estimation of the inhibitory effect:

Technique and the Agar well diffusion method had been performed by means of taking 0.1 ml from each and every bacterial suspension ( $1.5 \times 10^8$  cfu) which used to be inoculated to the Muller Hinton agar and spread via the usage of an L. shape glass spreader and left for 30 minutes to confirm suspension diffusion.

To evaluate the influence of these probiotics on the isolates of bacterial study, a disc (6 mm) from filter paper (Whitman No 1) was prepared and later autoclaved, 0.1 ml of “each probiotics” cultured into each 10 discs to be saturated with it (12, 13). Later the discs were cultured on the Muller Hinton agar, (use 0.1 ml from each probiotics used to be used to prepare identical 10 discs to measure the antagonism of these probiotics together) and was once incubated for about 14-16 hours at 37°C, the effects were revealed by size of the inhibition zone which was obtained at the areas around the discs which have been saturated by Probiotics of current study in the culture media (Muller Hinton agar) (14). Similar approach was also applied concerning agar well diffusion (The 6-mm or 7-mm wells were bored in each plate) but without the use of discs, 0.1 ml of the isolates were directly inoculated in the well (15).

## Results

The effect of the current study revealed that probiotic *Bifidobacterium* and probiotic which are a combination of *Bifidobacterium* and *Lactobacillus acidophilus* (MBL) were able to distinguish inhibition activity on the bacterial study (*Pseudomonas spp* and *Proteus spp* and *Streptococcus spp*, they had 25, 22mm, 28,30 mm inhibition zone for *Pseudomonas spp*, 23,25 mm, 26-27mm inhibition zone for *Proteus spp* and 22,20 mm, 33,29 mm inhibition zone for *Streptococcus species* by using the methods of disc and agar well diffusion respectively.

The results related to probiotic *Lactobacillus acidophilus* showed that it had susceptible inhibitory action on *Pseudomonas spp* 0-3 mm and *Streptococcus species* 1-7 mm by using the method of disc and agar well diffusion respectively. On the other hand, probiotic *Lactobacillus acidophilus* was found to have available zone of inhibition on the *Proteus spp* bacteria, 24 mm, 24 mm through the disc and agar well diffusion respectively. Shows, Tables 1 and Figure (1), Pictures (1, 2, 3, 4, 5, 6).

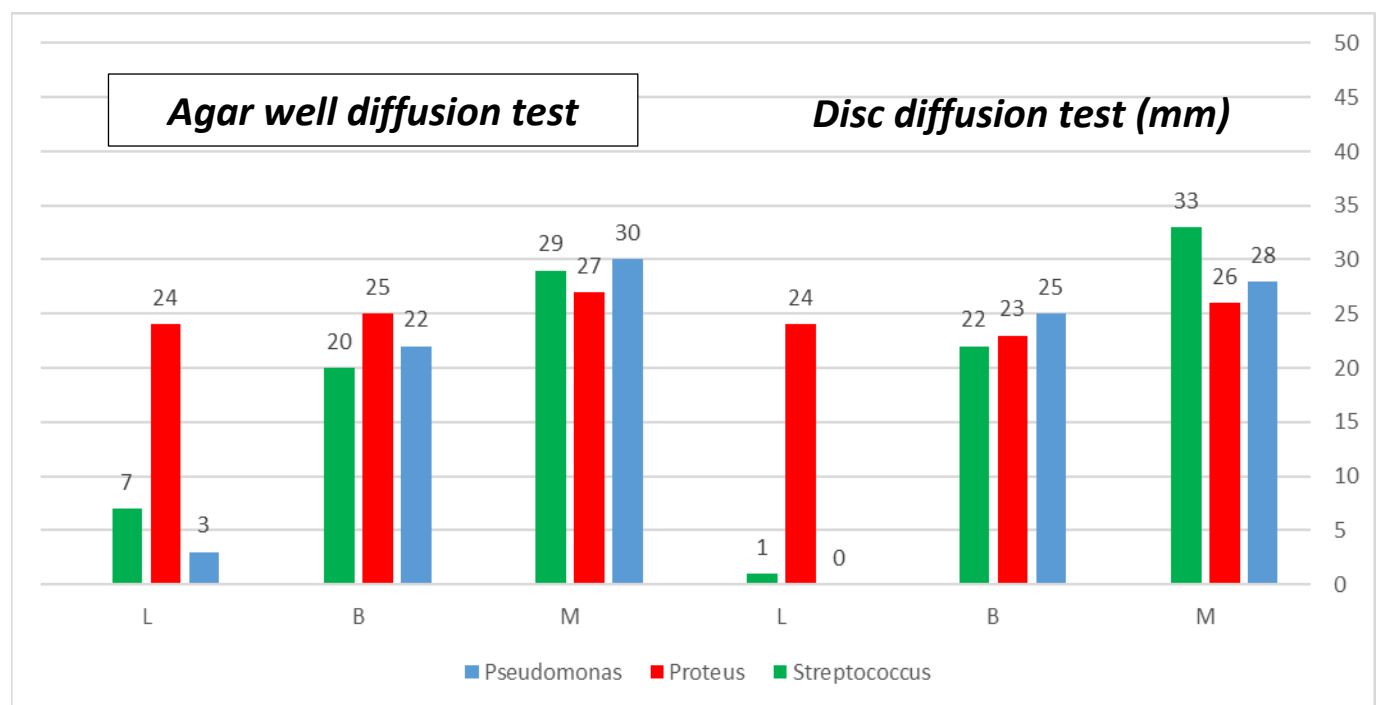
**Table (1) : The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on bacterial isolates is indicated by Inhibition Zone (mm).**

Bacterial Species	Disc Diffusion Test (mm)			Agar well diffusion test (mm)		
	L	B	M	L	B	MLB
<i>Pseudomonas</i> spp	0	25	28	3	22	30
<i>Proteus</i> spp	24	23	26	24	25	27
<i>Streptococcus</i> spp	1	22	33	7	20	29

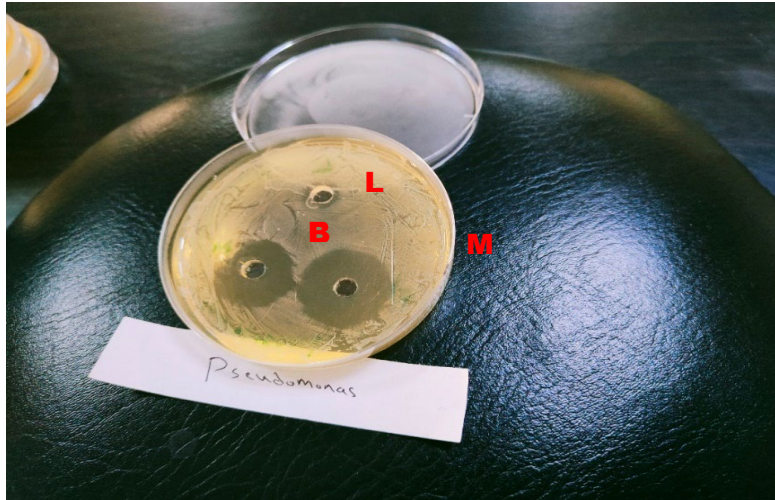
L – *Lactobacillus acidophilus*.

B – *Bifidobacterium* spp.

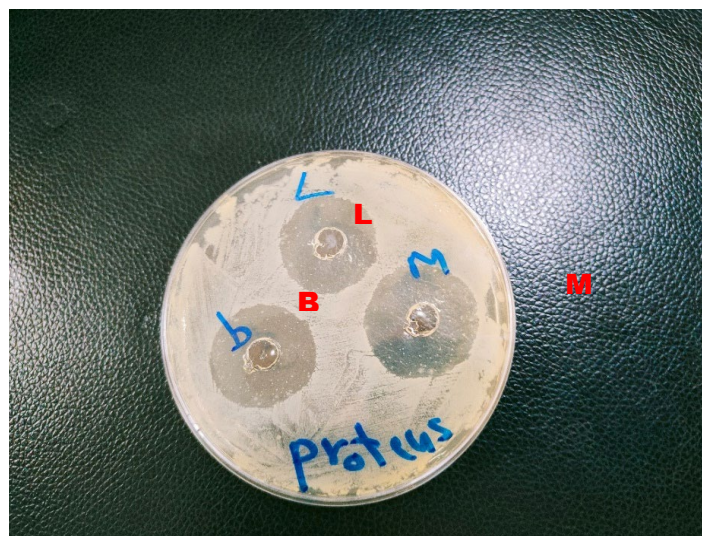
MLB – Mixture of *Lactobacillus* and *Bifidobacterium*.



**Figure (1): The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on bacterial study isolates**

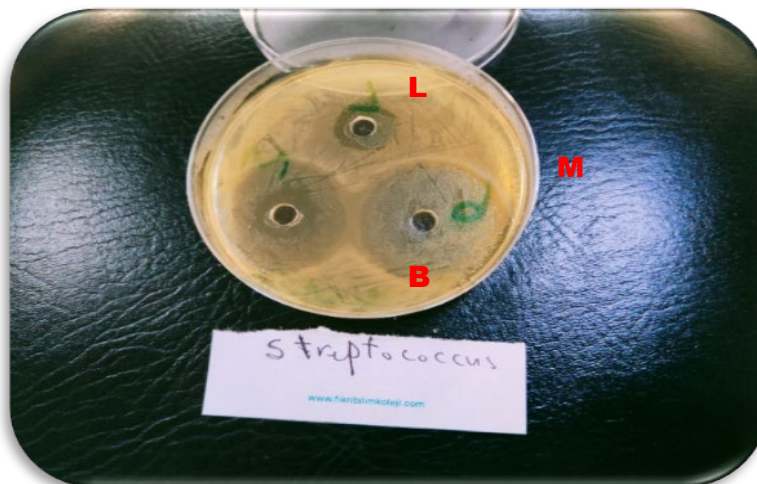


**Picture (1):** The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Pseudomonas spp* culture by using agar well diffusion test.

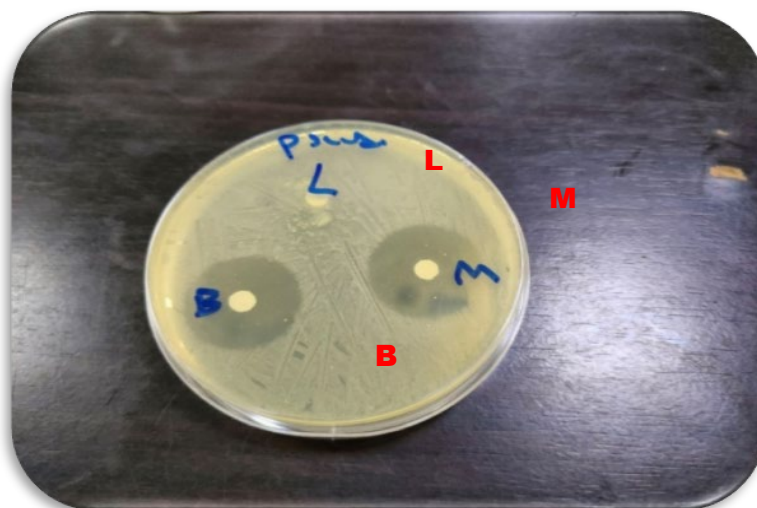


**Picture (2):** The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Proteus spp* culture by using agar well diffusion test.

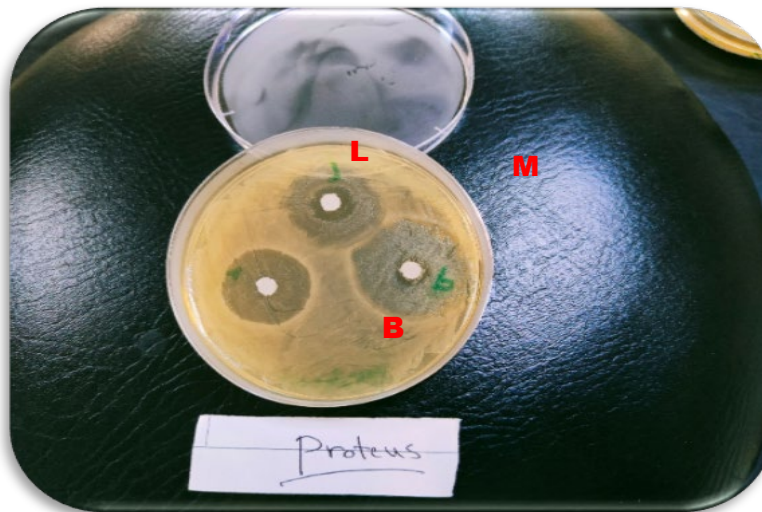




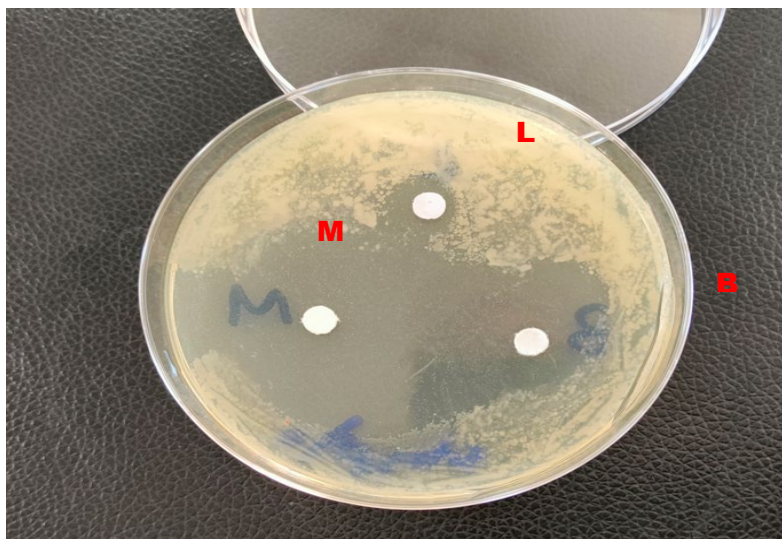
Picture (3): The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Streptococcus spp* culture by using agar.



Picture (4): The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Pseudomonas spp* culture by using agar Disc diffusion test.



**Picture (5):** The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Proteus spp* culture by using agar Disc diffusion test.



**Picture (6):** The effect of Probiotics *Bifidobacterium*, *Lactobacillus*, and the mixture (MBL) on *Streptococcus spp* culture by using agar Disc diffusion test

## Discussion

The Probiotic approach aims for repair of the deficiencies in the microflora and restoration of the animals' resistance to disease and it is now replacing the chemical growth promoters for farm animals (16, 17 ).

Several in vitro studies suggest that probiotic lactic acid bacteria for many enteric bacterial infections may inhibit the growth of food-borne pathogenic microbes (18).



In the present study, it was noticed that Probiotic *Lactobacillus* possess weak antimicrobial action on the *Streptococcus* 1-7 mm (Gram positive bacteria) and *Pseudomonas* bacteria 0-3 mm (Gram negative bacteria) by disc and agar well diffusion test respectively, and these outcomes are different with the study done by (18). in general, and also differ with the study done by (3). in relation with *Pseudomonas* spp. It was also observed that *lactobacillus* revealed the strongest inhibitory action on *Proteus* spp bacteria (24-24 mm) by using disc and agar well diffusion test respectively, these results agree with the study done by (18, 8).

The small intestine represents an important site of infection in humans as well as animals (19), Probiotics are thought to modulate the indigenous intestinal microbiota and improve health through different mechanisms of action, including the direct preventing of the growth of enteric pathogens via decreasing luminal pH, and the secretion of bactericidal proteins (20). The selection of *Lactobacilli* as a potential probiotic which promotes health in food as well as pharmaceutical Preparations necessitate to be screened in vitro to confirm certain criteria, that involve antibiotic tolerance, bile tolerance, growth inhibition

of other microorganisms as well as gastric juice that permit them to be established in the intestine (21 and 22).

Zone of inhibition of probiotic *Bifidobacterium* (B) has good inhibitory efficacy on bacterial study, *Streptococcus* 22-20 mm, *Pseudomonas* 25-22 mm and *Proteus* spp, 23-25 mm by using disc and disc and agar well diffusion test respectively, the proportion results of the *Bifidobacterium* in the present study was in agreement with those reported in different studies done such as (17), who studied Gram negative bacteria. Also is agree with a study about *pseudomonas* spp done by (21) by using agar well diffusion.

The results were interpreted by measure the zone around the discs and compared with the break points of CLSI (clinical laboratory institute 2015) (23).

*Lactobacillus* are important group of probiotic organisms that play an important role in human health by inhibiting pathogenic bacteria growth, induce immune response (24). The strains which are presently used as a type of probiotics, belong to both *Bifidobacterium* and *Lactobacillus*, are naturally exist as a form of microbiota of human intestine and has the ability to produce metabolites of antimicrobials like organic acids, hydrogen

peroxide, ethanol, diacetyl, acetaldehyde, saturated or unsaturated form of free fatty acids and compounds like peptides and bacteriocins (25 and 26). On the other hand, the outcomes of mixture MBL which had inhibitory action on bacterial study *Streptococcus* 33-29 mm, *Pseudomonas* 28-30 mm and *Proteus* spp, 26-27 mm by using disc and disc and agar well diffusion test respectively, these results agreed with the study performed by (25). Previous studies confirmed the antimicrobial activity of probiotic strains cultures on pathogenic bacteria but few studies reported influences as it was gained from co-cultured strains of probiotic (25).

## Conclusions

In conclusion, the results of the present study showed that the Probiotics had good and active inhibitory action on Gram-positive microorganisms (*Streptococcus*) and gram-negative microorganisms (*Pseudomonas* and *Proteus*) in vitro by using disc and agar well diffusion tests. Furthermore, the present study proved that the combination of two Probiotics MBL, were of more potent inhibitory action than each separate one of probiotics.

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### التأثير التثبيطي للبروبيوتيك على بعض أنواع البكتيريا الموجبة والسالبة الجرام

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### الخلاصة

كان الهدف من الدراسة الحالية هو تقييم النشاط المثبط للبروبيوتيك *Bifidobacterium* و *Lactobacillus acidophilus* (تم الحصول عليه من مديرية البحوث الزراعية ، وزارة العلوم والتكنولوجيا ، العراق) وعمل خليط من مزيج بين البروبيوتيك المذكورين مع نوعين من البكتيريا السالبة الجرام (*Pseudomonas spp*) و (*Proteus spp*) ونوع واحد من البكتيريا موجبة الجرام (*Streptococcus spp*) في المختبر. تم الانتهاء من الاختبارات المطلوبة للتحقق من نقاء البروبيوتيك ، وتم تقييم



العزلات البكتيرية المستخدمة في الاستقصاء الحالي باستخدام المقاييس البيوكيميائية ووسط الاستزراع الانتقائي (الخصائص الزرعية و المجهرية). بالإضافة إلى ذلك ، تم تقييم الفعالية التثبيطية للبروبيوتيك التي تم فحصها في أنواع مختلفة من البكتيريا موجبة وسالبة الجرام عن طريق اختبار حساسية الدواء (اختبار انتشار القرص وكذلك اختبار الانتشار في حفر الأجار). أكدت بيانات الدراسة الحالية نشاطاً مثبطاً ممتازاً لكل بكتيريا (*Bifidobacterium* (B) وخليط اثنين من البروبيوتيك (MLB) عن طريق قياس منطقة التثبيط ، وكان لديهم 25 ، 22 ملم ، 28 ، -30 ملم منطقة تثبيط لـ *Pseudomonas* spp ، منطقة تثبيط 23 ، 25 ملم ، 26-27 ملم لأنواع *Proteus* spp ، و 20،22 ملم ، 29،33 ملم ربع تثبيط لأنواع *Streptococcus* ، عن طريق استخدام طرق الانتشار بالقرص و الانتشار في حفر الاكار على التوالي. حيث كان نشاط التثبيط الضعيف لـ *Lactobacillus acidophilus* (L) على *Pseudomonas* spp ، 0-3 ملم وأنواع 1-7 *Streptococcus* ملم عن طريق استخدام القرص والانتشار في حفر الاجار على التوالي. من ناحية أخرى ، كان لدى البروبيوتيك (*Lactobacillus acidophilus*) منطقة تثبيط متاحة على بكتيريا *Proteus* spp ، والتي كانت 24 ، 24 ملم من خلال القرص والانتشار في حفر الأجار على التوالي. في الختام: وجد أن البروبيوتيك لها تأثير مثبط جيد وفعال على الكائنات الحية الدقيقة إيجابية الجرام (*Streptococcus*) والكائنات الحية الدقيقة سالبة الجرام (*Pseudomonas* and *Proteus*) في المختبر عن طريق استخدام اختبار الانتشار بالقرص و حفر الأجار ، والجمع بين الاثنين البروبيوتيك MBL في الدراسة الحالية ، كان لها تأثير مثبط أقوى من كل واحدة من البروبيوتيك المنفصلة المدروسة.

**الكلمات المفتاحية:** بروبايوتك , لاكتوباسيلس , التأثير المثبط , اختبار الحساسية الدوائية