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Determination of the inhibitory ability of the metabolites of lactic acid bacteria against of Steptococcus pyogenes and Escherichia coli bacteria isolated from different infections from incoming and recumbent patients in Salah Al-Din General Hospital in Tikrit city

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Abstract

This study aims to isolate and diagnose the bacterial species *Streptococus. pyogenes* and *Escherichia. coli* from different infections, and to study the inhibitory ability of the metabolic products of lactic acid bacteria against the isolated bacteria. The study was conducted on 50 samples from patients with tonsillitis, urinary tract infections, and wound infections, for both sexes and in different age groups, 32 isolates of *Streptococus. pyogenes* and 16 isolates of *Escherichia. coli* were obtained. In this study, certain types of lactic acid bacteria, including *Lactobacillus. acidophilus* and *Bifidobacterium. bifidum* collected from dairy products from local markets and homes in the city of Tikrit, where the filtrate of *Lactobacillus. acidophilus* and *Bifidobacterium. bifidum* bacteria was used separately, and the interaction of these two species with each other at certain concentrations (10, 20, 40) was used to determine the inhibitory ability against bacteria and *E. coli* using the well diffusion method. The results showed that the metabolites of *Lactobacillus. acidophilus* and *Bifidobacterium. bifidum*, both of which together possess a more efficient inhibitory capacity against isolates of *Streptococus. pyogenes* and *Escherichia. coli* when added to the holes at a concentration of 40 mg/ml compared to other concentrations of 10 and 20 mg/ml of These bacterial types, at a concentration of 40 mg/ml, led to the inhibition of *Streptococus. pyogenes* bacteria with diameters of inhibition of 17.5, 18.6, and 22.0 mm. As for *Escherichia. coli* bacteria, the diameters of inhibition were as follows: 16.0, 17.5, and 20.5 mm for *Lactobacillus. acidophilus*, *Bifidobacterium. bifidum*, and both together respectively. In synergy, the effectiveness of *L. acidophiles* and *B. bifidium* was high against *Streptococus. pyogenes* and *Escherichia. coli* Compared each separately. The concentration 40mg/ml was the best concentration for producing the metabolites of lactic acid bacteria, and the effect of the metabolites of *B. bifidium* bacteria on the *S. pyogenes* and *E. coli* isolates was wider than the metabolites of *L. acidophiles* bacteria.

Introduction

The term Probiotic refers to non-pathogenic microorganisms such as bacteria and yeasts that confer health benefits to the host when ingested and reach the host's intestines in sufficient numbers [1]. The organism must possess many attributes to be used as a probiotic,

including adhesion ability, the ability to produce acid and hydrogen peroxide H_2O_2.[2], bile tolerance, antibacterial activity, and immunomodulatory activity [3,4]. Microorganisms considered as probiotics for Humans include *Bacilli*, yeasts, *E. coli*, *Enterococci*, and the most common and used are *Bifidobacteria* and Lactic acid Bacteria, such as *Lactobacilli*, *Lactococci*, and *Streptococci* [5].

In this study, two species *L. acidophiles* and *B. bifidium* of lactic acid bacteria (LAB) were used, where *L. acidophiles* belongs to the group of obligatory fermenting homozygous bacteria within the genus *Lactobacillus*, where the name of this species is derived *Lactobacillus acidophilus* two syllables to the first is *Lactobacillus*, which means *lactobacilli*, while the second syllable, *acidophilus*, means acidophilic[6].

The American scientist Moaro is considered the first to be able to isolate this type from feces of infants in 1900, when it was known at that time as *Bacillus acidophilus*, but its name was changed a year later to *Lactobacillus acidophilus*, and in 1901 the scientist Cahn reported that *Lactobacillus acidophilus* bacteria are present in the stools of formula-fed infants as well as infants who are breastfed, Weiss also reported that *lactobacilli*, similar to the *Lactobacillus acidophilus* discovered by the scientist Moaro, increased when consuming milk in abundance [7].

These bacteria are characterized as slender rods with a circular end, gram-positive, do not form spores, grow better in anaerobic conditions or in 5-10% of carbon dioxide instead of aerobic conditions, and the optimum temperature for their growth is 35-38 degrees Celsius, but it does not grow at a temperature below 20 degrees Celsius, and its optimum pH is 5.5-6.0. It is resistant to bile and acids, and has biological importance for humans and animals, including reducing the risk of cardiovascular diseases, and these bacteria have a role. It is important in treating diseases of the digestive system, regulating the body's immune system, and preventing and treating cancer [8].

Bf. bifidium bacteria belong to the genus *Bifidobacterium*, which is considered a member of the *Bafidobacteriaceae* family [9]. It is usually found in the digestive system of humans and animals. In 1900, the first strain of *Bifidobacterium* was isolated by the scientist Tissier from a stool sample of a breastfed infant called *Bacillus bifidus*. In 1924, the Danish microbiologist Orlajensen classified it as a separate species within the genus *Bafidobacterium*, and currently this genus includes 48 recognized classifications [10]. It is an anaerobic microorganism, positive for Gram stain, usually appears in V or Y-shaped pairs, non-spore-forming, non-pathogenic, and its presence in the intestines of infants is an indicator of health [11].

These bacteria present in the intestinal flora of the human digestive system represent 91% of the feces of children who depend on breast milk for their nutrition, 75% of the feces of children who depend on artificial feeding (formula milk), and 3% of the feces of adults [12]. It is considered one of the probiotics that have a major role in improving or maintaining the microbial balance in the intestine, and works to inhibit the types of pathological bacteria present in the digestive system by preventing the formation of enzymes secreted by pathological bacterial species, and thus prevents the formation of toxins and other carcinogens, and has the ability of sticks to the host's intestines, in addition to reducing the sensitivity of some consumers to consuming lactose, and produces organic acids that can

lower the pH, and has the ability to tolerate (resist) bile salts, and reduces cholesterol in the body, and this genus *Bafidobacterium* is able to produce inhibitory substances that inhibit living organisms Pathogenic microbes are therefore used to preserve foods [13].

Tonsillitis, urinary tract infection, and wound infections are among the most common diseases in medical practices that affect people of all ages, from the youngest to the elderly. Tonsillitis is one of the common respiratory diseases that affect both children and adults. It is a case of tonsillitis. palatine tonsils, pharyngeal tonsils, tubal tonsils, and lingual tonsils [14]. Tonsillitis is inflammation caused by a bacterial or viral infection [15]. Most of the common causes of tonsillitis may be due to viruses such as adenovirus, rhinovirus, corona virus, respiratory syncytial virus, influenzae, Epestein-Barr virus, Herpes simplex virus, and human immunodeficiency virus (HIV). The most common bacterial cause is group A beta-haemolytic *streptococcus*, which is referred to as strep throat [16,17]. Tonsillitis occurs frequently in children. However, the condition rarely occurs in children younger than 2 years of age. Tonsillitis caused by streptococcus species usually occurs in children between the ages of 5-15 years, although viral tonsillitis is more common in younger children. Tonsillitis is considered a contagious disease that is transmitted from one person to another through coughing and sneezing, and also when sharing household items such as cups and spoons, especially if they are not sterilized [18].

Urinary tract infection occurs mainly due to pathogen invasion of the urinary tract, leading to an inflammatory response to the urinary tract [19]. Studies have indicated that the incidence of urinary tract infections in women is more common than in men as a result of several physiological and anatomical causes in the urinary tract among women and men [20]. Moreover, the anatomical length of the urethra in men (20 cm) acts as a barrier against microorganisms, but in women the urethra is short (5 cm) through which microorganisms easily pass. This is another reason for reducing infection in males [19]. Urinary tract infections (UTI) are common in pregnant women and are at increased risk of infection due to the weight of the fetus and the dilation of the ureters and renal pelvis [21], Where there are many factors that help bacteria in women enable them to recur, , which is the cells lining the wall of the urinary system that help in the adhesion of bacteria and their transmission to the urinary system.[22] Urinary tract infection (UTI) is common in childhood. In the first year of life, the infection is more common in boys at a rate of 3.7% compared to 2% in girls. After childhood, this infection is more common among girls during pre-puberty, where the infection rate in girls is 3% compared to 1% in boys [23].The incidence of infection in uncircumcised children is higher than in circumcised children [24]. The individual's marital status, age, gender, and parental education level are considered the most important factors that contribute to the incidence of urinary tract infection [25]. As well as the imbalance in the urinary system in some individuals causes obstruction or blockage of the passage of urine, which causes stagnation of urine and an opportunity for bacteria to multiply, and individuals who use catheter tubes are considered one of the main sources causing urinary tract infections to help them enter bacteria into the bladder [26]. Congenital malformations of the urinary system and chronic diseases, including chronic kidney disease, the presence of stones and diabetes,contribute significantly to the incidence of infection [27,23]. In elderly patients, the increased incidence of urinary tract infections is due to their immune status and the anatomical and physiological changes that are associated with the aging process. *E. coli* is one

of the most common bacterial pathogens responsible for complicated and uncomplicated urinary tract infections [28].

The skin is the body's first defensive barrier against microbial pathogens, and among the injuries that the skin is exposed to are wounds, where the wound represents any physical damage, rupture in the epithelial layer, or a breach in the natural tissue that the individual suffers from due to exposure to some accidents, accidental occurrence, or due to operations surgical interventions will lead to cellular imbalances, and the severity of the wound ranges from slight to life-threatening in some cases [29], where wound infections are considered one of the biggest health problems that the patient is exposed to, and these infections may occur directly after the injury or may occur after several days from injury.

Materials and working methods

Sample collection

Tonsil, urinary tract and wound samples from patients

Fifty samples of different infections (tonsils, urinary tract, wounds) were collected from incoming and recumbent patients at Salah Al-Din General Hospital in Tikrit using sterile cotton swabs. These swabs were cultured on the different media, and the culture media as Blood agar, MaCconkey agar, Mannitol agar, Eosin methylene blue, Nutrient agar were incubated in the incubator, and after observing the growth on the plates, a Sub Culture was done for the single colony appearing on the plate to obtain a single pure colony, and they were diagnosed with the biochemical tests for each bacterial type and the Vitek2 System Compact system.

Dairy product samples

Samples were collected from fermented milk in the local markets of the city of Tikrit, of which five samples bore the “Al Safi” brand, three were from the “University Dairy” brand, and six samples were artisan-made in homes. They were placed in sterilized flasks of 250 ml and kept in the refrigerator at 4 degrees Celsius and reactivate it every four weeks until they were used to isolate lactic acid bacteria.

Results and discussion

Diagnosis of bacterial isolates

After collecting patients' pathological samples following certain conditions, the samples were cultured on culture media and the phenotypic characteristics of each bacteria were observed, including the shape, color, and size of the colonies, after the isolates were stained with gram stain to distinguish between gram-positive and gram-negative bacterial species. Observe the arrangement and shape of the colonies of each bacteria under microscope. Various biochemical tests were performed for the bacterial isolates, including catalase, oxidase, and IMVIC tests, and the obtained results were compared with the references used to diagnose bacterial isolates, also a Vitek2 System Compact system was used to confirm the diagnosis. Two isolates of S.*pyogenes* and *E.coli* were obtained.

S. pyogenes bacteria showed β-hemolysis on blood agar, and their shape under the microscope was cocci like strept, linked together in the form of strings resembling pearl necklaces. It was negative for the Catalase and Oxidase tests. To distinguish the *S. pyogenes*

bacteria from other *Streptococci* species, the antibiotic Bacitracin was used, and it was sensitive to the antibiotic [30], as shown in Table 1.

Table 1 : Results of biochemical tests for the diagnosis of *Streptococcus pyogenes*.

Bacteria / Test	*S.Pyogenes*
Number of isolates	32
Hemolysis on blood agar	β-Hemolysis
Catalase	-
Oxidase	-
Bacitacin	S

(-)The result is negative , (S) The result is sensitive

Colonies of *E. coli* bacteria appeared gray in color on Blood agar medium, and pink in color on MaCconkey agar medium, The reason is because it fermented the sugar lactose, and their colonies were dry, solid, and medium in size, while on Eosin Methylene Blue agar (EMB) medium, their colonies appeared with a bright metallic green color, positive for the Catalase test and negative for the Oxidase test. *E. coli* bacteria showed positive for the Indole and Methyl Red tests and negative for the Voges Proskauer and Citrate Utilization tests. As for Triple Sugar Iron, it showed A/A, producing gas and not producing hydrogen sulfide, [31], as is Shown in Table 2.

Table 2: Results of biochemical tests for diagnosing *E. coli* bacteria isolated from different infections

Bacteria / Test	*E. coli*
Number of isolates	16
Catalase	+
Oxidase	-
Indole test	+
Methyel Red	+
Voges Proskauer	-
Citrate Utilization	-
Lactose fermentation	+
Triple sugar iron	A/A, gas, no H_2S

(+)The result is positive , (-)The result is negative , (A) Acid

Diagnosis of dairy products samples

Lactic acid bacteria (LAB) which who isolated from dairy products obtained two isolates *L. acidophiles* and *B. bifidium* base on negative catalase test and positive Gram staining. *L. acidophiles* and *B. bifidium* isolates were isolated using MRSA media and characterized by morphology of colony including colony color, colony shape, colony edge shape and colony size. The colonies appeared white to cream, small, circular in shape, smooth, and convex with varying diameters. To confirm the diagnosis, biochemical tests were performed and the Vitek2 System Compact was used, as shown in Table 3 below.

Table 3: Phenotypic and biochemical tests for lactic acid bacteria (LAB).

Bacteria / Test	*L. acidophiles*	*B. bifidium*
Gram Stain	Gram positive	Gram positive
Motility Test	-	-
Catalase Test	-	-
Production of acetone from Glucose	-	-
Production of dextran from Sucrose	+	-
The fermentation of Sugars		
Cellobiose	+	-
Galactose	+	+
Fructose	+	+
Lactose	+	+
Mannitol	-	-
Maltose	+	-
Melibiose	-	-
Sorbitol	-	-
Trehalose	-	-
Xylose	-	-
Ribose	-	-
Arabinose	+	-

(+)The result is positive , (-)The result is negative

Bacterial isolates

After diagnosing 32 isolates of *S. pyogenes* bacteria and 16 isolates of *E. coli* bacteria from different infections, it was found that *S. pyogenes* bacteria are the dominant bacteria in tonsillitis, as they represented the largest percentage of the 26 isolates (81.3%). This study agreed With a study [32], where *S. pyogenes* was detected in 40 (63.5%) of throat cultures, as the bacteria was prevalent among hospital staff in Turkey as a result of transmission from one person to another and through food as a result of hand pests and mouth secretions of food service staff, and the only difference was the number of isolates collected from patients by researchers was more numerous than those collected by us from our patients, and *S. pyogenes* bacteria was found in small numbers in both wound and urinary tract infections, at a rate of 15.6% and 3.1%, respectively. while *E. coli* bacteria were the dominant bacteria in urinary tract infection, with a percentage of 72.2% of the total number of isolates, and the results of our study were consistent with the study Researcher [33], where *E. coli* bacteria represented the largest percentage of isolates in Iran, which is 74.6%, and it agreed with the results of the researcher's study [34], where the most prevalent isolates were *E. coli* bacteria with a rate of 72% of the total number of isolates.

The reason for the high percentage of *E. coli* isolates among the family of intestinal bacteria that causes urinary tract infections may be due to their possession of resistance to many antibiotics and their possession of the β-lactamase enzyme and of many virulence factors, including the production of the hemolysin enzyme, the production of toxins , and the capsule.

These bacteria are present as a normal flora in large numbers in the gastrointestinal tract of individuals [35], While *E. coli* bacteria were found in small numbers in wound infections at a rate of 18.8%, no isolation of this bacteria was found in tonsillitis. as shown in Table 4.

Table 4: Total numbers and percentages of *S. pyogenes* and *E. coli* isolates according to the type of infection.

Bacterial type	Type of infection	The total number	Percentage
S. pyogenes	Wound	5	15.6%
	Tonsils	26	81.3%
	Urinary tract	1	3.1%
The total		32	100%
E. coli	Wound	3	18.8%
	Tonsils	0	0.0%
	Urinary tract	13	72.2%
The total		16	100%

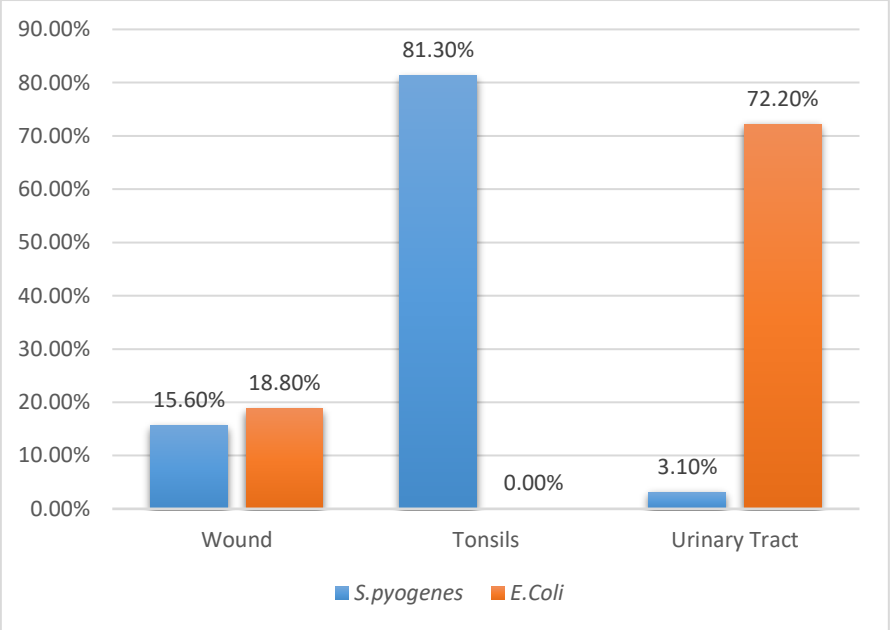

Fig. 1: Percentages of *S. pyogenes* and *E. coli* bacteria isolated from different infections.

Determining the inhibitory ability of the metabolites of lactic acid bacteria against the two isolates of S. *pyogenes* and *E. coli*:

Table 5 below shows that the inhibitory capacity of the metabolites of types of lactic acid bacteria *L. acidophiles* and *B. bifidium* and the interaction of these two types with each other at the following concentrations of 10, 20 and 40 mg/ml and on the culture medium Mueller Hinton agar against two types of bacteria, *S. pyogenes* and *E. coli* bacteria under ideal culture conditions at pH 7.0 and temperature 35°C for 24 hours.

Table 5: Inhibitory capacity of lactic acid bacteria filtrate in *S. pyogenes* and *E. coli* isolates

Lactic acid bacteria	Concentrations used (mg/ml)	*S. pyogenes* isolate	*E. coli* isolate
		Diameter of the inhibition zone in mm	Diameter of the inhibition zone in mm
L. acidophiles	10	4.2	4.0
	20	7.5	8.0
	40	17.5	16.0
B. bifidium	10	4.3	4.2
	20	10.6	9.5
	40	18.6	17.5
L. acidophiles + *B. bifidium*	10	3.9	4.0
	20	8.0	8.4
	40	22.0	20.5

The results showed that the metabolites of *L. acidophiles* and *B. bifidium* species, both of which together possess a more efficient inhibitory capacity against isolates of the *S. pyogenes* bacteria and *E. coli* bacteria when added to the holes at a concentration of 40 mg/ml compared to the other concentrations of 10 and 20 mg/ml of the bacterial type, and at a concentration 40 mg/ml, it led to the inhibition of *S. pyogenes* bacteria with diameters of inhibition of 17.5, 18.6, and 22.0 mm. As for *E. coli* bacteria, the diameters of inhibition were as follows: 16.0, 17.5, and 20.5 mm for *L. acidophiles* and *B. bifidium* and both together, respectively.

At a concentration of 20 mg/ml of the metabolites of the following species, *L. acidophiles*, *B. bifidium*, and *L. acidophiles* with *B. bifidium*, the diameters of inhibition for the *S. pyogenes* were 7.5, 10.6, and 8.0 mm, respectively, while the diameters of The inhibition against *E. coli* bacteria, is 8.0, 9.5, and 8.4 mm, respectively, but at a concentration of 10 mg/ml, it was noted that the inhibitory effectiveness became less against these bacterial pathogens, *S. pyogenes* and *E. coli*, as the diameters of inhibition for *S. pyogenes* bacteria were 4.2, 4.3, and 3.9 mm, while the inhibition diameters for *E.coli* bacteria are 4.0, 4.2, and 4.0 mm for *L. acidophiles*, *B. bifidium*, and *L. acidophiles* with *B. bifidium*, respectively.

These results agreed with what was reported by the researcher [36], who found an inhibitory effect of lactic acid bacteria on *E. coli* bacteria, as well as with the researcher's study [37], where *L. acidophiles* bacteria showed an inhibitory effect on *E. coli* bacteria.

It agreed with what was stated by the researchers [38] who proved that lactic acid bacteria have the ability to produce hydrogen peroxide, H_2O_2, which has the ability to inhibit pathogenic microbial species, as well as with the study of the researcher [39], where they confirmed that these bacteria have an inhibitory effect by creating an undesirable environment. It is suitable for microbial pathogens through the secretion of antimicrobial factors such as bacteriocin, defensin, and H_2O_2, and it worked to change the pH of the environment by producing acetic acid, lactic acid, and fatty acids. It also has a role in interacting with toxins derived from microbial pathogens.

Our study showed that the *B. bifidium* has an inhibitory role against Gram-positive and Gram-negative microbial pathogens and agreed with the findings of researchers[40], who

confirmed that these bacteria have an inhibitory mechanism for Gram-positive and Gram-negative microbial pathogens by producing acids such as acetate and lactate, and also have the ability to It exerts more than one mechanism of inhibition and thus may be important in the prevention of gastroenteritis.

Conclusion

Scientific facts can be concluded from the above results of our study that the two types of lactic acid bacteria, *L. acidophiles* and *B. bifidium*, have an inhibitory effect on the growth of *S. pyogenes* and *E.coli*. In synergy, the effectiveness of *L. acidophiles* and *B. bifidium* was high and gave inhibition values of 3.5 and 4.0 mm at a concentration of 10 mg/ml, 8.0 and 8.4 mm at a concentration of 20 mg/ml, while at a concentration of 40 mg/ml the inhibition zones were 22.0 and 20.5 mm. Compare each separately. The concentration 40mg/ml of *L. acidophiles* and *B. bifidium* was the best concentration for producing the metabolites of lactic acid bacteria, and the effect of the metabolites of *B. bifidium* bacteria on the *S. pyogenes* and *E. coli* isolates was wider than the metabolites of *L. acidophiles* bacteria.

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تحديد القدرة التثبيطية للنواتج الايضية لبكتيريا حمض اللاكتيك تجاه عزلتي المكورات العقدية والاشريكية القولونية المعزولة من المرضى الوافدين والراقدين في مستشفى صلاح الدين العام لمدينة تكريت

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البحث مستل من رسالة ماجستير الباحث الاول

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بكتيريا حمض اللاكتيك، بكتيريا العصية اللبنية الحمضية، بكتيريا المشقوقة، بكتيريا المكورات العقدية، بكتيريا الاشريكية القولونية، المعززات الحيوية، منتجات الالبان، التهاب اللوزتين، الجروح، التهاب المسالك البولية.

معلومات المؤلف

الايميل:
الموبايل:

الخلاصة:

تهدف هذه الدراسة الى عزل وتشخيص الأنواع البكتيرية *S. pyogenes* و *E. coli* من أخماج مختلفة ، ودراسة القدرة التثبيطية للنواتج الأيضية لبكتيريا حمض اللاكتيك ضد الأنواع المعزولة، وقد أجريت الدراسة على 50 عينة من المرضى المصابين بالتهاب اللوزتين وأخماج المسالك البولية وأخماج الجروح، ولكلا الجنسين وبفئات عمرية مختلفة، فقد تم الحصول على 32 عينة من بكتيريا المكوات العقدية *S. pyogenes* و16 عينة من بكتيريا الاشيركية القولونية *E. coli* .استخدمت أنواع معينة من بكتيريا حمض اللاكتيك منها *L. acidophilus* و *B. bifidum* التي جمعت من منتجات الالبان من الأسواق المحلية والبيوت المنزلية لمدينة تكريت، حيث أستخدم راشح بكتيريا *L. acidophilus* و *B. bifidum* كل على حدة وكذلك استعمل تداخل هذين النوعين مع بعضهما عند تراكيز معينة (40,20,10) ملغم/مل لمعرفة القدرة التثبيطية ضد بكتيريا المكورات العقدية *S. pyogenes* وبكتيريا الاشيريكية القولونية *E .coli* باستعمال طريقة الحفر. أظهرت النتائج امتلاك النواتج الايضية لنوعي *L. acidophiles* و *B. bifidium* وكلاهما معاً قدرة تثبيطية أكفأ ضد عزلتي بكتيريا المكورات العقدية *S. pyogenes* وبكتيريا الاشيركية القولونية *E. coli* عند إضافتها الى الحفر بتركيز 40 ملغم / مل مقارنة مع التراكيز الأخرى 10 و 20 ملغم/مل من النوع البكتيري، وعند تركيز 40 ملغم / مل أدى الى تثبيط بكتيريا *S. pyogenes* بأقطار تثبيط 17.5 و18.6 و22.0 ملم أما بكتيريا *E.coli* فقد كانت أقطار التثبيط كالاتي 16.0 و17.5 و20.5 ملم للأنواع *L. acidophiles* و *B. bifidium* وكلاهما معاً على التوالي. في التآزر، كانت فعالية *L. acidophiles* و *B. bifidium* عالية ضد بكتيريا *S. pyogenes* وبكتيريا *E. coli* مقارنة كل على حدة. كان التركيز 40 ملغم/مل هو التركيز الأفضل لانتاج النواتج الايضية لبكتيريا حمض اللاكتيك، كان تأثير النواتج الايضية لبكتيريا *B. bifidium* على عزلات *S. pyogenes* و *E. coli* أوسع من تأثير النواتج الايضية لبكتيريا *L. acidophilus*.