

Isolation and Evaluation of Antibacterial Activity of Bacteriocins Produced by Bifidobacteria sp Against Staphylococcus aureus

Hanaa Khaleel, Moaed Hannon

Public health department, college of Veterinary medicine- Basrah University, Iraq

Hanaakhli878@yahoo.com

Abstract:

This study included detection of bacterial antimicrobial activity produced from Bifidobacteria sp on isolates of Staphylococcus aureus. Eighty sample different bovine species (feces, milk, soil and vaginal swab) were collected isolates to Bifidobacteria sp and Fifty five sample different bovine (nasal discharge, wounds and ear swab) isolates to Staph. aureus in northern Basra province. culture characteristics of the colonies, microscopic examination and biochemical tests showed that 10 isolates belonging to Bifidobacteria sp. The Bifidobacteria sp isolates were tested for fermentation the results showed the dominance of Bifidobacteria. longum of the other species we represented Bifidobacteria bifidum, Bifidobacteria reve, Bifidobacteria adreventis, Bifidobacteria thermoacidophilium and Bifidobacteria sp. The results showed that most of the isolates of Bifidobacteria sp were effective against Staph.aureus isolates using the diffusion technique of hole and disc agar. The diameter of the inhibition zones ranged from 10-25 mm and tested the ability of Bifidobacteria sp isolates to produce bacteriocines against Staph. aureus. Staph. aureus with the diffusion technique and the isolation (Bifidobacteria bifidum2) was higher (P <0.05). It was observed that the highest production of bacteriocins at the development of isolates in the media of liquid MRS at pH 6 and at 37 ° C .

Key word : Bifidobacteria sp, bacteriocin, Staph. aureus

I. INTRODUCTION

The microbes are of a protein nature produced by elite large bacteria have an inhibitory effect, killer or position(1) to grow towards its. Sensitive bacteria and often have a genetic affinity or link with the produced bacteria . Bacteriosin is known as lactic acid bacteria Peptides with low molecular weights and effective anti-bacterial and often extends its range to species other species are closely related to the produced bacteria (2). Most bacteriosin are non-lactic acid bacteria affect the gram negative bacteria as they do not affect the yeasts and perfumes as well as their effect in some gram positive bacteria with the possibility of presence non-sensitive cells within the strain(3,4). Sensitive Bactericosins are protein compounds with high molecular weight. (5) . The discovery of bacteriocines dates back to 1877, when researchers observed the phenomenon of bactericidal resistance between microorganisms, as some bacterial strains can inhibit the growth of other bacteria belonging to the same species antagonism (6) general microcins (5). However, there are bacteriocines whose production is controlled by the chromosome, especially whose genes are chromosome-dependent. Serratia marcescenc produced by bacteriocin 28b (7).

Bacteriosin is produced and stimulated in bacteria that possess a special plasmid for the production of bacteriocin only, and it is manufactured in the normal cases in small quantities (8, 9). Bifidbacter sp was first discovered by Taysir in 1990 and was described as anaerobic, positive for chromatography, immobile, not blackboard, producing acids and producing gas during growth, isolated from the feces of breastfed infants since its appearance is similar to Y that's, why it's named Bacillus bifidus then re-named and named Bifidobacterium bifidus (10) Bifidobacterium has been identified over the last century and many studies have indicated that these bacteria have therapeutic properties related to human and animal health (11). Isolated from the intestinal tract of animals such as rats, rabbits, poultry, pigs and cattle (12).

The species of this genus was first discovered in 1899 by Tissier and was referred to as Bacillus bifidus, but later was classified as Bifidobacterium bifidum. For their resemblance with lactobacilli in many morphological and physiological aspects, at first they were collectively referred to as Lactobacillus bifidus. In 1960s, the 'bifid shunt', a specific Bifidobacterium hexose fermentation pathway with help of a fructose-6-phosphate phosphoketolase (F6PK) was discovered (13). Culturing is the conventional method of Bifidobacterium identification and enumeration and many selective media have been developed for that purpose(14) .Bifidobacteria sp has the ability to synthesize organic acids and other microbial antibodies, bacteriocins, although some research has indicated organic acid production through heterogenous fermentation processes responsible for bifidobacteria sp and some bifidobacteria sp have the ability to produce bacteriocins (15) Bifidobacterium isolates are unable to produce catalase, because they are anaerobic growth,

losing the chain of electron transport, which leads to the accumulation of hydrogen peroxide in the cell due to the effectiveness of the enzyme NADH Oxidase flavoprotein Oxidase (16). Bacteriocins are labeled as peptides or proteins that create ribosomes and are produced by bacteria and have the efficacy of either bacteriostatic or bactericidal inhibitors against other species(17). Meghrou et al., (1990) are the first showed that Bifidobacteria sp have the ability to produce antimicrobial agents against other types of bacteria for the gram-like form of bactericin. On the other hand, the production of bacteriosin by the gram-positive bacteria will act on adhesion to the outer membrane and then lead to the destruction of bacterial cells (18).

II. MATERIAL AND METHODS

1. Sample collection

All 80 different samples were collected to isolate the Bifidobacteria sp and 55 samples of Staph. aureus in north of Basra city as described in table (1) .

Table (1): Sample types and number collection isolated type bacteria Bifid bacteria and Staph. aureus

Isolation bacteria	Type specimen	N. sample collectio
Bifid bacteria sp	Cow feces	٢٠
	Cow milk	٢٦
	Animal soil	١٣
	Cow vaginal swab	٢١
Isolation bacteria	Type specimen	N. sample collectio
Staphylococcus aureus	Cow nasal discharge	١٥
	cow swabs of wounds	١٠
	Cow ear swab	٢٠
	Cow vaginal swab	١٠

2. Bacterial Isolation

To identify bifidobacteria sp samples were culture on the m-MRS medium The MRS liquid medium ,as culture on the Bifidobacterium media contains 23gm pepton, 5gm NaCl, 5gm glucose, 1gm soluble starch , 0.3gm L-Cysteine hydrochloride and 15gm agar. These components dissolved in liter of distilled water and adjusted to pH to 6.8± 0.2 , sterilized in autoclave at 121°C for 15 mint and incubated under anaerobic conditions at 37°C for 48-72 h. (19). To identify Staph. aureus samples were culture on the Mannitol Salt Agar and incubated at 37°C for 24 h .

3 . Microscopic examination

Microscopic examination of the isolates showed a positive gram stain and a bacilli branch like shape Y or V (20). While, Staph. aureus showed positive gram stain and a spherical shape similar to grape clusters (21).

4. Biochemical Test

a. Catalase test: Place part of the bacterial growth of both types of Bifidobacteria sp and S. aureus on a glass slide and add to it a drop of the hydrogen peroxide reagent the appearance of bubbles evidence of positive testing(22).

b. Oxidase test: One pure colony protected on the 24-h. nutrient agar was transferred to a pre-wetted filtration paper with an oxidase enzyme detector using sterile wooden chopsticks that turned the colony into a dark violet directly(23).

c. Methyl red test: Inoculated the tubes containing the methyl red media Bifidobacteria sp and incubated tubes at 37°C for 48-72 h. After the incubation period, the methyl red reagent change to the red color evidence of the positive test (24).

d. Indole test : Poured in 5ml peptone water in tubes , sterilized by autoclave, and incubated at 37°C for 24 h . After the incubation period two drop (Kovac's reagent) was added to each tube to identify Bifidobacteria sp (25).

e. Citrate utilization test : Poured in glass tube (5ml for each one), autoclaved put in slant position to harden inoculated with bacteria and incubated at 37°C for 24 h. Changing the color from green to blue indicates a positive test (24).

f. Urease test: Inoculated tubes containing urease agar for bacteria Bifidobacteria sp way stabbing and streaking on the slant, incubated at 37°C for 24 h. Changing the color to pink indicates a positive test (25).

g. Coagulase test : This test was used to distinguish the strain of Staph. aureus produced by the enzyme from those non- productive strain . Performed the test according slide coagulase test (22).

h. Gelatin liquefaction : Prepared was test from dissolved 12 gm from gelatin in 100ml from nutrient slant broth and distributed in tubes and sterilized autoclave . This media was used to detect the susceptibility of bacteria to gelatin synthesis by producing gelatinase enzyme(24).

j. Capsule production detection : This test was done by used India ink stain to detect susceptibility to capsule production of Staph. aureus (26).

k. B-hemolysin : The test was done to investigate the susceptibility of the bacteria B- hemolysin production .which works on the whole blood analysis (25).

4. Sugar fermentation test : Inoculation test tube containing on sugar fermentation prepared from dissolution 10 gm peptone, 1gm beef extract , 5gm NaCl 0.08 gm phenol red in 1000ml distal water and adjust the pH to 7.4 added carbohydrate solution after sterilization with Millipore filter (0.22 µm) to give final concentration 1%. This test was done to investigate the susceptibility of bacteria to carbohydrate fermentation (25) .

5. Study the antimicrobial activity Bifidobacteria sp against resistance Staph. aureus

a. Cup agar : Culture bacteria Bifidobacteria sp in streaking away on MRS agar the pH 5.5 ±2 incubation condition anaerobic at 37°C for 24-48 hours .After incubation, discs were worked with hole diameter of 5 mm from this medium and placed on Muller Hinton agar cultured S. aureus and incubation at 37°C for 24hours .The diameter of the inhibition area around the discs was then measured (27).

b. Broth well- diffusion method : culture Bifidobacteria sp in tubes containing on MRS broth the pH 5.5±2 incubation tubes at 37°C for 24 h. under condition anaerobic, the diffusion method was used in the drill to detect the inhibitory activity Bifidobacteria sp and so on culture Petri dish on MHA by 0.1 ML of the Staph. aureus isolated vaccine and worked 5 mm hole using a filled cork hole 50 µl of the liquid culture Bifidobacteria sp after, incubation plate 37°C for 24 h. and diameter of the inhibition area around hole was then measured (28).

6. Extraction Bacteriosin from Bifidobacteria sp

All Bifidobacteria sp isolates were used to extract the Bacteriosin production as follows:

The bacteria were cultured on liquid MRS the pH 5.5 ±2 incubated at 37°C for 48-72 h. under condition anaerobic. The centrifuge tubes were then discarded (6000 cycle per minute for 30 minute), then the supernatants was taken away and the precipitate was neglected and the mixture was properly balanced pH to 7 by (1N) from NaOH to completely eliminate the antibacterial activity of the organic acid after sterilization of the liquid through filters Millipore diameter 0.22µm and incubation 4°C until use(29).

7. Study of the inhibitory activity of bacteriocin produced from Bifidobacteria sp towards Staph. aureus

The diffusion method was used in the well to detect the inhibitory activity of Bifidobacteria sp and so on culture plates containing on MHA by 0.1 ml of inoculation isolated S. aureus sizes 1.5×10^8 cell/ml well work diameter 5 mm using the cork hole was filled 50µm of bacteriocin raw Bifidobacteria sp. After incubation plates at 37°C for 24 h. the diameters were measured around the hole (29).

III. RESULT AND DISCUSSION

Ten *Bifidobacteria* sp. samples were isolated from 80 samples collected from different sources in Basrah city include (20 cow milk, 26 cow feces, 13 animal soil and 21 cow vaginal swab).

The colonies *Bifidobacteria* sp. when culture in the media of m-MRS agar in the conditions of anaerobic color of non-transparent and convex shape circular and most colonies near to the surface and a smaller size and this matches to the description(30), has been cultured on *Bifidobacterium* agar to disperse them *Lactobacillus*. The results of the microscopic examination of the slides prepared from the colonies of *Bifidobacteria* sp using the gram positive stain, short bacillus of shape taken in the form of Y or V as described in Figure (1)

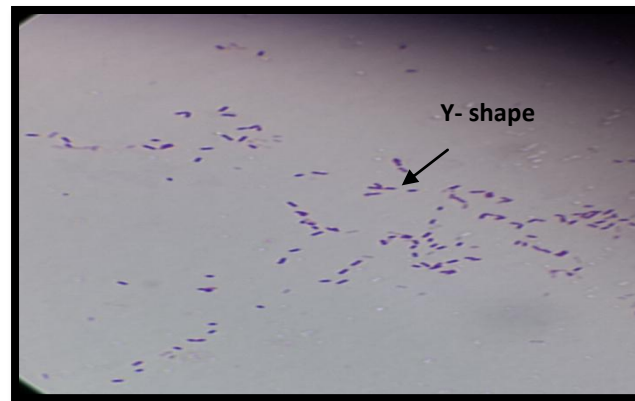


Figure (1): *Bifidobacteria* sp. showing gram positive stain

As for *S. aureus*, 55 samples were collected from different sources included (15 cow swabs from the nasal, 10 cow swabs of burns and wounds, 20 cow swab of ear and 10 cow vaginal swab). The isolates *Staph. aureus* able to grow on an aerobic on MSA color change by changing the pink to yellow medium due to its ability to ferment the mannitol sugar and some isolates, the color of the medium has not changed because it cannot ferment the mannitol sugar (21). While, the microscopic examination showed that their positive spherical cells or bunches of grapes this matches with (31) as described in Figure (2).

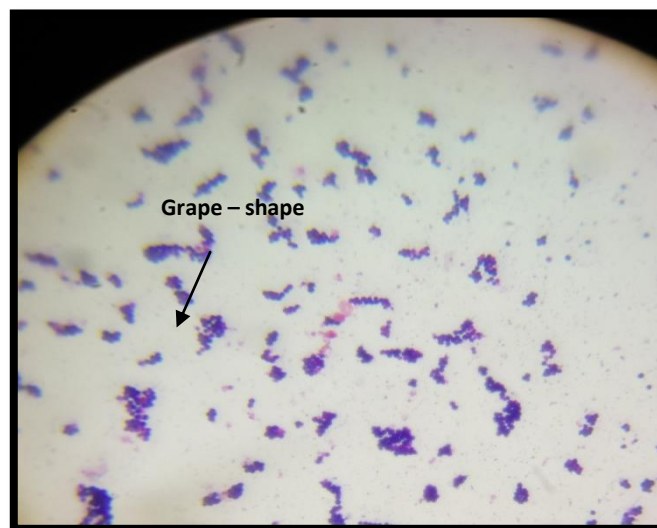


Figure (2): microscopic examination Showing of *Staph. aureus* Gram stain

The biochemical tests of *Bifidobacteria* sp and *S. aureus* obtained from various sources showed that all isolates *Bifidobacteria* sp was characterized by being negative for the test of catalase, oxides and urea table (2). This finding is in a greenmt with previous study (32).

Results have recorded that all isolates Staph. aureus was characterized by being positive for the test of catalase because it is an aerobic or anaerobic facultative. It can through this capacity of the production of catalase enzyme, protects it from the toxic effect of hydrogen peroxide produced during the metabolic processes and inability to produce the enzyme oxidase (33,34). That showed a positive result of coagulase enzyme.

Table (2) Biochemical test Bifidobacteria sp and s. aureus

Bacteria	Bifidobacteria sp	S.aureus
Biochemical test		
Gram stain	+	+
Catalase	-	+
Growth 37°C	+	+
Citrate utilization	-	
Methyl Red	+	
Idol test	-	
Urease test	-	
Oxidase test	-	-
Capsule formation		+
Gelatin hydrolysis		+
Coagulase test		+
β-hemolytic		+

4. Carbohydrate fermentation test for diagnosis of bacterial species Bifidobacteria

Differential biochemical tests are important in the diagnosis of Bifidobacteria sp isolates from other bacteria. The ability of these bacteria to ferment and represent carbohydrate sources is an important feature of distinguishing between Bifidobacteria sp, this test was used to demonstrate the ability of Bifidobacteria sp to consume carbonic sources and sugar fermentation medium used sugars to test the ability of bacteria to ferment and gave the isolates a taste and a clear variety in their ability to ferment sugars. That was shown in the table which indicated the results of fermentation test of carbohydrate sources of bacteria Bifidobacteria sp. The results showed that some isolates (Bif 7, Bif 5) were fermented for arabinose sugar. While, isolates (Bif 1, Bif 4) did not ferment ribose sugar isolates (Bif 3, Bif 8) did not ferment sugar. This finding in agreement with previous study (35) to distinguish bacterial species B. breve, B. bifidum and B. longum. While, isolates (Bif 9, Bif 2) were fermented for mannitol sugar (36) to distinguish B. adolescentis, while isolation Bif 6 showed non-fermented lactose sugar. This finding agrees (37) to diagnosis B. thermoacidophilum.

Table(3): Testing the fermentation of sugars to distinguish between Bifidobacteria sp

Sugars	B. Bifidum	B. longum	B. breve	B. adolescentis	B. thermoacidophilum
Galactose	+	+	+	+	

Sucrose	+	+	-		
Arabinos	-	+	-	-	W
Lactose	+	+	+	+	-
Ribose	-	+	+	-	W
Mannitol	-	-	±	+	
Fructose	+	+	+	+	
Maltose	+	-	+		
Mannose	-	-	+	+	
Glucose	+	+	+	+	
Xylose	-	-	-		-

± : both results positive and negative, W : a weak results

Capsule production Detection

The susceptibility of 15 isolates from *Staph. aureus* was investigated for its capsule production by examination microscopy of bacteria using Indian ink, The results showed that all the isolates were producing the capsule. Therefore these isolates are fierce because the capsule is one of the factors of virulence. This is confirmed by studies, which have shown that the capsule has an important role in the disease (38) and the presence of the microcapsule cannot bacteria from the movement and give it the character of ferocity (39).

Investigation of the viability of *Staph. aureus* on the production of gelatinase

All isolates were investigated for the production of gelatinase. The results showed only 13 isolates on the enzyme production by incubation period between 24-48 days. While, the isolates 2 did not produce this enzyme even after incubation period of more than 48 days . Each of the (40), it has been fouled that the gelatinase has the ability to break the components of the membrane such as collagen, gelatin and proteoglycan constituents of the extra cellular matrix during the outbreak of cancer tumors. It has been fouled showed the results that some isolates possess the ability to gelatin hydrolysis. This finding is in agreement with previous study (41). Therefore, the enzyme gelatinize is an important enzymes that increase the ferocity of the bacteria as it works to break down gelatin to the basic units of amino acids (42).

Investigation of the susceptibility of *Staph. aureus* to the production of haemolysin

The susceptibility of 15 isolates *Staph. aureus* was tested on haemolysin production by the cultured of bacteria on container plates on blood agar, The results showed that only 13 isolates were blood hemolytic for the appearance of a transparent halo around the colonies indicating the ability of these isolates to produce β- hemolysis match with (43), which shows about 85.1% of isolation *Staph.aureus* productive of type β- hemolysis, the ability of bacteria to produce β- hemolysis varies according to several factors. The most important of which is the source of red blood cells used in the culture medium in which the detection of the ability of bacteria to produce β- hemolysis, as well as the test method used, and the presence of serum and cholesterol in the blood used to inhibit the process of analysis (44) ,thus the *Staph. aureus* has the ability to produce haemolysin due to the osmosis property it possesses to analysis the blood cells or because of the pore formation or cytotoxic cell to solve the types of human blood cells (45).

Antimicrobial activity Bifidobacteria sp and bacteriosin to ward *Staph. aureus*

The study used 10 isolates of Bifidobacteria sp and 10 bacterial isolates of *Staph. aureus* , Two methods of pore and disc agar were used to determine the effect Bifidobacteria sp and bacteriosin to word isolates *S. aureus*.The diffusion method was detected by well in the liquid medium of Bifidobacteria sp. without extracting bacteriocin efficiently in inhibition of most isolates compared to the method of disc agar and bacteriocin as shown in tables (3,4,5), this can be due to the liquid nature of the medium, which makes it easier for the lactic acid bacteria cells to obtain the nutrients that are easy to consume and then to produce the best microbial against in the liquid medium. They also

determined their effectiveness by measuring the inhibition diameter (Fig3), antimicrobial activity of Bifidobacteria sp. and bacteriocin two way disc agar and diffuse of well to ward Staph. aureus.



Figure 3 : Antimicrobial activity isolation Bifidobacteria sp and bacteriocin to word Staph. aureus by way disc agar and well diffusion

Differentiation of the inhibitory effect of bifidobacteria sp isolates towards Staph. aureus isolates about between 10-25 mm. The effect of each isolation of Bifidobacteria sp isolates was different to different isolates of the bacterium Staph. aureus.

The inhibitory efficacy of Bifidobacteria sp isolates towards Staph. aureus isolates showed significant differences ($P < 0.005$), for most of the isolates used from Bifidobacteria sp bacteria towards Staph. aureus (Table 4).

Table 4: Antimicrobial activity Bifidobacteria sp against Staph. aureus in a way agar

Diameter inhibited toward isolation Staph. aureus compared mm										Bifidobacteria sp
S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	
20	18	21	22	20	20	15		10.8	10.5	B.brevei 1
20	19	22		20	18	20.5	10	14	20	B.bifidium 1
13	10	25	20	15	10.9	17	20	16	13	B. longum 1
23	20		15.5	22	17	20	14		18	B.adolescentis 1
12.9	10.5	13	10.9		10.8	15.5	11	10.3	10	B.thermoacidophilu
10.5	14	19	13.6		10	10.7	11	18	20	B.brevei 2
11	18	14.8	18.5	21		24	20	15.5	13	B. longum 2
24	18.8	23	18	22	16.5	10	16	20		B.bifidium 2
24	19.6		24	14.9	10.8	20	17.5	20	18	B. longum 3
10	20	12.8	16	10		15	10.5	18		B.adolescentis 2

As for the inhibitory effectiveness of Bifidobacteria sp isolates towards Staph. aureus isolates in the broth method, the results showed there was significant differences ($P < 0.005$) (Table 5).

Table 5 : Antimicrobial activity isolates Bifidobacteria sp against Staph .aureus in a way broth

Diameter inhibited toward isolation Staph. aureus compared mm	Bifidobacteria sp
---	-------------------

S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	
10.5	14	19	13.6	20	10	10.7	11	18	20	B.brevei 1
11	18	14.8	18.5	21	18	24	20	15.5		B.bifidium 1
10	20	12.8	16	10	11	15		18	13	B. longum 1
23	25	11.5	15.5	22	17	20	14	10	19	B.adolescentis 1
10	12	11	17	11	16	10.7	20		10	B.thermoacidophilum
20	19	22	20	20		20.5	10	10	12	B.brevei 2
12.9	10.5	13	10.9	17	10.8	15.5	11	10.3	10	B. longum 2
20	18	21	22	20	20	15		10.8	10.5	B.bifidium 2
24	19.6	18	24	14.9	10.8	20	17.5	22	20	B. longum 3
10.5	18	21	11		20	20	15.7	22	18	B.adolescentis 2

For the inhibitory efficacy of bacteriocine produced from Bifidobacteria sp towards Staph. aureus isolates, there was significant differences (P<0.005).

Table 6 : Antimicrobial activity isolates Bifidobacteria sp in a way bacteriocin

Diameter inhibited toward isolation Staph. aureus compared mm										Bifidobacteria sp
S10	S9	S8	S7	S6	S5	S4	S3	S2	S1	
10	20	12.8	16	10	11	15	10.5	18	13	B.brevei 1
14	20		24	18	20	23	16	19.5	10	B.bifidium 1
20	18	21	22	20	20	15	10.4	10.8	10.5	B. longum 1
	18	21	11	19	20	20	15.7	22	18	B.adolescentis 1
12.9	10.5	13	10.9	17	10.8	15.5	11	10.3		B.thermoacidophilum
20	18	21	24		20	22.5	23	17	11	B.brevei 2
24	19.6	18	24	14.9	10.8	20	17.5	22	20	B. longum 2
20	17	11	17	16.5	23	18	22	10		B.bifidium 2
	14	19	13.6	20	10	10.7	11	18	20	B. longum 3
11	18	14.8	18.5	21	18	24	20	15.5	13	B.adolescentis 2

The results showed that Bifidobacteria sp. were resistant to Staphylococcus aureus. Bifidobacteria sp. may be due to organic and inorganic acids such as lactic acid and acetic acid. The inhibitory effect of these acids is due to their non-disintegrating form by their ability to penetrate and impede the transport of nutrients as they are weak organic acids and partial disintegration of solutions hydrolysis and possessing a lipid-soluble property, which makes them free to spread freely through the plasma membrane to the cytoplasm (46).

The results were also agreed with(47), who have proven that Bifidobacteria sp. have an inhibitory effect on Staph. aureus and other negative and Gram positive bacterial strains. The inhibitory effect is due to the ability of organic acids produced from Bifidobacteria sp. to penetrate the cellular membranes by spreading and removing proteins in the cell leading to increased cytoplasmic acidity (48), it has a signal of both (49) , to the differing views of researchers on the mechanism of the inhibitory effect of bacteria Bifidobacteria sp. towards pathogenic bacteria, some of which was caused by the effect of the acidic organic and lactic acid produced by these bacteria in the inhibition of pathogenic bacteria, in part due to the possession of the protein produced by bacteria Bifidobacteria sp and its inhibition. The researchers explained that the production of organic acids and reduction of pH is not the main factor in the inhibition of pathogens because of the presence of some pathogens such as E. coli, which has the ability to resist pH and the greater inhibitory effect of the other inhibitory substances produced by Bifidobacteria sp , in addition to the role of lactic acid bacteria in reducing pH, oxidative stress reduction and nutrient competition, as well as its production of microbial antibodies of bacteriocin (50). These results are match with both (51,52) in inhibiting the growth of S. aureus and other bacterial pathogens.

As mentioned (53) Bifidobacteria sp. have inhibitory effect on pathogens that produce other inhibitory substances such as bacteriocin, which has an antimicrobial action and is a key inhibitor of pathogenic bacteria and bacteria that cause food damage. It has also been shown that the broth culture of Bifidobacteria sp. have inhibitory effect

against the negative and positive bacteria of Gram stain as well as yeasts (54). The inhibitory action of bacteriocin is due to having a deadly act and the ability to bind to the receptors of the specialized cell. The cytoplasmic membrane is the primary target of bacteriocin. The treatment of cells has the uncommon flow velocity of amino acids and positively charged ions, exploding the cell membrane and thus killing sensitive cells (55).

IV. CONCLUSIONS

The possibility of isolating *Bifidobacteria* sp from different samples of animals in the northern city of Basra, *Bifidobacteria* sp. have a large inhibitory capacity for *Staph. aureus*, which opens the way for the study of their use as biocomponent agents for the treatment of *Staph. aureus* for resistance to most antibiotics, the bacteriocin product of *Bifidobacteria* sp. is effective against *Staph. aureus*.

REFERENCES

1. Hanlin, M.B.; Kalchayanand, N.; Ray, P. and Ray, B. (1993). Bacteriocins of lactic acid bacteria in combination have greater antibacterial activity. *J. of food protection*, vol. 50, No. 3, 252 - 255.
2. Helander, I.M.; Wright, A.V. and Mattial - sandholm, T.M. (May 1997). Potential of lactic acid bacteria and novel antimicrobials against Gram - negative bacteria. *Trends in food science and technology*, vol. 8, p.146-150.
3. Harady, K. (1986). *Bacterial plasmid*. 2nd Edition. American society for Microbiology - U.S.A.
4. Riley, M. A. (2002). Bacteriocin-Mediated Competitive Interactions of Bacterial Populations and Communities in "Prokaryotic Antimicrobial Peptides: From Genes to Applications" Drider, D. and Rebuffat, S. (eds.), Springer Scienc.
5. Ridley, H. and Lakey, J. H. (2015). Antibacterial toxin colicin N and phage protein G3p compete with TolB for a binding site on TolA. *Microbiology*, 161:503-15.
6. Vidotto, M. C.; Furlaneto, M. C. and Perugini, M. R. E. (1991). Virulence Factors of *Escherichia coli* in Urinary Isolates. *Brazilian J. Med Biol. Res.*, 24: 365- 73.
7. Harry, C.O. and Walker, D. (2013). Cytotoxic activity of colicin E1, E3 and E9 against *E. coli* BW25113 in the planktonic and biofilm states. *Int. J. Curr. Res. Aca. Rev.* 1 (2):55-71.
8. Cursino, L.; Smarda, J.; Chartone, E. and Nascimento, A. (2002). Recent Updated aspects of Colicins of Enterobacteriaceae. *Braz. J. Microbiol.*, 33: 196-217.
9. Pugsley, A. P. and Schwartz, M. (1983). A genetic approach to the study of Mitomycin- induced lysis of *E. coli* K-12 strains which produce colicin E2. *Mol. Gen. Genet.*, 190: 366- 72.
10. Tannock, G. (2010). Analysis of *Bifidobacterial* population in bowel ecology studies. In B. Mayo and D. van Sinderen. ed. *Bifidobacteria. Genomics and Molecular Aspects* Caister Academic Press. pp. 1-15.
11. Aldra, Omar Abboud Aldroush, Amer Khalaf Aziz Obeidi, Fares Abd Ali (2005). The effect of fermented dairy in the level of cholesterol for the blood of rats and thair on the normal level of cholesterol. *Journal of Iraqi Agricultural Sciences* 2(36) 165-172.
12. Biavati, B. and P. Mattarelli. (2005). The family *Bifidobacteriaceae*. In *The prokaryotes an evolving electronic resource for the microbiological community*. M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer and E. Stackebrandt (eds.). 3rd ed. release 3.2. Springer. New York.
13. de Vries W., Stouthamer A. (1967). Pathway of glucose fermentation in relation to the taxonomy of *bifidobacteria*. *J. Bacteriol.*, 93, 574-576.
14. Ferraris L. et al. (2010). New selective medium for selection of *bifidobacteria* from human feces. *Anaerobe* (16), 469-471.
15. Bruno, F.A. and Shah, N.P. (2002). Inhibition of pathogenic and putrefaction microorganism by *Bifidobacterium* sp. *Milchwiss.* 57:617-621.

16. Kaufman, P.; Pfeffernkorn, A.; Teuber, M. and Meile, L.(1997). Identification and quantification of Bifidobacterium species isolated from food with genus-specific 16S rRNA- targeted probes by colony hybridization and PCR. *Appl. Environ. Microbiol.* 63:1268-1273.
17. Balciunas, EM.;Martinez, FAC.; Todorov, SD.; de Melo Franco, BDG.; Converti, A.; de Souza Oliveira, RP.(2013). Novel biotechnological applications of bacteriocins .*Rev.Food. Control.* 32:134-142.
18. Brook, I. (1999). Bacterial interence. *Crit .Rev. Microbiol.* 25:155-172.
19. Atlas, R.M.(2004). *Handbook of Microbiology Media* . 3rd ed. Parks, L.C.(Ed), CRC Press, Boca Raton.
20. Scardovi V. (1986). Genus Bifidobacterium Orla-Jensen 1924,472 al ..In: Seath P.H.A., Mair N.S., Sharpe M.E., Holt J.G., eds, *Bergeys Manual of Systemic Bacteriology* . Williams and Wilkins , Baltimore, pp. 2: 1418-1434.
21. Schleifer, K. and Bell,J.A. (2009). Staphylococcusea. In , *Bergeys Manual of Systemic Bacteriology* . Parte, A.C., Whitman, W.B., Vos, P.De, G. M. Garrity, Dorothy Jones, Krieg, N.R., Ludwig, W., Rainey, F.A., Schleifer K. and Whitman, W.B.,ed *Biological Sciences Building University of Georgia Athens, GA-USA.P:* 392-420.
22. Fordes, A.; Sahm, D. and Wessfeld, A. (2007). *Diagnostic microbiology.* 12th ed. Elsevier. Texas.
23. Harly, J.P. and Prescott, L.M. (2007). *Laboratory Exercises in Microbiology* . 7th ed. McGraw- Higher Education . New York.
24. Macc Fadin, j.K.(2000). *Biochemical test for identification of medical bacteria* . 3th ed. Lippincott Williams and Winkins . Awolter Klumer Company . Philadelphia Baltimor. New York.
25. Collee, j.G.; Fraser, A.G.; Marmino, B.p; and Simons , A. (1996). *Mackin and McCartney Practical Medical Microbiology.* 14th ed. The Churchill Livingstone . Inc. U.S.A.
26. Bottone , E.J.; Patel, L.; Patel, P. and Robin, T.(1998). Mucoid encapsulated Enterococcus faecalis an emerging morphotype isolated from patients with urinary tract infections .*Diagen. Microbiol. Infect. Dis.* 31: 429-430.
27. Martinez. Gonzalez, B.; Erioton, E.; zopoulos. G; Tsalcalidon, E. and Mentis. A.(2004). In Vitro and in vivo inhibition Helicobacter pylori by Lactobacillus casei strain shirota. *APPL. Envir . Microbiol.* (790) 19: 518-526.
28. Annuk, H.; Shchepetova, J.; Kullisaar, T.; Songisepp, E.; Zilmer, M. and Mikelsaar, M.(2003) Characterization of intestinal Lactobacilli as putative probiotic candidates.*J. Appl. Microbiol.* 94: 403- 412.
29. Zinedine, A.and Faid,M. (2007). Isolation and characterization of strains of Bifidobacterum with probiotic oroprieties In Vitro. *Worl. J. Dairy . Food. Sci.* 2:28-34.
30. Dubey, U.K. and Mistry, V.(1996). Growth characteristics of Bifidobacteria in infant formula. *J.Dairy. Sci.*79:1146-1155.
31. Benson, J.H.(2002). *Microbiological applications: Laboratory manual in general microbiology.* 8th ed. McGraw Hill companies . New York.
32. Leahy, S.C.; Higgins, D.D.; Fitzgerald, G.F. and Van Sinderen, D.(2005). Getting better with Bifidobacteria .*J. Appl. Microbiol.* 98: 1303- 1315.
33. Schneewind,O. and Missiakas, D.(2009). Staphylococcus aureus and Releted Staphylococci. In: Goldman E. and Green L. H.(ed). *Practical handbook of microbiology.* Taylor and Francis Group, an informa business.
34. Mack, D.; Horskotte, M.A.; Rohde, H. and Knobloch, J.K-M.(2009). Coagulase negative staphylococci . in: *biofilms, infection, antimicrobial therapy.* Pace, J.L. Rupp, M.E. and Finch, R.G. 109- 132. Taylor and Francis. NewYork.
35. Mahomoudi, F.; Miloud, H.; Bettach, G. and Mebrouk, K.(2013). Identification and physiological Properties of bifidobaterium Strains Isolated from Different Origin. *University of el-menaouer. J.Food. Sci.Engine.* 3:196-206.

36. Toure , R.; Kheadr, E.; Lacroix, C.; Moroni, O. and Fliss, I.(2003). Production of antibacterial substances by Bifidobacterial isolates from infant stool active against *Listeria monocytogenes*. University of Alexandria , Egypt. *J. Appl. Microbiol.* (95): 1058- 1069.
37. Dong, X.; Xin, Y.; Jian, W.; Liu, X. and Ling, D.(2000b). *Bifidobacterium thermacidophilum* sp. Nov., isolated from an anaerobic digester. *Int. Syst. Evol. Microbiol.* 50 : 119- 125.
38. Gemmell, CG. (1986). Coagulase – Negative Staphylococci . *J. Med.Microbiol* . 22: 282- 295/
39. Holt, J.G.; Krieg, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T.(1994). *Bergeys manual of determinable bacteriology* 9th ed. William and Wilkins, Baltimore.
40. Estefania, M.A.; Beatriz, G.S.; Carlos, A.; Cristina, C.; Rosa, C.; Pablo, E.H.; Carmen, H. and Luis, M.C.(2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of Lactic Acid Bacteria of aquatic origin intended for use as probiotics in aquaculture. *BMC Microbiol.* 13 (15): 1-22.
41. Stock, I. and Wiedemann. (2001). Natural antibiotic susceptibility of *Klebsiella pneumonia* , *K. oxytoca*, *K. planticola*, *K. ornithinolytica* and *K. terrigena* strains . *J. Med. Microbiol* . 50 : 396- 409.
42. Pickett , M.J.; Greenwood, J.R.; Harvey, S.M. (1991). Test for detecting degradation of gelatin : Comparison of five methods. *J. Clin. Microbiol* . 29(10): 2322-2325.
43. Al- Junadi, A. S.(2005). Immunological study on TSS-1 toxin extracted from *Staphylococcus aureus* isolated from infected wounds. Ph. D. Thesis . College of Science / Al- Mustansyria University. Iraq.
44. Hellerstein , S (2002). Urinary tract infection in children : pathophysiology, risk factor and management . *Infect . Med.* 19: 554- 560.
45. Al- Chalabi , R.; AL- Ubaidy, A. and AL-Ibabi , M.(2010). Dtection of Urovirulence Genes (*ea*, *E-hly*, α -*hly*)of Uropathogenic *Escherichia coli* by Specific PCR. *J. Biot. Res. Center.* 4(1): 44-54.
46. Rodrigues , E., Arques, J. and Rodrigues, R. (2012). Antimicrobial properties of probiotic strains isolated frunc. *Food.* 4 : 542 – 551.
47. Kozhakhmetov , S.; Oralbayeva, S. S.; Kushugulova, A. R.; Almagambetov, K.; Abzhalelov, A. K. and Raman – kulov, E. M. (2009).Creation of the probiotic consortium on the Base of Strains of *Bifidobacterium* spp., Malaysian . *J.Microbiol* . 5(2) : 67 – 72.
48. Ogawa, M.; Shimizu, K.; Nomoto, K.; Tanaka, R.; Yamasaki, S.; Takeda, T. and of in vitro growth of Shiga toxin-producing *Escherichia coli* O157: H7 by probiotic *Lactobacillus* strain due to production of Lactic Acid . *Int. J.Food . Microbiol.* 68: 135- 140.
49. Cheikhyousssef , A.; pogori , N. and Zhang, H. (2007a). Study of the inhibition effects of *Bifidobacterium* supernatant against *Bacillus cereus* and *Escherichia coli* . *Int J. Dairy. Sci.* 2 : 116- 125.
50. Palaria, A.; Kanada, I. and Sullivan, D. (2011). Effect of asymbiotic yogurt on levels of fecal *Bifidobacterium*, *Clostridia* and *Enterobacteria*, *Appl. Environ. Microbiol.* 78(4): 933- 940.
51. Chuayana, E.L.; Cabrera, Ponce, C.V.; Rivera, R.B. and Cabrera , E. C.(2003). Atimicrobial activity of probiotics from milk products. *Phil. J. Microbiol . Infect. Dis.* 32 : 71- 74.
52. Reyed, R.M. (2007). The role of *Bifidobacteria* in Health. *Research of J. Med. And Med . Sci.* 2(1) : 14 – 24.
53. Lievin , V.; Peiffer, I.; Hudault, S.; Rochat, F. and Servin, A.L.(200). *Bifidobacterium* strain from resident infant human gastrointestinal microflora exert antimicrobial activity. *GUT.* 47(5): 646-652.
54. Collado, M.C.; Hernandez, M. and Sanz, Y. (2005). Production of bacteriocin- like inhibitory compounds by human fecal *Bifidobacterium* strain. *J. Food . Prot.* 68: 1034- 1040.
55. Mishra , C. and Lambert, J. (1996). Production of antimicrobial substance by probiotics. *Asia pacific . J. Clin. Nutr.* 5 : 20- 24.