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FACTORS AFFECTING THE ANTIBACTERIAL ACTIVITY OF PROBIOTIC BACTERIA AGAINST CERTAIN PATHOGENS

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ABSTRACT

This study was carried out to determine the effect of various factors influencing the antibacterial activity of fermented milk with *Bifidobacterium* spp. 420 (bifidus) and with *L. acidophilus* 145 (acidophilus) against eight test bacteria, *viz Staphylococcus aureus, Escherichia coli 0157:H7, Pseudomonas fluorescens, Klebsiella pneumoniae, Enterobacter cloacae, Listeria monocytogenes, Serratia marcescens* and *Salmonella infantis* were determined. The agar diffusion technique was used to determine the antibacterial activity. There was a significant variation ($P \leq 0.05$) in the antibacterial activity of bifidus and acidophilus made of various types of milk (buffaloes, cows, goats, ewes and camels' milk). Goats and camels fermented milk had a greater antibacterial activity than control (MRS medium) and other types of milk. The Gram-positive bacteria (*L. monocytogenes* and *Staphylococcus aureus*) took an opposite trend compared with tested Gram-negative pathogens. Antibacterial activity against Gram-positive were higher at pH 5 and pH 4.8 than pH 4.6, while antibacterial activity increased against tested Gram-negative pathogens at lower pH values. Statistically, no significant differences were observed between control and 15% sucrose concentration against all tested pathogens, except *Escherichia coli* 0157:H7 and *Serratia marcescens*, which were significant inhibition at 15% sucrose. Generally, there were not statistically significant differences between antibacterial activity in control and 0.3 % sodium chloride (NaCl) or between 0.6 and 0.9 % NaCl, however, 0.6 and 0.9% resulted in significant inhibition against the tested pathogens than 0.3 % NaCl.

Keywords: probiotic, pathogenic bacteria, the antibacterial activity

INTRODUCTION

The possible prophylactic and/or therapeutic properties of yoghurt and related products have been the subject of much speculation. As a result of that, there has been a contemporary trend to enhance such properties of fermented milk by inclusion of therapeutic bacteria in the composition of starter. These bacteria involve *Lactobacillus casei* subsp *casei* biovar shirota, *L..acidophilus and Bifidobacterium* spp. (Dong *et al.* 1987). A lot of research has referred to the health benefits of those bacteria, which could be summarized by Mercenier (1999).

The need for better control of food borne pathogens has been paramount in recent years. Within the last contract, considerable interest has been developed in the world with respect to use of Bifidobacteria and lactobacilli as a biopreservatives in food. Probiotic have the abillty to suppress the growth of pathogenic bacteria by producing organic acids such as lactic and acetic acids, other antimicrobial compounds such as hydrogen peroxide and bacteriocins. Lactic and acetic acids account for more than 90% of the acids produced in small quantities which include citric, hippuric, orotic and uric acids (Shah, 2001).

Lactic acid bacteria including *Bifidobacterium* spp. and *Lactobacillus acidophilus* produce bacteriocins, this inhibitory substance are proteinaceous in nature and can be antagonistic either to Gram-negative bacteria or role species within the genera (Marshall and Tamime, 1997). The effects of certain factors influencing the antibacterial activity of bifidus milk against four pathogenic bacteria were investigated (Misra and Kuila, 1992). The antimicrobial activity as well as growth decrease in the presence of high concentration of bile salts, there were not different at pH of the fermentation medium at the end of 24 and 48 h, the antimicrobial activity increased after 24 h of fermentation (Custy and Khem, 1988).

The objective of this study was to investigate the influence of the type of milk, NaCl concentration, pH of fermentation and concentration of sucrose on antibacterial activity against certain pathogenic bacteria.

MATERIALS AND METHODS

Milk, NaCl and sucrose:

Fresh buffaloes, cows, and goats' milk were obtained from El- Serow Station for Animal Production Research and spray dried skim milk powder, low heat, of France origin was used during this work. Ewes and camels' milk were purchased from local market as well as NaCl and sucrose.

Bacterial strains:

Pure lyophilized culture of *L. acidophilus* 145 and *Bifidobacterium* spp. 420 were obtained from Laboratorium wiesby, Niebull, Germany. *Bifidobacteria* spp. and *L. acidophilus* were separately transferred into sterile skim milk containing 10 g dextrose and 1g yeast extract /L, then incubation was carried out at 37 °C until coagulation, while Bifidobacteria was incubated anaerobically at 37 °C (in all experiments in this study) until coagulation. Further activation was achieved by three similar successive transfers in the same medium (Beena and Prasad, 1997).

The following strains were obtained from The Center for Food Safety and Quality Enhancement, Department of Food Science and Technology, The University of Georgia. *Staphylococcus aureus, Escherichia coli 0157:H7, Pseudomonas fluorescens, Klebsiella pneumoniae, Enterobacter cloacae, Listeria monocytogenes* and *Serratia marcescens* while *Salmonella infantis* was kindly supplied by Mr. Papoff. Institute Pasteur, Paris, France. These pathogenic strains were reactivated twice using brain heart infusion (BHI) broth (Difco) at 35 °C for 24 h befcre usé in this study and were transferred weekly. Intermediate culture was prepared by transferring stock culture into Tryptose Broth (TB) (Difco Manual, 1977), which was then incubated quiescently for 24 h at 35 °C. Working cultures were prepared by transferring intermediate culture into TB and incubating it under the previous conditions for 48 h.

Effect of type of milk on the antibacterial activity of probiotics against certain pathogens:

100 ml fresh milk (buffaloes, camels, cows, ewes or goats' milk) were standardized 3% fat and heated to 95 °C for 15 min and immediately cooled to 37 °C, separately inoculated with 6% (v/v) of high activated

cultures of *L. acidophilus* 145 or *Bifidobacterium* spp. 420 and were incubated at 37°C until fully coagulation, acidophilus and bifidus milks were transferred directly to the refrigerator, sterile MRS broth medium was inoculated with the same cultures and incubated at 37 °C for 18 h as control (Karthikeyan and Santhosh, 2009).

Effect of pH on the antibacterial activity:

Six flasks each containing 100 ml sterile reconstituted skim milk (10%), three flasks were inoculated with 2% (v/v) of highly activated cultures of *L. acidophilus* 145 and the other three with *Bifidobacterium* spp. 420, and were incubated at 37°C, samples of acidophilus and bifidus milks were withdrawn at different values of the pH (5, 4.8 and 4.6) to determine the antibacterial activity.

Effect of NaCl on the antibacterial activity:

Eight flasks each containing 100 ml reconstituted skim milk (10%) and 0.0, 0.3, 0.6 and 0.9% NaCl were sterilized. Four flasks were inoculated with 2% (v/v) of highly activated cultures of *L. acidophilus 145* and the other with *Bifidobacterium* spp. 420, and were incubated at 37°C for 18 h. samples of fermented milks were withdrawn to determine the antibacterial activity.

Effect of sucrose concentration on the antibacterial activity:

Eight flasks each containing 100 ml reconstituted skim milk (10%) and 0.0, 5, 10 and 15% sucrose were sterilized. Four flasks were inoculated with 2% (v/v) of highly activated cultures of *L..acidophilus* 145 and the other with *Bifidobacterium* spp. 420 and were incubated at 37 °C for 18 h, samples of fermented milks were taken to determine antibacterial activity.

Determination of the antibacterial activity:

The agar diffusion technique described by Singh and Laxminarayana (1973) was adopted. The resultant-fermented milks were centrifuged at 4000 rpm for 15 min, and the supernatant whey was collected. The latter should be clearing as much as possible for facilitating the filtration afterwards, so it was recentrifuged when appeared unclear. The clear supernatant was sterilized by passing thought sterile 0.45 µm syringe filter for obtaining cell-free filter (CFF), the antibacterial activity of each culture or fermented milks was detected by pouring an amount of 20 ml of sterile nutrient agar into sterile petri dishes, after solidification, a 24 h slant culture of previously different pathogenic bacteria were swabbed by sterile cotton tipped applicators which was then used to inoculate 9 ml of sterile saline for each strain. After agitation another sterile cotton tipped applicators were immersed into the inoculation saline and used for inoculating the entire surface of the nutrient agar dishes in three directions approximately 60 degrees from each other. Then, sterile filter paper discs (2 cm), each of which was previously immersed into of filtrates of 2-3 sec, were placed on the surface of each the other half of the dishes numbers, then petri dishes were kept in the refrigeratcr for 2 h for diffusion, and then incubated at 37°C for 24 h before the examination for zones of inhibition.

Statistical analysis:

The data from all experiments were subjected to analysis of variance. The differences among means of the studied traits were judged by Duncan's multiple range tests according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect of type of milk on the antibacterial activity of Probiotic:

Scientific reports on bifidobacterial growth in goats' milk are rare (Slacanac *et al.*, 2004), as well as in camels and ewes' milk. The results obtained in this work suggest that *Bifidobacterium* spp. and *L. acidophilus* grow better in goats' milk than in other milks (Table1), data in it show the pH-values and the fermentation time of fermented milks. PH-values decreased rapidly in goats' milk followed by Buffaloes, Camels, Ewes' milk then cows' milk. These results agreed with Pavlovic *et al.* (2006). The higher fermentation activity of probiotic bacteria in goats' milk might be back to its specific composition. The possible other reasons, the higher amount of some minerals and short chain fatty acids, as well as the easier protein digestibility (Alichandis and Polychroniadou, 1997).

Table (1): Coagulation Time and pH of fermentation of fermented milks with *Bifidobacterium* spp. *or L. acidophilus* in different types of milk

Type of milk	Fermented milk by *L. acidophilus*		Fermented milk by *Bifidobacterium* spp.	
	pH	Time /h	pH	Time /h
Buffaloes	4.82	8.0	4.85	8.0
Camels	4.80	9.0	4.83	8.5
Cows	4.87	9.5	4.92	10.0
Ewes	4.86	9.0	4.90	9.0
Goats	4.81	7.0	4.85	7.0

Although strong antibacterial activity has been indicated, little is known about the influence of fermented goats, camels and ewes' milk on pathogenic and potentially pathogenic organisms. The results presented in Table (2) exhibited that a greater Inhibitory effect of *Bifidobacterium* spp. 420 on the growth selected pathogenic strains than *L. acidophilus* 145. However, goats and camels' milk have a distinct antimicrobial impact. Slacanac *et al.* (2004) attributed this to their specific composition may result in the increased the antimicrobial compounds. These results were confirmed with Slacanac *et al* (2005), who reported that higher metabolic activity of *Bif. longum Bb-46* in goats' milk than in cows' milk, *Bif. longum Bb-46* grew better in goats than in cows' milk, pH values decreased more rabidly during the fermentation of goats' milk., also acetic and short fatty acids were more in goats' milk than cows' milk. Generally, the lowest antibacterial activity was observed in the control and there were not significant differences between the control and some types of milk against certain pathogens. It is worth noting that, there is no statistically significant difference between the type of milk on the inhibition Gram-positive and

Gram-negative pathogenic bacteria. It is quite clear from Table (2) that regarding, the antibacterial activity of therapeutic fermented milks against pathogens, bividus and acidophilus goats' milk had the greatest antibacterial activity against all pathogens followed by bividus camels' milk, acidophilus camels' milk, bividus buffalos, acidophilus buffalos' milk then fermented cows' milk, the antibacterial activity of fermented ewes' milk was the lowest among all fermented milks samples. Furthermore, bifidus milk had a higher antibacterial activity than acidophilus milk toward the most pathogens. However, there was no statistically significant difference between the antibacterial activities of bifidus and acidophilus milks on *Staph. aureus*, *Sal. Infantis*, S. *marcescens* and K. *pneumoniae* in fermented goats and camels' milk. In the contrary, the antibacterial activity of *Bifidobacterium* spp. Was more significant than *L.acidophilus* against all pathogens in buffaloes' milk, the same trend was obsarved toward all pathogens, except *S. marcescens* in the control, also in cows' milk against *L. monocytogenes* and Staph. *aureus*.

Effect of PH on the antibacterial activity of propiotic:

Bifidobacterium spp. result in a clear significantly advantage in the inhibition of the all tested pathogens more than *L. acidophilus* on the tested PH values (Table 3). The most important observation is that the Gram-positive bacteria (*L. monocytogenes* and *Staph. aureus*) took an opposite trend. Antibacterial activity against them was higher at pH 5 and pH4.8 than pH 4.6, while the antibacterial activity increased against all Gram- negative bacteria at the low pH values, it could also be noticed that Gram- negative bacteria were affected by increasing the acidity of fermented milk, inhibition zones increased by decreasing pH values. These results were in agreement with those reported by Lefteris and Luc (2006) who confirmed that the inhibitory mechanism for Gram-negative bacteria was shown to be dependent on the lowering of the pH of the medium and the production of organic acids, in particular, acetic and lactic acid. Also El-Sharoud(1999), indicated that the antagonistic action wasn't just to acid produced by the lactobacilli since inhibition was also obtained when the associative culture maintained at pH 6.5.

Bacteriocin production was strongly depended on pH, nutrient source and incubation temperature as claimed by Todorov and Dicks (2004). Who proved that Maximum activity noted at pH 5 and the Gram-positive bacteria were more influenced by bacteriocin production than increasing acidity, this consistent with the results of the current search. Didn't observe significant variation between the impact of pH5 and pH 4.8 against *L. monocytogenes*, *S. marcescens* and *E. coli* 0157:H7. On the other hand, when examining the effect of two types of probiotic against tested pathogens at different pH values, there weren't significant variations between *Bifidobacterium* spp. and *L. acidophilus* antibacterial activity on all tested pathogens at pH 5 and pH 4.8, except *Staph. aureus*, *Sal. infantis* ,*P.seudomonas* and *L. monocytogenes*, *Bifidobacterium* spp. shows more antibacterial activity at all pH values than *L. acidophilus* against the last four pathogens. Although, *Bifidobacterium* spp. shows significant superiority more than *L. acidophilus* at pH 4.6 against all pathogenic bacteria

Table (2): Antibacterial activity of *L. acidophilus* 145 and *Bifidobacterium* spp. 420 toward certain pathogenic bacteria in different types of milks

Treatments		*Ent. cloacae*	*E. coli*	*K. pneumoniae*	*L. monocytogenes*	*P. fluorescens*	*Sal. infantis*	*S. marcescens*	*Staph. aureus*
A		4.17 b	4.17 b	3.48 b	3.27b	3.20 b	3.47 b	3.27 b	3.46 b
B		4.77 a	4.94 a	3.68 a	3.87 a	3.92 a	4.08 a	3.75 a	3.50 a
F. test		**	**	*	**	**	**	**	**
Control		3.1 d	3.5 d	2.10 e	2.62 c	2.5 e	2.52 e	2.78 c	2.87 d
Buffalos' milk		3.2 d	3.4 d	2.45 d	2.45 c	2.52 e	2.82 d	2.73 c	2.72 e
Camels' milk		6.0 b	6.1 b	5.50 a	5.2 a	4.9 b	5.35 b	4.65 a	4.83 b
Cows' milk		4.15 c	4.2 c	3.20 b	3.3 b	3.5 c	3.65 c	3.43 b	3.35 c
Ewes' milk		3.25 d	3.5 d	2.65 c	2.45 c	2.8 d	2.7 de	2.7 c	3.00 d
Goats' milk		7.1 a	6.5 a	5.60 a	5.4 a	5.1 a	5.62 a	4.75 a	5.00 a
LSD (1%)		0.162	0.199	0.121	0.238	0.108	0.262	0.158	0.141
A	Control	3.0 g	3.2 f	2.20 fg	2.4 f	2.4 h	2.2 g	2.83 de	2.83 ef
	Buffalos' milk	3.2 fg	3.8 e	2.00 g	2.8 e	2.6 g	2.8 ef	2.73 ef	2.9 e
	Camels' milk	5.5 c	5.5 c	5.20 b	4.80 c	4.37 c	4.9 b	4.23 b	4.53 b
	Cows' milk	3.4 f	3.9 e	2.50 ef	2.8 e	2.80 f	3.1 de	2.9 de	2.8 ef
	Ewes' milk	3.0 g	3.0 f	2.40 ef	2.1 g	3.23 e	2.50 fg	2.57 f	2.63 f
	Goats' milk	6.5 b	6.8 a	5.80 a	5.6 b	5.40 b	5.8 a	5.07 a	5.13 a
B	Control	3.9 e	3.8 e	3.00 d	2.9 e	3.83 d	3.4 d	3 d	3.23 cd
	Buffalos' milk	4.4 d	4.6 d	3.40 c	3.7 d	2.8 f	3.9 c	3.87 c	3.47 c
	Camels' milk	6.6 b	6.0 b	5.40 b	4.9 c	4.20 c	5.23 b	4.30 b	4.70 b
	Cows' milk	3.5 f	3.7 e	2.60 e	2.4 f	2.8 f	2.8 ef	2.73 ef	3.2 d
	Ewes' milk	3.0 g	3.20 f	2.70 de	2.50 f	2.60 g	2.60 f	2.67 ef	2.8 ef
	Goats' milk	7.6 a	6.9 a	5.80 a	5.9 a	6.0 a	6 a	5.2 a	5.3 a
LSD (1%)		0.376	0.226	0.366	0.295	0.191	0.399	0.235	0.249

A =. *Lactobacillus acidophilus* 145 B = *Bifidobacterium* spp. 420

Means are the average of three replicates.*the diameter of the inhibition zone in mm and the diameter of the filter paper disc were excluded. Values with different letter are significantly different (P≤0.05)

Table (3): Effect of pH of bifidus and acidophilus milks on the antibacterial activity against certain pathogens

Treatments		*Ent. cloacae*	*E. coli*	*k. pneumoniae*	*L. monocytogenes*	*P. fluorescens*	*Sal. infantis*	*S. marcescens*	*Staph. aureus*
L .acidophilus		3.56 b	3.67 b	3.76 b	3.80 b	3.46 b	3.52 b	3.61 b	3.69 b
Bifidobacterium spp.		3.87 a	3.87 a	3.90 a	4.84 a	3.84 a	4.02 a	3.90 a	4.78 a
F. test		**	*	*	**	*	*	**	**
pH 5		3.43 c	3.58 b	3.53 c	4.58 a	3.38 c	3.47 c	3.65 b	4.67 a
pH 4.8		3.67 b	3.70 b	3.82 b	4.58 a	3.55 b	3.68 b	3.52 b	4.53 b
pH 4.6		4.03 a	4.05 a	4.13 a	3.80 b	4.02 a	4.17 a	4.10 a	3.80 c
LSD (1%)		0.154	0.154	0.144	0.148	0.072	0.136	0.169	0.104
L .acidophilus 145	pH 5	3.33 c	3.53 d	3.50 c	4.17 bc	3.17 e	3.17 d	3.53 bc	4.17 c
	pH 4.8	3.50 c	3.60 cd	3.77 b	4.00 c	3.33 d	3.53 c	3.53 bc	3.87 d
	pH 4.6	3.83 b	3.93 b	3.97 b	3.23 d	3.87 b	3.87 b	3.77 b	3.03 e
Bifidobacterium spp.420	pH 5	3.53 c	3.63 cd	3.53 c	5.00 a	3.60 c	3.77 b	3.77 b	5.17 a
	pH 4.8	3.83 b	3.80 bc	3.87 b	5.47 a	3.77 b	3.83 b	3.50 c	5.20 a
	pH 4.6	4.23 a	4.17 a	4.30 a	4.37 b	4.17 a	4.47 a	4.43 a	4.57 a
LSD (1%)		0.218	0.218	0.204	0.209	0.101	0.193	0.239	0.148

These results the average of three replicates. *The diameter of the inhibition zone in mm and the diameter of the filter paper disc were excluded. Values with different letter are significantly different (P≤0.05).

Effect of sucrose concentration on antibacterial activity of bifidus and acidophilus milks:

Since bifidus and acidophilus sour milk tastes too bitter for infants, sweetened bifidus and acidophilus milk were prepared using different concentrations of sucrose, also sucrose is added to ice milk and frozen yoghurt Abd El-Rahman *et al.* (2000). It could be appeared from Table (4) that there was significant variation between probiotc for *Bifidobacterium* spp. against all tested pathogens, except *K. pneumoniae* which was unaffected by two types of probiotcs bacteria in this experiment. This obvious significant superiority agreed with the findings of Shady *et al.* (1999). Statistically, no significant variations between control and sucrose concentration in inhibition zones against *Staph. aureus* and *K. pneumoniae* and between control and 15% sucrose against all tested pathogens, except *E. coli 0157:H7* and *S. marcescens*, the 15% sucrose had significantly effect on inhibition these bacteria . At 5 and 10% Sucrose no statistically significant differences found against all tested pathogens except *S. marcescens* and Sal. *infantis*, their inhibition zones increased with increasing sucrose concentration, this could be due to special vulnerability of these bacteria or may return to physical and chemical properties of the metabolic products of probiotic which affected those pathogens that may have a particularly sensitive under these conditions.

At interaction type of probiotic and concentrations of sucrose, it was observed that there was significant variation in the antibacterial activity of probiotic for *Bifidobacterium* spp. to *L. acidophilus* against all tested pathogens at all sucrose concentrations, except against *E. coli* 0157:H7 at 15% sucrose, *P. fluorescens* at 10 and 15% sucrose and *K. pneumoniae* at all sucrose concentrations. It could be noticed the absence of significant difference among antibacterial activity of probiotic and sucrose concentrations against those pathogens. Generally, inhibition zones by acidophilus were lower than that of bifidus. It was observed that as the level of sucrose addition increased there wasn't significant variation in the antibacterial activity. This was confirmed by Misra and Kuila (1992).

Effect of NaCl concentration on antibacterial activity of bifidus and acidophilus milks:

Results presented in Table (5) clearly illustrate that the *Bifidobacterium* spp. resulted in significant superiority to *L. acidophilus* when studying the effect of probiotic on inhibition of pathogenic bacteria in the presence of very low levels of NaCl (0.3, 0.6 and 0.9%), but there was not significant variation between probiotcs toward *Staph. aureus* and *P. fluorescens* in this position. On the other hand, there wasn't significant variation observed between control and 0.3% NaCl neither in inhibition zones nor between 0.6% and 0.9 % NaCl against all tested pathogens. However, 0.6 and 0.9% NaCl resulted in significant inhibition against the tested pathogens than 0.3 % NaCl These results agreed with Karthikeyan and Santhosh (2009), who found maximum antibacterial activity (bacteriocin production) by *L. acidophilus* at 0.9 % NaCl against *Staph. aureus, Sal. .typhimurium, paratyphi 'B', E. coli, Klebsiella* sp., *S. marcescens* and *P.* aeruginosa.

Table (4): Effect of sucrose concentration on the antibacterial activity of bifidus and acidophilus milks against certain pathogens

Treatments		*Ent. cloacae*	*E. coli 0157:H7*	*K. pneumoniae*	*L. monocytogenes*	*P. fluorescens*	*Sal. infantis*	*S. marcescens*	*Staph. aureus*
L .acidophilus		4.03 b	4.10 b	3.55 a	3.10 b	3.43 b	3.26 b	3.36 b	3.48 b
Bifidobacterium spp.		4.72 a	4.52 a	3.53 a	4.07 a	3.63 a	4.08 a	4.06 a	3.97 a
F. test		*	*	ns	**	**	**	**	**
Sucrose 0%		4.18 b	4.12 b	3.42 b	3.50 b	3.43 b	3.62 b	3.63 b	3.67 a
Sucrose 5%		4.50 a	4.38 a	3.62 ab	3.68 a	3.65 a	3.63 b	3.60 b	3.77 a
Sucrose 10%		4.52 a	4.35 a	3.63 a	3.65 ab	3.53 ab	3.83 a	3.80 a	3.70 a
Sucrose 15%		4.30 b	4.38 a	3.48 ab	3.50 b	3.50 ab	3.60 b	3.80 a	3.77 a
LSD (1%)		0.178	0.217	0.217	0.177	0.152	0.152	0.102	0.210
L .acidophilus 145	Sucrose 0%	3.77 c	3.77 d	3.33 b	2.97 b	3.23 c	3.20 c	3.23 e	3.50 b
	Sucrose 5%	4.17 b	4.20 bc	3.63 ab	3.20 b	3.47 b	3.23 bc	3.43 d	3.50 b
	Sucrose 10%	4.20 b	4.12 c	3.77 a	3.20 b	3.53 b	3.43 b	3.40 d	3.43 b
	Sucrose 15%	4.00 bc	4.27 abc	3.47 ab	3.03 b	3.50 b	3.17 c	3.37 de	3.50 b
Bifidobacterium spp. 420	Sucrose 0%	4.60 a	4.47 abc	3.50 ab	4.03 a	3.63 ab	4.03 a	4.03 b	3.83 a
	Sucrose 5%	4.83 a	4.57 a	3.60 ab	4.17 a	3.83 a	4.03 a	3.77 c	4.03 a
	Sucrose 10%	4.83 a	4.53 a	3.50 ab	4.10 a	3.53 b	4.23 a	4.20 a	3.97 a
	Sucrose 15%	4.60 a	4.50 ab	3.50 ab	3.97 a	3.50 b	4.03 a	4.23 a	4.03 a
LSD (1%)		0.251	0.307	0.306	0.251	0.216	0.216	0.144	0.286

These results the average of three replicates. *The diameter of the inhibition zone in mm and the diameter of the filter paper disc were excluded. Values with different letter are significantly different (P≤0.05)

Table (5): Effect of NaCl concentration on the antibacterial activity of bifidus and acidophilus milks against certain pathogens

Treatments		*Enr. cloacae*	*E. coli 0157:H7*	*K. pneumoniae*	*L. monocytogenes*	*P. fluorescens*	*Sal. infantis*	*S. marcescens*	*Staph. aureus*
L .acidophilus		4.66 b	4.60 b	3.58 b	3.40 b	3.71 a	3.79 b	3.50 b	3.73 a
Bifidobacterium spp.		5.19 a	5.32 a	4.07 a	4.37 a	3.95 a	4.46 a	4.24 a	3.82 a
F. test		**	**	*	**	ns	**	**	ns
NaCL 0.0%		4.52 b	4.32 b	3.50 b	3.48 b	3.65 b	3.68 b	3.48 b	3.50 b
NaCL 0.3%		4.41 b	4.50 b	3.53 b	3.48 b	3.77 b	3.80 b	3.60 b	3.50 b
NaCL 0.6%		5.43 a	5.43 a	4.18 a	4.33 a	4.13 a	4.50 a	4.23 a	4.00 a
NaCL 0.9%		5.33 a	5.58 a	4.07 a	4.23 a	4.12 a	4.52 a	4.17 a	4.08 a
LSD (1%)		0.116	0.255	0.178	0.210	0.189	0.170	0.164	0.150
L .acidophilus 145	NaCL 0.0%	4.23 d	4.00 e	3.20 d	3.03 e	2.97 d	3.17 d	3.00 c	3.40 b
	NaCL 0.3%	4.03 e	4.23 e	3.43 cd	2.80 e	3.60 c	3.57 c	3.20 c	3.50 b
	NaCL 0.6%	5.23 b	5.03 bc	3.87 b	3.83 d	4.03 ab	4.23 b	3.97 b	3.97 a
	NaCL 0.9%	5.13 b	5.13 b	3.80 b	3.93 cd	4.23 a	4.20 b	3.83 b	4.03 a
Bifidobacterium spp.420	NaCL 0.0%	4.80 c	4.63 d	3.80 b	3.93 cd	3.63 c	4.20 b	3.97 b	3.60 b
	NaCL 0.3%	4.80 c	4.77 cd	3.63 bc	4.17 c	3.93 b	4.03 b	4.00 b	3.50 b
	NaCL 0.6%	5.63 a	5.83 a	4.50 a	4.83 a	4.23 a	4.77 a	4.50 a	4.03 a
	NaCL 0.9%	5.53 a	6.03 a	4.33 a	4.53 b	4.00 ab	4.83 a	4.50 a	4.13 a
LSD (1%)		0.164	0.360	0.252	0.297	0.267	0.240	0.231	0.212

These results the average of three replicates. *The diameter of the inhibition zone in mm and the diameter of the filter paper disc were excluded. Values with different letter are significantly different (P≤0.05).

While at the interaction of the NaCl concentration and probiotic, the antibacterial activity increased significantly from *Bifidobacterium* spp. than *L..acidophilus* against all pathogens with the exception of *K. pneumoniae* at 0.3% NaCl, *P. fluorescens* at 0.6 and 0.9% NaCl and *Staph. aureus* at all levels of NaCl.

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العوامل المؤثرة على التأثير المضاد للبكتريا المحفزة حيويًا على بعض البكتريا الممرضة

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أجريت هذه الدراسة لتحديد العوامل المؤثرة على التأثير المضاد للبكتريا المحفزة حيويًا على بعــض البكتريا الممرضة ولقد استخدمت البكتريا الممرضة:

Staph. aureus, E. coli 0157:H7, P. fluorescens, K. pneumoniae, Ent.cloacae, L. monocytogenes, S. marcescens and *Sal. infantis*،كما استُخدمت كل من بكتريا البروبيوتك *Bifidobacterium* spp. 420 و *L. acidophilus* 145 ، أظهرت نتــائج التحليــل الاحــصائى عنــد (P≤0.05) أن هناك فروقًا معنوية للنشاط المضاد لبكتريا البيفيدوبكتريم عن الأسيدوفيلس أمــا بخــصوص أنواع الألبان ، تُصدر لبن الماعز القائمة محققا فرقا معنويًا على جميع أنواع الألبان المختبــره تجــاه تلــك الميكروبات الممرضة المختبرة تلاه لبن الثوق ثم اللبن البقرى ثم اللبن الجاموسى فالنعاج ، ولم يكن هنــاك فروقا معنوية بين النشاط المضاد للبكتريا الناتج عن بعض انواع لبن البيفيدوس والاسيدوفيلس وبين ذلك الناتج من بيئة الكنترول (MRS) تجاه البكتريا الممرضة ، وأن هناك تفاوت كبير بين *Bifidobacterium* spp. و *L. acidophilus* 145 لصالح البيفيدوبكتريم فى تأثيرها على البكتريا الممرضة ، كما لوحظ أن البكتريا الموجبة لصبغ جرام تم تثبيطها على pH ٥ ، ٤,٨ أكثر من pH ٤,٦ ، لكن البكتريا السالبة لصبغ جرام تأثرت أكثر بزيادة الحموضة ، الملاحظة الهامة الأخرى انه لم يوجد فروقا معنوية بين النشاط البكتيري المضاد بــين الكنترول (لبن فرز مختمر بالبيفيدوبكتريم والاسيدوفلس – خالي من السكروز) وبين نفس اللبن فى وجود ١٥ ٪ سكروز تجاه كل البكتريا المرضية المختبرة ما عــدا *S. marcescens* و *E. coli* 0157:H7 خالفــت ذلك. أما بخصوص تأثير NaCl ، لم تُسَجل أى فروق معنوية بين الكنترول وبين تركيز ٠,٣ ٪ ولا بــين تركيز(٠,٦ و ٠,٩ ٪) ولكن تفوق التركيزان الآخران معنويًا على الكنترول وتركيز ٠,٣ ٪ فــي النــشاط البكتيري المضاد للبكتريا الممرضة.

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