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INFLUENCE OF SOME NUTRIENTS AND BILE SALT ON THE PRODUCTION OF ANTIMICROBIAL AGENTS BY BIFIDOBACTERIA

BY

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SUMMARY

Seven strains of bifidobacteria were screened for their inhibitory activity. *Bif. bifidum* ATCC29521, *Bif. bifidum* ATCC15696, *Bif. longum* NCFB2299, *Bif. longum* BL-04, *Bif. infantis* ATCC15567, *Bif. catenulateum* ATCC18371 and *Bif. lactis* BL-01 produced antimicrobial substances with a wide spectrum of antimicrobial activity. Cell free culture of all bifidobacteria strains inhibited strongly the growth of *E. coli* ATCC69337, *Staphylococcus aureus* ATCC20231, *Bacillus cereus* ATCC33018, *Pseudomonas aeruginosa* ATCC9027, *E. coli* 0157-H7, *Staph, aureus, Bacillus cereus, Salmonella typhi* and *Salmonella enteritidis* and slightly the growth of lactic acid bacteria. Supplementation of media with cysteine + glycine, inulin, hydrolyzed whey protein, whey protein hydrolysate, tomato juice and carrot juice increased the production of antimicrobial agents by all bifidobacteria strains, while bile salt decreased their production. The increase or decrease of antimicrobial agents production was proportional to the rate of supplementation. *Bif. longum* BL-04 and *Bif. bifidum* ATCC 29521 exhibited the highest inhibitory activity. It seems that the production of antimicrobial agents by bifidobacteria is species and strain dependent.

Key words: Antimicrobial agents, bifidobacteria, nutrients, fortification, bile salt.

INTRODUCTION

Bifidobacteria are well known to beneficially affect human health by improving the balance of intestinal micro-flora, improving mucosal defenses against pathogens, enhancing immune response, reducing serum cholesterol, reducing ammonia and free serum phenol in patients with liver disease and improving of lactose-tolerance. Additional health benefits include vitamin synthesis, anti-carcinogenic activity and anti-bacterial activity (Kebary, 1995; Badawi and El-Sonbati, 1997; Brassert and Schiffrin, 2000; Lourens-Hattingh and Viljoen, 2001 and Wright *et al.*, 2002). It is estimated more than 90 probiotic products containing bifidobacteria are produced worldwide (Shah, 2000). They include fermented milk, butter milk, sour cream, frozen dessert, cheese, baby foods,

pharmaceutical preparations and livestock feed supplements (Kebary, 1996; Kebary *et al.*, 1998; Hussein and Kebary, 1999; Badran *et al.*, 2004; Boylston *et al.*, 2004; Hamed *et al.*, 2004; Kebary *et al.*, 2004 and Moussa *et al.*, 2004).

Several factors have been claimed to affect the survival and growth of bifidobacteria such as bile salt, amino acids, tomato and carrot juices, hydrolyzed whey proteins, inulin and sweeteners (Kamaly, 1997; Dave and Shah, 1998; Ibrahim, 2001; Hamed *et al.*, 2004; Ibrahim *et al.*, 2004; Kebary *et al.*, 2004 and Picot and Lacroix, 2004). The production of antimicrobial agents by bifidobacteria have been reported (Kebary, 1995; Badawi and El-Sonbaty, 1997; Hussein and Kebary, 1999; Kim *et al.*, 2001 and Saleh and El-Sayed, 2004).

It seems there is a lack of detailed information in the literature about the effect of nutrients and other factors on the production of antibacterial agents by bifidobacteria.

In view of the aforementioned, the objectives of this study were to investigate the ability of some bifidobacteria strains to produce antimicrobial substances, determine their antagonistic effect on some pathogenic bacteria and some lactic acid bacteria and study the effect of nutrients, such as amino acids, whey protein hydorlyzates, carrot and tomato juices and bile salt on the production of antimicrobial substances by bifidobacteria strains.

MATERIALS AND METHODS

Sources of bacterial strains used in this study and the media on which were grown are listed in Table (1). Dry cultures of lactic acid bacteria and bifidobacteria were activated by three successive transfers in sterile 10% reconstituted skim milk. Stock cultures were also prepared in the same milk. Stock cultures of bacterial strains were activated by two successive transfers in the broth of the media listed in Table (1) and incubated for 24 h at optimum temperatures (30°C for *Lb. casei, Lactococcus lactis* subsp. *lactis* and *Bacillus cereus*; 37°C for *Bifidobacteria* spp., *Lb. helveticus, Lb. rhamnosus, Lb. acidophilus, Staphylococcus aureus, E. coli, Salmonella* spp. and *Pseudomonas aeruginosa*; 40°C for *Lb. bulgaricus* and *Streptococcus thermophilus*). Solid media were used to determine the inhibitory activity of bifidobacteria strains against the listed bacterial strains by adding 1.5% agar to the broth media (Table 1).

Screening of antimicrobial agents production by bifidobacteria:

Tubes containing 9.5 ml of sterile modified lactobacilli MRS broth (Ventling and Mistry, 1993) were inoculated with 0.5 ml of fresh bifidobacteria strains cultures. All tubes were incubated anaerobically using the Baltimore Biological Laboratories (BBL) gas pak (BBL, Cockeysville, MD, USA) at 37°C for 36 h. The cultures were centrifuged at 8000 rpm for 30 min. at room temperature to obtain the cell free broth, which was used to measure the inhibitory activity by the disc assay procedure (Pulusani *et al.*, 1979). The target strains were seeded on the appropriate solid media (Table 1). The inhibitory activity was determined by

measuring the diameter of inhibition zone in mm. *Staphylococcus aureus* ATCC 20231 was used as test organisms to study the effect of various factors on the production of antimicrobial agents because of its higher sensitivity towards antimicrobial agents produced by bifidobacteria.

Factors affecting antimicrobial agents production:

The effect of fortification of modified MRS medium with amino acids, whey protein hydrolysate (degree of hydrolysis 10), hydrolyzed whey protein (degree of hydrolysis 20), inulin, bile salts, tomato and carrot juices on the growth of bifidobacteria and their inhibittory activity against *Staphylococcus aureus* ATCC 20231 were studied.

A mixture of two amino acids; cysteine and glycine were filter sterilized and added to modified MRS medium to give a final concentration from each amino acid of 0.05, 0.075 and 0.10% (w / v). Whey proteins, inulin and bile salts were sterilized by steaming for three successive days. Whey protein hydrolysate and hydrolyzed whey protein (Proteint, Mountain Lake, MN, USA) were added separately to the medium at the rate of 0.5, 1.0 and 1.5% (w / v). Also, inulin (Orafti, Tienen, Belgium) was added at the rate of 0.5, 1.0 and 1.5% (w / v). Modified MRS medium was fortified with bile salt (Sigma Chemical Comp., ST. Louis, MO, USA) at the rate of 0.5, 1.0 and 1.5% (w / v).

Tomato juice was prepared by blending the ripe tomato which was then filtered through cheese cloth followed by Whatman No. 1 filter paper. The supernatant was adjusted to pH 6.6 and autoclaved at 121°C for 15 min (Ibrahim, 2001). Sterile tomato juice was added to the modified MRS at the rate of 0.5, 1.0 and 1.5% (v / v). Carrot juice was centrifuged at 3000 rpm for 5 min, then filtered through Whatman No. 1 filter paper and autoclaved at 121°C for 15 min. Sterile carrot juice was added to the modified MRS at the rate of 0.5, 1.0 and 1.5% (v / v). The concentration of these nutrients were chosen according to the literature (Kamaly, 1997; Ibrahim, 2001). Flasks containing 95 ml of the fortified modified MRS were inoculated with 5 ml of fresh bifidobacteria strains. All flasks were incubated anaerobically at 37°C for 36 h (Ventling and Mistry, 1993). Samples from each culture were taken to monitor the bacterial growth by measureing the optical density at 600 µm (Liao *et al.*, 1993) using Jenway 6305 uv/vis spectrophotometer (Jenway Ltd., Felsted, Dunmow, England). The rest of the cultures were centrifuged at 8000 rpm for 30 min at room temperature to get the cell free broth, which was used to determine the inhibitory activity by the disc assay method against *Staph. aureus* 20231. *Staph. aureus* was seeded on staphylococcus medium 110.

Table (1): Baterial strains and media used in this study.

Bacterial strains	Source of strains	Media
Bifidobacterium longum BL-04 *Bifidobacterium lactis* BL-01	Rhodia, Madison, WI, USA	Modified lactobacilli MRS (MRS + 0.05% L-cysteine-HCl) according to Ventling & Mistry (1993).
Lactobacillus rhamnosus LR-32		Lactobcilli MRS (Difco Manual, 1984, Difco Laboratories Detroit ML, USA).
Lactobacillus helveticus CNRZ 53 *Lactobacillus casei* NIH 334	Prof. M. El-Soda Dairy Sci. Dept. Fac. of Agric. Alex. Univ., Egypt.	Lactobcilli MRS (Difco Manual, 1984, Difco Laboratories Detroit ML, USA).
Lactobacillus delbrueckii subsp. *bulgaricus* LbRR	Prof. Larry L. McKay, Dept of Food Sci. and Nutrition Univ. of Minnesota, MN, USA.	Lactobcilli MRS (Difco Manual, 1984, Difco Laboratories Detroit ML, USA).
Streptococcus salivarius subsp. *thermophilus* STES		Yeast lactose agar (Skinner & Quessal, 1978)
Bifidobacterium infantis ATCC 15567	Prof. Joellen Feirtag, Dept. of Food Sci. and Nutrition, Univ. of Minnesota, MN, USA.	Modified lactobacilli MRS (MRS + 0.05% L-cysteine-HCl) according to Ventling & Mistry (1993)
Bifidobacterium catenulateum ATCC 18371		
Lactococcus lactis subsp. *lactis* ATCC 11454	American Type culture collection, Rockvile, Md, USA.	Modified M_{17} (Terzaghi and Sandine, 1975).
Lactobacillus casei ATCC 393		Lactobcilli MRS (Difco Manual, 1984, Difco Laboratories Detroit ML, USA).
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 1184		

Table (1): Continued.

Bacterial strains	Source of strains	Media
Lactobacillus delbrueckii subsp. *bulgaricus* ATCC 479		
Lactobacillus acidophilus ATCC 4356		
Bifidobacterium bifidum ATCC 15696 *Bifidobacterium bifidum* ATCC 29521 *Bifidobacterium longum* NCFB 2299	Prof. Linda J. Brady. Dept. of Food Sci. and Nutrition, Univ. of Minnesota, MN USA	Modified lactobacilli MRS (MRS + 0.05% L-cysteine-HCl) according to Ventling & Mistry (1993).
Escherichia coli ATCC 69337	Prof. Baraka A. Abd El-Salam. Dairy Research Institute. Agriculture Research Center. Cairo. Egypt	Mackoneky broth (Oxoid LTD. Basingstode. Hampshire GB)
Staphylococcus aureus ATCC 20231		Staphylococcus medium 110 (Difco Manual, 1984)
Bacillus cereus ATCC 33018		Mannitol egg yolk polymyxin broth (Harmon & Goepfert, 1984).
Psendomonas aeruginosa ATCC 9027		Nutrient broth (Abd El-Salam *et al.*, 2004).
E. coli 0157 H7	Prof. G. A. Ibrahim. Dairy Sci. and Tech. Dept., National Research Center. Dokki, Cairo, Egypt.	Mackoneky broth (Oxoid LTD. Basingstode. Hampshire GB).
Staphylococcus aureus		Staphylococcus medium 110 (Difco Manual, 1984)
Bacillus cereus		Mannitol egg yolk polymyxin broth (Harmon & Goepfert, 1984).
Pseudomonas aeruginosa		Nutrient broth (Abd El-Salam *et al.*, 2004).
Salmonella typhi	Prof. A. M. Mettwally. Dept. of animal medicine. Faculty of Veterinary Medicine. Alex. Univ.	Mackoneky broth (Oxoid LTD. Basingstode. Hampshire GB).
Salmonella enteritidis		

RESULTS AND DISCUSSION

The inhibitory activity of cell free culture broth of different strains of bifidobacteria against the various bacterial strains is presented in Table (2) and Fig. (1). It is obvious from these data that the inhibitory activity of different strains of bifidobacteria was most evident against the pathogenic bacterial strains (Table 2), therefore incorporation of bifidobacteria or their free cell culture broth in different foods might help to preserve these foods, and at the same time improve their nutritional and health benefits. Cell free cultures of bifidobacteria strains had lower inhibitory activity against lactic acid bacteria, so bifidobacteria could be used as adjunct in the manufacture of different dairy products and other foods without affecting the growth of lactic acid bacteria used as starters for these products. *Streptococcus thermophilus* was more sensitive than lactobacilli (Table 2). These results are in agreement with those reported by Collins and Hall (1984); Kebary *et al.* (1995); Badawi and El-Sonbati (1997); Hussein and Kebary (1999); Kim *et al.* (2001) and Saleh and El-Sayed (2004). *Bif. longum* BL-04 and *Bif. bidium* ATCC 29521 exhibited the greatest inhibitory activity.

Bifidobacteria strains were grown in modified MRS medium fortified with cysteine and glycine to assess their effect on cell growth and production of antimicrobial agents (Table 3). It is evident from the presented data that addition of the mixture of amino acids enhanced the growth of all bifidobacteria strains and increased their inhibitory activity against *Staph. aureus* ATCC 20231 that was used as indicator bacteria (Table 3). This increase of bacterial growth and antimicrobial agents production was increased with the increase of the amount of amino acids added (Table 3). The effect of supplementation of modified MRS with amino acids on both, growth of bifidobacteria strains and production of antimicrobial agents followed similar trends (Table 3). It has been reported that cysteine had a stimulatory effect upon the growth of bifidobacteria (Collins and Hall, 1984; Hunger and Peitersen, 1992; Kamaly, 1997; Shah, 1997 and Dave and Shah, 1998) which might be due to the reduction of redox potential. Moreover, Murad *et al.* (1997) found that supplementation of buffalos' milk with individual amino acids lysine, glycine and cysteine enhanced the growth of *B. bifidum* and their ability of acid development. Kamaly (1997) reported that adding of a mixture of glycine and cysteine was more effective to increase the growth of bifidobacteria and acid production than adding each one separately.

The impact of supplementation of modified MRS medium with whey proteins on the growth of bifidobacteria and production of antimicrobial substances is presented in Table (4). The obtained results revealed that adding of whey proteins enhanced the growth of all bifidobacteria strains and the production of antimicrobial agents and this increase was proportional to the rate of adding whey proteins (Table 4). Hydrolyzed whey protein (HWP) (degree of hydrolysis 20) was more effective to stimulate the growth of all bifidobacteria strains and increase the production of

antimicrobial agents by these bacteria than corresponding whey protein hydrolysate (WPH) (degree of hydrolysis 10) (Table 4). These results are in agreement with those reported by Badran *et al.* (2004) and Hamed *et al.* (2004), who found that replacing of non-fat dry milk with whey proteins during manufacturing of frozen yoghurt enhanced the growth of bifidobacteria. Also, Dave and Shah (1998) found that supplementation of yoghurt with whey protein concentrate enhanced the growth of bifidobacteria. This stimulatory effect of whey protein might be due to the presence of cysteine.

Fortification of modified MRS with inulin stimulate the growth of all bifidobacteria strains and increased their inhibitory activity against *Staph. aureus* ATCC 20231. This increase of bifidobacteria growth and production of antimicrobial agents was proportional to the amount of added inulin (Table 5). These results confirmed previous studies by Gibson *et al.* (1995), Roberfroid *et al.* (1998) and Ibrahim *et al.* (2004) who have shown that a product supplemented with inulin provide an effective means to enhance the growth of bifidobacteria.

Supplementation of modified MRS with either tomato or carrot juice increased slightly the growth of all bifidobacteria strains and production of antimicrobial agents (Table 6). There were positive correlations between the amount of juices added and bacterial growth and production of antimicrobial agents (Table 6). This enhancement of bifidobacteria growth and production of antimicrobial agents might be due to the presence of some vitamins and mineral salts, those have been proved to promote the growth of bifidobacteria (Hunger and Peitersen, 1992).

Adding of bile salts to modified MRS decreased the growth of all bifidobacteria strains and the production of antimicrobial agents and this decrease was proportional to the rate of adding bile salts (Table 7). It has been reported that bile salt retard or suppress the growth of bifidobacteria and this effect is species and strain dependent (Lankaputhra and Shah, 1995; Shah, 1997).

Bif. longum BL-04 and *Bif. bifidum* ATCC 29521 exhibited the highest inhibitory activity at any concentration and type of nutrient and bile salts against *Staph. aureus* ATCC 20231.

It could be concluded that tested bifidobacteria strains exhibited antibacterial activity that was more evident against pathogenic bacteria, but had less inhibitory ability against lactic acid bacteria. Fortification of media with amino acids, whey proteins, inulin, tomato and carrot juices enhanced the growth of bifidobacteria and increased the production of antimicrobial agents, while addition of bile salt suppressed both, the bacterial growth and production of antimicrobial agents. It seems that the production of antimicrobial agents is species and strain dependent.

Table (2): Antimicrobial spectrum of cell free cultures of bifidobacteria.

Target bacteria	Diameter of inhibition zone (mm)[b]						
	A[a]	B	C	D	E	F	G
a- Gram positive							
Lactobacillus helveticus	8	8	8	8	8	8	8
Lactobacillus bulgaricus LbRR	9	8	10	8	10	8	10
Lactobacillus casei	8	8	8	8	8	8	8
Lactobacillus casei ATCC 393	9	8	8	8	10	8	10
Lactobacillus acidophilus ATCC 4356	8	8	8	8	8	8	8
Lactobacillus delbruechii subsp. *lactis* ATCC 4797	8	7	8	8	8	7	8
Lactobacillus delbruechii subsp. *bulgaricus* ATCC 11842	8	7	7	8	8	7	9
Lactobacillus rhamnosus	7	6	6	7	8	6	7
Streptococcus thermophilus stES	12	10	10	10	12	10	12
Lactococcus lactis subsp. *lactis* ATCC 11454	10	10	8	8	10	8	10
Staphylococcus aureus ATCC 20231	23	21	20	20	26	23	24
Bacillus cereus ATCC 33018	18	16	16	18	21	18	20
Staphylococcus aureus	22	22	21	22	26	23	24
Bacillus cereus	18	17	16	18	21	19	20
b- Gram negative							
Escheichia coli ATCC 69337	20	18	18	19	23	20	21
Pseudomonas aeruginosa ATCC 9027	20	20	21	20	22	20	22
Escherichia coli 0157 – H7	20	19	19	20	22	19	21
Pseudomonas aeruginosa	20	19	20	20	22	19	22
Salmonella typhi	19	18	20	20	22	19	21
Salmonella enteritidis	19	19	20	21	23	19	22

a = bifidobacteria strains. A = *Bif. bifidum* ATCC 15696. B = *Bif. longum* NCFB 2299. C = *Bif. infantis* ATCC 15567. D = *Bif. catenulateum* ATCC 18371. E = *Bif. longum* BL – 04. F = *Bif. lactis* BL – 01. G = *Bif. bifidum* ATCC 29521.

b = All measurements including disc diameter of 6 mm.

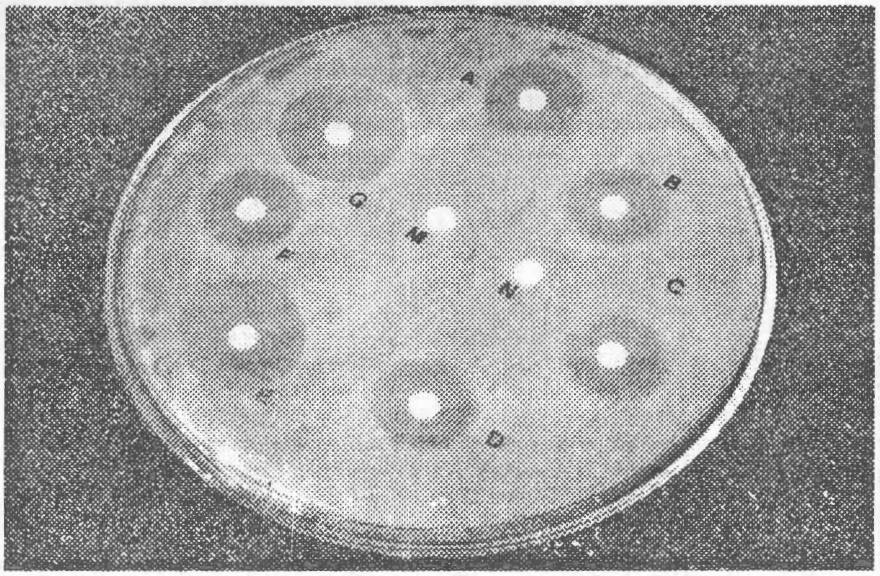

Fig. (1). Light photograph showing the inhibitory activity of cell free culture broth of bifidobacteria against *Staph. aureus* ATCC 20231. A, B, C, D, E, F and G are cell free culture of bifidobacteria strains (see Table 2), M: modified MRS medium, N: free disc.

Table (3): Effect of fortification of growth medium with a mixture of cysteine and glycine on growth of bifidobacteria (optical density at 600 μm) and production of antimicrobial agents (zone inhibition mm) against *Staphylococcus aureus* ATCC 20231.

Bacterial strains	Concentration of cysteine and glycine (%)			
	0.0	0.05	0.075	0.1
	Optical density at 600 μm			
A*	1.128	1.142	1.152	1.193
B	1.122	1.150	1.168	1.192
C	1.122	1.163	1.176	1.204
D	1.124	1.169	1.188	1.213
E	1.132	1.183	1.207	1.236
F	1.126	1.172	1.192	1.221
G	1.135	1.194	1.225	1.243
	Zone inhibition (mm)[a]			
A	19	20	22	23
B	17	18	20	22
C	17	18	20	22
D	18	20	21	23
E	21	23	25	26
F	19	20	22	22
G	21	23	25	26

* See Table (2)

a. Diameter of inhibition zone including disc diameter of 6 mm

Table (4): Effect of fortification of growth medium with hydrolyzed whey protein (HWP) and whey protein hydrolysate (WPH) on growth of bifidobacteria (optical density at 600 μm) and production of antimicrobial agents (zone inhibition mm) against *Staphylococcus aureus* ATCC 20231.

Bacterial strains	HWP %				WPH %			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
	Opitcal density at 600 μm							
A*	1.128	1.163	1.174	1.279	1.128	1.166	1.179	1.264
B	1.122	1.160	1.171	1.243	1.122	1.160	1.175	1.232
C	1.122	1.173	1.182	1.249	1.122	1.181	1.192	1.221
D	1.124	1.181	1.191	1.251	1.124	1.179	1.196	1.234
E	1.132	1.192	1.202	1.273	1.132	1.187	1.192	1.253
F	1.126	1.187	1.195	1.251	1.126	1.179	1.185	1.241
G	1.135	1.192	1.235	1.291	1.135	1.193	1.205	1.274
	Zone inhibition (mm)*							
A	19	22	25	26	19	21	23	24
B	17	19	22	24	18	20	22	22
C	17	18	22	24	18	20	22	23
D	18	20	23	24	19	21	22	22
E	21	24	26	28	24	22	24	26
F	19	22	24	26	19	22	24	24
G	21	25	27	28	21	23	24	26

* See Table (2).

a: Diameter of inhibition zone including disc diameter of 6 mm

Table (5): Effect of fortification of growth medium with inulin on growth of bifidobacteria (optical density at 600 μm) and production of antimicrobial agents (zone inhibition mm) against *Staphylococcus aureus* ATCC 20231.

Bacterial strains	Inulin (%)			
	0.0	0.5	1.0	1.5
	Optical density at 600 μm			
A*	1.128	1.149	1.163	1.175
B	1.122	1.163	1.173	1.186
C	1.122	1.168	1.182	1.196
D	1.124	1.177	1.196	1.203
E	1.132	1.182	1.203	1.225
F	1.126	1.176	1.199	1.210
G	1.135	1.193	1.215	1.236
	Zone inhibition (mm)*			
A	19	21	24	25
B	17	19	21	23
C	17	19	21	24
D	18	20	22	24
E	21	23	24	25
F	19	21	23	24
G	21	23	24	26

* See Table (2).

a: Diameter of inhibition zone including disc diameter of 6 mm

Table (6): Effect of fortification of growth medium with tomato and carrot juice on growth of bifidobacteria (optical density at 600 µm) and production of antimicrobial agents (zone inhibition mm) against *Staphylococcus aureus* ATCC 20231.

Bacterial strains	Tomato juice (%)				Carrot juice (%)			
	0.0	0.5	1.0	1.5	0.0	0.5	1.0	1.5
	Opitcal density at 600 µm							
A*	1.128	1.134	1.146	1.154	1.128	1.130	1.139	1.142
B	1.122	1.136	1.140	1.149	1.122	1.130	1.139	1.146
C	1.122	1.139	1.146	1.158	1.122	1.136	1.141	1.149
D	1.124	1.146	1.152	1.164	1.124	1.139	1.148	1.153
E	1.132	1.158	1.169	1.174	1.132	1.147	1.158	1.162
F	1.126	1.148	1.153	1.163	1.126	1.145	1.149	1.153
G	1.135	1.167	1.182	1.198	1.135	1.159	1.173	1.182
	Zone inhibition (mm)[a]							
A	19	19	21	22	19	19	20	20
B	17	19	20	21	17	18	19	19
C	17	18	20	20	17	18	20	20
D	18	20	21	22	18	20	21	21
E	21	22	23	24	21	22	22	23
F	19	20	20	22	19	20	20	22
G	21	22	22	23	21	22	22	22

* See Table (2).

a: Diameter of inhibition zone including disc diameter of 6 mm.

Table (7): Effect of fortification of growth medium with bile salt on growth of bifidobacteria (optical density at 600 µm) and production of antimicrobial agents (zone inhibition mm) against *Staphylococcus aureus* ATCC 20231.

Bacterial strains	Bile salt			
	0.0	0.5	1.0	1.5
	Optical density at 600 □m			
A*	1.128	1.113	1.003	1.001
B	1.122	1.108	1.003	1.002
C	1.122	1.106	1.005	1.001
D	1.124	1.109	1.001	1.001
E	1.132	1.113	1.108	1.003
F	1.126	1.103	1.001	1.002
G	1.135	1.116	1.109	1.102
	Zone inhibition (mm)[a]			
A	19	12	10	8
B	17	12	10	8
C	17	12	10	8
D	18	12	10	8
E	21	16	12	10
F	19	12	10	8
G	21	15	12	10

* See Table (2).

a: Diameter of inhibition zone including disc diameter of 6 mm

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تأثير بعض المواد الغذائية وأملاح الصفراء على إنتاج المواد المضادة للبكتريا بواسطة بكتريا الـ Bifidobacteria

يهدف هذا البحث لدراسة تأثير بعض المواد الغذائية المضافة للبيئة على كل من سرعة نمو وحيوية بكتريا الـ Bifidobacteria وإنتاجها للمواد المضادة .

ولقد أوضحت النتائج المتحصل عليها أن سبع سلالات من بكتريا Bifidobacteria وهى *Bif. bifidum* ATCC29521, *Bif. bifidum* ATCC15696, *Bif. longum* NCFB2299, *Bif. longum* BL-04, *Bif. infantis* ATCC15567, *Bif. catenulateum* ATCC18371, *Bif. lactis* BL-01 لها القدرة على إنتاج مواد مضادة للبكتريا حيث ثبطت مستخلصات المزارع الخالية من البكتريا لهذه السلالات من البكتريا الآتية *E. coli* ATCC69337, *Staphylococcus aureus* ATCC20231, *Bacillus cereus* ATCC33018, *Pseudomonas aeruginosa* ATCC9027, *E. coli* 0157-H7, *Staph. aureus, Bacillus cereus, Salmonella typhi, Salmonella enteritidis* بينما كان تأثيرها بسيطا على بكتريا حمض اللاكتيك .

وقد أدى تدعيم البيئة بواسطة كل من مخلوط السستيئين والجليسين والأنيولين وبروتينات الشرش المحللة وعصير الطماطم والجزر إلى زيادة إنتاج المواد المضادة بواسطة بكتريا Bifidobacteria بينما أدى إضافة أملاح الصفراء إلى انخفاض المقدرة على إنتاج هذه المواد المضادة .

ولقد أظهرت سلالات *Bif. longum* BL-04, *Bif. bifidum* ATCC 2952 أعلى مقدرة على تثبيط البكتريا .

وتُوضح النتائج أن إنتاج المواد المثبطة يعتمد على نوع وسلالة البكتريا .