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EFFECT OF BIFIDOBACTERIUM SPP. ON SOME PATHOGENS

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ABSTRACT: The bacteriological assessment of tested Bifidus-milk samples during incubation time showed gradual increase in bifidobacteria counts in all treatments. However, bifidobacteria counts attained the maximum population at the fresh state and then gradually decreased as extending storage period.

On the other hand, the counts of either E. coli or Staph. aureus reached their maximum on the second hour of incubation, then decline gradually as the incubation time progressed and also throughout storage period.

Moreover, samples artificially contaminated with E. coli or Staph. aureus possessed the lowest content of diacetyl as compared with those of control.

Additionally, Bif. bifidum showed great antagonistic effect against both pathogens where their counts were sharply reduced and were completely absent from resultant product after 10 days of storage.

INTRODUCTION

In the past three decades many authors reported the beneficial effects of bifidobacteria on human health (Kurmann and Rasic, 1991; Dinakar and Mistry, 1994; Blanchette and Roy, 1995 and Blanchette et al., 1996).

However, many investigators measured the antimicrobial activity of Bifidobacterium against several pathogens (Anand et al., 1985; Misra and kuila, 1992; Kebary, 1995; Badawi and El-Sonbaty, 1997; Sarker and Misra, 1998 and Abd-El-Rahman, 2000). The bioactivity of bifidobacteria may be based on the following effects: (a) competitive antagonisms against invading pathogens; (b) production of organic acids and possibly, by some strains, other antimicrobial substances; (c) lowering the activity of some harmful bacterial enzymes and, consequently, decreasing the formation of harmful products (amines, ammonia, nitrosamines, etc.); (d) probiotic effect, contribution to the balance of the intestinal microflora.

Therefore, because of these nutritional and therapeutic effects many efforts have been devoted to incorporate bifidobacteria in dairy products (Rasic and Kurmann, 1983 and Hunger and Peitersen, 1992).

Thus, the main objective of this work was to be sure wether bifidobacteria cultures (Bif. longum and Bif. bifidum) of Bifidus-milk products can inhibit the growth of the pathogenic microorganisms involved E. coli and Staph. aureus.

MATERIALS AND METHODS

Materials:

Milk source:

Fresh whole buffaloe's milk was obtained from the herd of Faculty of Agriculture, Al-Azhar University.

Starter cultures:

Bifidobacterium bifidum ATCC-15696, Bifidobacterium longum ATCC-15708, were secured from Cairo Microbiological Resources Center (Cairo MIRCEN),Cairo, Egypt .

Pathogenic bacteria:

Staphylococcus aureus ATCC-6538 and Escherichia coli O157 : H7 ATCC-51657 were obtained from Botany and Microbiology Department, Faculty of Science, Al-Azhar Univ., Assiut, Egypt.

Methods:

Bifidus milk manufacture:

Two equal portions of fresh skimmed buffaloeís milk (3 + 0.1 % fat) were heated at 90 ± 1.0°C for 15 minutes, cooled to 45 ± 1°C., each portion was divided into three equal parts, the first part was inoculated with 10 % of either Bif. bifidum or Bif. longum to serve as control, while the second and third parts were inoculated with about 106 cfu/ml. of E. coli and S. aureus respectively, each part of the previous cultured milk was separately distributed into plastic cups (200ml.), covered with aluminium foil, incubated at 42 ± 1°C. till curdling and then stored in the refrigerator for 15 days.

Chemical analysis:

The titratable acidity (TA) of tested samples was adopted according to Ling (1963). The pH value was measured using a laboratory pH meter (Model 68 ESD 19713, USA), as described by Ling (1963). Diacetyl was determined as described by Westerfeld (1945).

Microbial counts:

The total plate count technique outlined in the Standard Methods for Examination of Dairy Products (A.P.H.A., 1978) was adopted. Serial dilutions of each sample in citrate buffer were plated on modified MRS agar medium (m-MRS) according to Dave and Shah (1996), The plates were incubated at 37oC for 48 hours.

For E. coli count, Violet red bile agar (VRBA) medium was used as recommended by Klein and Fung (1978) and incubated at 37°C for 24 hours. While, Staph. aureus count was estimated on Baird-Parkerís egg yolk tellurite agar medium (Baird-Parker, 1962). The plates were incubated at 37°C for 24 hours.

RESULTS AND DISCUSSION

Some chemical and bacteriological analysis of Bifidus-milk cultured with Bif. bifidum were carried out and presented in Table (1) and Fig. (1 and 2).

It was obvious that there was gradual increase in acidity till the end of incubation time. Moreover, Bifidus-milk artificially contaminated with E. coli or S. aureus had the highest titratable acidity throughout incubation time, as compared with that of plain Bifidus-milk (Control).

This statement may be attributed to ability of both contaminants to produce acid from lactose (Banwart, 1981 and Badawi and El-Sonbaty, 1997).

Contrary to the developed acidity, pH values of all tested samples slightly decrease with the advancing of incubation time. Such a decrease in the pH of Bifidus-milk is mainly due to the developed lactic acid by lactose fermentation.

Moreover, bacteriological analysis of Bifidus-milk samples revealed a gradual increase of bifidobacteria count throughout incubation time. Also, it was evident from data obtained in Tables (1) & (2), that the bifidobacteria count of all tested samples was gradually de-

creased during storage period. However, the count of E. coli or Staph. aureus reached their maximum on the second hour of incubation, then decreased gradually as the incubation time progressed. This finding may be due to the production of antimicrobial agents by bifidobacteria which suppress the growth of E. coli and Staph. aureus (Wijsman et al., 1989 and Kebary, 1995). However, our result was in complete agreement with that of Badawi and El-Sonbaty (1997).

Moreover, Tables (3 and 4) showed the changes in some compositional and bacteriological analysis in different treatments of Bifidus-milk during storage for 15 days at 10 + 1oC. As could be expected, there was gradual increase in titratable acidity in different treatments throughout storage period. In addition, Bifidus-milk contaminated with E. coli possessed the highest lactic content either at fresh stage or at the end of storage, actually 0.88 % and 1.28 % respectively, which may due to the ability of the pathogen to produce acid from lactose (Banwart, 1981).

Furthermore, the pH values of the same samples exerted contrary trend. However, at the end of storage period (15 days) the pH values of stored samples markedly reduced and varied from 3.76 to 3.90. At this pH range most pathogens could be inhibited, which makes the resultant products biologically safe (Walestra et al. 1993).

The present results declared that diacetyl content of all Bifidus-milk samples gradually increased during the storage period. Continuously, it was clear that plain Bifidus-milk attained the highest level of diacetyl either in the fresh or stored samples. Fresh sample possessed diacetyl of 108 mg/100g, which increased to 149 mg/100g after 15 days of storage.

Also, as could be expected, Bifidus-milk contaminated with either E. coli or Staph. aureus possessed lower values for diacetyl, actually 100 and 99 mg/100g at fresh stage and 125 and 123mg/100g at the end storage respectively. This phenomenon could be a result for diacetyl reductase activity, which originated from microbial contaminant (Seitz et al., 1963). In this connection, Wong and Frank (1981) stated that 45 % of diacetyl content reduced by E. coli in stored buttermilk.

However, it is clear from the obtained data that using of Bif. bifidum minimized the capability of E. coli and Staph. aureus to reduce diacetyl content and an improvement in diacetyl concentration in contaminated Bifidus-milk was observed.

It was evident from the data presented in Tables (3) & (4) that Bif. bifidum showed greatest antagonistic effect against E. coli and Staph. aureus. However the counts of both pathogens in contaminated Bifidus-milk sharply reduced and could not be detected in any of the tested samples after 10 days of storage. This finding may be attributed to the production of antimicrobial agents by bifidobacteria which suppress the growth of both pathogens (Wijsman et al., 1989 and Kebary, 1995).

Generally, from the foregoing results, it could be concluded that the using of Bifidobacterium bifidum as starter culture in the manufacture of dairy products not only greatly improved the chemical composition of the resultant product, but also showed greatest antagonistic effect against either E. coli or Staph. aureus, which led to a great decline in the values of diacetyl reduction (%).

Therefore, Bif. bifidum could be successfully used in the manufacture of Bifidus-milk product not only to suppress the growth of pathogenic and undesirable organisms, but also for their beneficial role in improving the organoleptic properties (flavor).

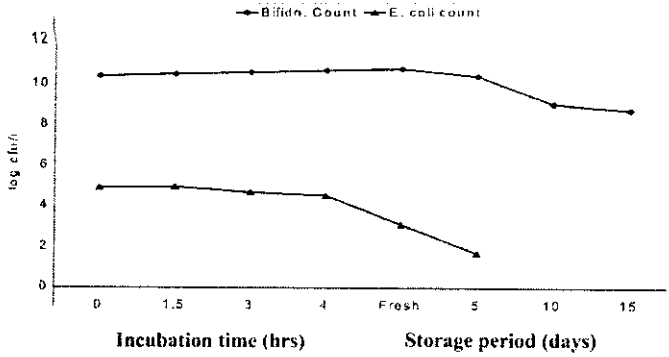

Fig. (1): Log counts of *Bif. longum* and *E. coli* counts in Bifidus-milk.

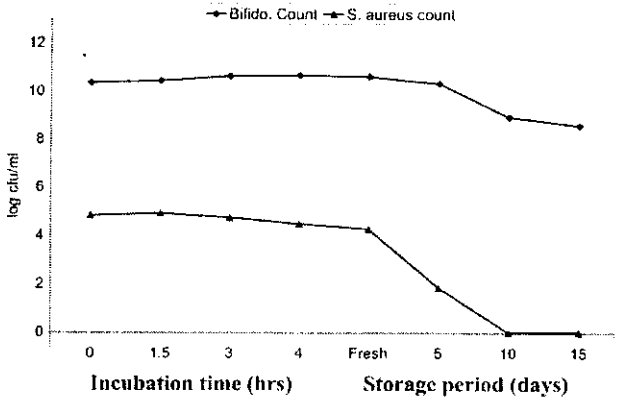

Fig. (2): Log counts of *Bif. longum* and *S. aureus* counts in Bifidus-milk.

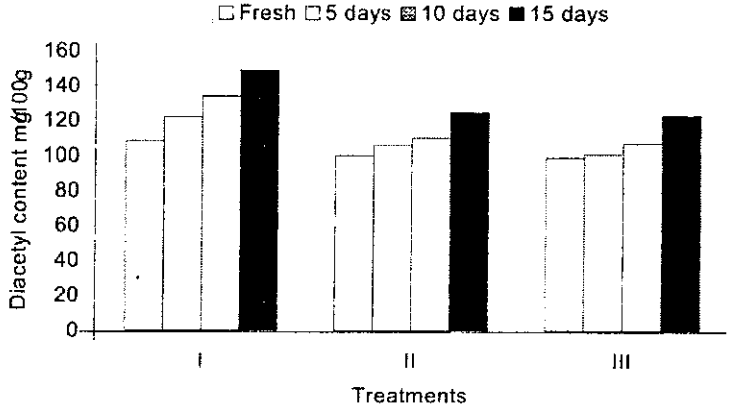

Fig. (3): Diacetyl content of different treatments of Bifidus-milk made with *Bif. longum* during storage periods.

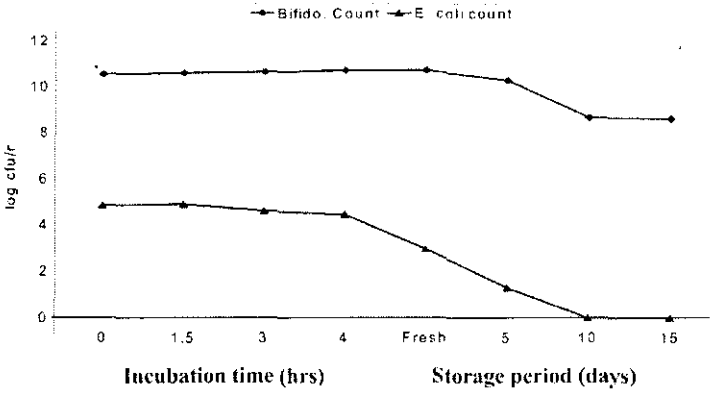

Fig. (4): Log counts of *Bif. bifidum* and *E. coli* counts in Bifidus-milk.

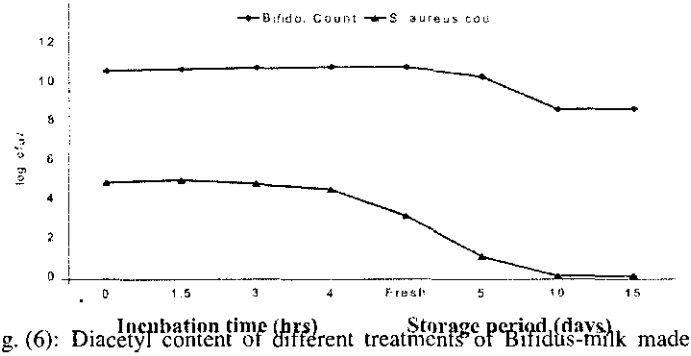

Fig. (6): Diacetyl content of different treatments of Bifidus-milk made with *Bif. bifidum* during storage periods.

Fig. (5): Log counts of *Bif. bifidum* and *S. aureus* counts in Bifidus-milk.

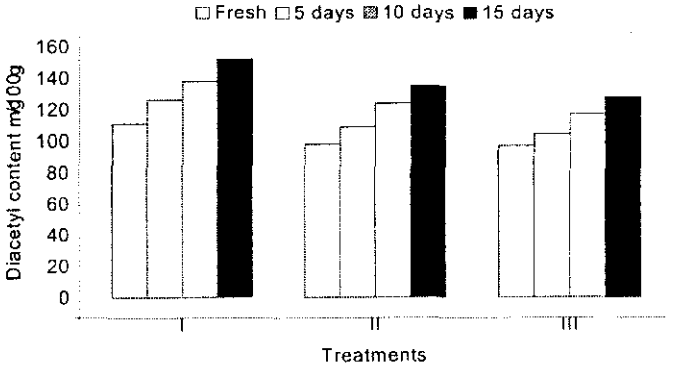

Table (1) Some chemical and bacteriological analysis of different treatments of Bifidus-milk during incubation time (average of 3 replicates):

Components	Treatments											
	I				II				III			
	Incubation time (hrs)											
	0	2	4	7	0	2	4	7	0	2	4	7
Acidity %	0.16	0.19	0.37	0.58	0.17	0.21	0.43	0.60	0.18	0.20	0.39	0.59
pH	6.64	6.48	5.60	4.93	6.62	6.45	5.51	4.89	6.63	6.46	5.52	4.90
Diacetyl (mg/100g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bif. count (Log cfu/ml)	10.36	10.48	10.58	10.65	10.36	10.46	10.54	1064	10.36	10.45	10.63	10.65
E. coli count (Log cfu/ml)	-	-	-	-	4.86	4.91	4.64	4.49	-	-	-	-
S. aureus count (Log cfu/ml)	-	-	-	-	-	-	-	-	4.81	4.91	4.37	4.48

ND: Not determined
I : Bifidus-milk cultured with *Bif. Longum* (Control)
II : Bifidus-milk cultured with *Bif. Longum* and artificially contaminated with *E. coli* .
III : Bifidus-milk cultured with *Bif. Longum* and artificially contaminated with *Staph. aureus*.

Table (2) Some chemical and bacteriological analysis of different treatments of Bifidus-milk during incubation time (average of 3 replicates):

Components	Treatments											
	I				II				III			
	Incubation time (hrs)											
	0	2	4	7	0	2	4	7	0	2	4	7
Acidity %	0.17	0.20	0.37	0.56	0.18	0.22	0.42	0.52	0.17	0.21	0.38	0.58
pH	6.63	6.51	5.60	4.94	6.62	6.46	5.51	4.89	6.63	6.50	5.52	4.90
Diacetyl (mg/100g)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Bif. count (Log cfu/ml)	10.54	10.59	10.63	10.71	10.54	10.60	10.64	10.72	10.54	10.59	10.65	10.71
E. coli count (Log cfu/ml)	-	-	-	-	4.86	4.90	4.62	4.46	-	-	-	-
S. aureus count (Log cfu/ml)	-	-	-	-	-	-	-	-	4.81	4.92	4.72	4.44

ND: Not determined
I : Bifidus-milk cultured with *Bif. bifidum* (Control)
II : Bifidus-milk cultured with *Bif. bifidum* and artificially contaminated with *E. coli* .
III : Bifidus-milk cultured with *Bif. bifidum* and artificially contaminated with *Staph. aureus*.

Table (3) Some chemical and bacteriological analysis of different treatments of Bifidus-milk during storage period (average of 3 replicates):

Components	Treatments											
	I				II				III			
	Storage period (days)											
	Fresh	5	10	15	Fresh	5	10	15	Fresh	5	10	15
Acidity %	0.85	0.99	1.12	1.26	0.88	1.11	1.20	1.28	0.88	1.10	1.18	1.21
pH	4.70	4.25	4.01	3.83	4.60	4.16	4.00	3.76	4.58	4.12	4.10	3.90
Diacetyl (mg/100g)	108	122	134	149	100	106	110	125	99	101	107	123
Bif. count (Log cfu/ml)	10.72	10.32	8.88	8.48	10.73	10.36	9.01	8.71	10.61	10.34	8.95	8.60
E. coli count (Log cfu/ml)	-	-	-	-	3.08	1.67	NG	NG	-	-	-	-
S. aureus count (Log cfu/ml)	-	-	-	-	-	-	-	-	4.26	1.85	NG	NG

NG: No growth
I : Bifidus-milk cultured with *Bif. Longum* (Control)
II : Bifidus-milk cultured with *Bif. Longum* and artificially contaminated with *E. coli* .
III : Bifidus-milk cultured with *Bif. Longum* and artificially contaminated with *Staph. aureus*.

Table (4) Some chemical and bacteriological analysis of different treatments of Bifidus-milk during storage period (average of 3 replicates):

Components	Treatments											
	I				II				III			
	Storage period (days)											
	Fresh	5	10	15	Fresh	5	10	15	Fresh	5	10	15
Acidity %	0.80	1.00	1.10	1.22	0.85	1.10	1.18	1.24	0.84	1.08	1.17	1.22
pH	4.72	4.26	4.10	3.93	4.66	4.21	4.11	4.00	4.63	4.22	4.11	3.87
Diacetyl (mg/100g)	111	126	138	152	98	109	124	135	97	104	117	128
Bif. count (Log cfu/ml)	10.70	10.23	8.32	8.20	10.72	10.28	8.65	8.60	10.69	10.22	8.53	8.56
E. coli count (Log cfu/ml)	-	-	-	-	2.95	1.30	NG	NG	-	-	-	-
S. aureus count (Log cfu/ml)	-	-	-	-	-	-	-	-	3.08	1.00	NG	NG

NG: No growth
I : Bifidus-milk cultured with *Bif. bifidum* (Control)
II : Bifidus-milk cultured with *Bif. bifidum* and artificially contaminated with *E. coli*.
III : Bifidus-milk cultured with *Bif. bifidum* and artificially contaminated with *Staph. aureus*.

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تأثير التضاد بسلالات من الـ Bifidobacterium على بعض البكتريا المرضية

أظهرت التحليلات البكتيرية للعينات المختبرة زيادة تدريجية في أعداد بكتريا الـ Bifidobacteriumأثناء فترة التحضين حيث وصلت إلى حدها الأقصى في المنتج النهائي ثم بدأت أعدادها في التناقص تدريجياً خلال مدة التخزين وذلك بتأثير ارتفاع درجة حموضة المنتج. كما أوضحت النتائج أن أعداد البكتريا المرضية تصل إلى حدها الأقصى بعد ساعتين من بداية عملية التحضين ثم تبدأ الأعداد في التناقص ويستمر هذا التناقص خلال مدة التخزين وذلك كنتيجة لتأثير المواد المضادة التي تفرزها بكتريا الـ Bifidobacterium والتي تثبط نمو البكتريا المرضية .

أظهرت النتائج أن العينات الملوثة ببكتريا E. coliأو Staph. aureusينخفض محتواها من مركب الداى أسيتايل مقارنة بالعينات الخالية من الملوثات ويعزى ذلك إلى قدرة البكتريا المرضية لإنتاج أنزيم Diacetyl-reductase والذي يختزل مركب الداى أسيتايل إلى مركب ٢،٣ بيوتيلين جليكول.

لوحظ أن استخدام بكتريا Bif. bifidum أدى إلى انخفاض شديد في أعداد البكتريا المرضية وفي غضون ١٠ أيام اختفت السلالات المرضية المستخدمة تماما من العينات المختبرة.