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ENHANCING OF LACTIC ACID BACTERI Grown with PROBIOTIC BACTERIA CULTURES

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ABSTRACT

Some Lactic acid bacteria (LAB) may act as probiotic bacteria *(Streptococcus thermophilus, lactobacillus delbrueckii subsp. bulgaricus, lactobacillus helveticus and lactobacillus acidophilus*) .enhancing the growth of these bacterial species being grown separately or in combination was attempted , so as to increase numbers to attain the squared healthy levels .examination was carried out to evaluate the effect of the examined cultures, whether separately or their combinations by evaluating the maximum turbidity being occurred in the examined selective growth broths . Intensive turbidity resulted in the combinations of the examined cultures, compared with the individual strains. Mean while, the examined single or combined culture varied due to the single species or involved in the mixed cultures. *Bifidobacterium bifidum* in combination with any of the examined lactic acid bacteria reached the maximum growth after shorter time of incubation ,compared with its growth simply ,and attained more growth and reached more members reuired for the healthy dose.

Keywords: lactic acid bacteria, bifidobacterium bifidum, interaction, growth rate.

INTRODUCTION

Interest in the role of beneficial gut microorganisms (probiotics) for promoting human health dates back to 1908 when Metchnikoff suggested that man should consume milk fermented with lactobacilli to prolong life (Hughes and Hoover 1991; O'Sullivan *et al*., 1992). At present, it is generally recognized that an optimum 'balance' in microbial population in our digestive tract is associated with good nutrition and health (Rybka and Kailasapathy, 1995). The health promoting effects of probiotic LAB include metabolic stimulation of vitamin synthesis and enzyme production, stabilization of gut microflora and competitive exclusion of enteric pathogens, enhancement of innate host defenses by production of antimicrobial substances, reduction of serum cholesterol by assimilation mechanisms, decreased risk or colon cancer by detoxification of carcinogens and tumor suppression by modulation of cell mediated immunity. Probiotic microorganisms commonly involve strains belonging to lactobacilli and bifidobacteria.

Bifidobacteria play a significant role in controlling intestinal pH through the liberation of lactic and acetic acids (produced in a molar ratio of 2 : 3, respectively). By controlling intestinal pH, it is possible to restrict the growth of putrefactive and pathogenic bacteria These probiotics are thought able to inhibit the growth of coliforms, enterococci and clostraidia, and

capable of correcting abnormal adult and elderly intestinal microflora amal El-saady (2010).

A variety of interactions was demonstrated. *Lb. delbrueckii subsp. bulgaricus* was found to be able to inhibit *S. thermophilus* strains. Among probiotic cultures, *Lb. acidophilus* was the sole species that was inhibited by the others (*Lb. casei* and *Bifidobacterium*). In general, probiotic bacteria proved to be inhibitorier towards lactic acid bacteria than vice versa since the latter did not exert any effect on the growth of the former, with some exceptions. The possible interactions among the strains selected to manufacture a probiotic fermented dairy product should be taken into account when choosing the best combination/s to optimize their performance in the process and their survival in the products during cold storage Vinderola *et al*., (2002). So, this work aims to determine the interaction among four strains of lactic acid bacteria and bifidobacterium bifidum and its effect on the viability of probiotic bacteria strains.

MATERIALS AND METHODS

USA skim milk powder (Fat 1.5%, Moisture 4%) was obtained from local market and reconstituted by the ratio of 10% in hot tab water (46 ^{o}C). Lactic acid bacteria *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus acidophilus 145(*Lb acid type 145), *Lactobacillus helveticus* and *Bifidobacterium species 420 Bifidum ssp*. strains obtained from the Microbiological Resources Center (Cairo MIRCEN) - Faculty of Agriculture Ain Shams University.

The optical density was measured at 650 nm as recommended by Ezzat and EL-Shafei (1991) using spectrophotometer shimad24 (UV-visible). Total bacterial counts of yoghurt were determined according to the American Public Health Association (1978) by plating the proper dilution in duplicates using nutrient agar medium (Difco manual, 1966).The count of bifidobacteria was determined according to Dave and Shah (1996) by using modified MRS agar supplemented with 0.05% L-cystein and 0.3 % Lithuim chloride. The plates were incubated at 37^{0}C for 48 hrs under anaerobic conditions. *L. acidophilus* counts were determined according to Gilliland and Walker (1990) by using modified MRS agar supplemented with 0.2 % oxagal. The plates were incubated at 37° C / 48 hrs before counting. Lactobacilli, counts were determined according to Dave and Shah (1996) by plating on M.R.S media. Probiotic bacteria count on (M.R.S) according to Dave and Shah (1996). Streptococci count on (M17) according to Terzaghi and Sandine (1975).

RESULTS AND DISCUSSION

Data in Table (1) show that the numbers of *streptococcus thermophilus* gained pronounced increase when it grew in a combination with bifidobacteria bifidum and any other lactobacilli spp., On the other hand, this increase wasn't pronounced or when *streptococcus thermophilus* grew with

lactobacilli spp. only. These data were in agreement with Chouraqui etal., (2004).

Table (1): Count of *streptococcus thermophilus* in solo and in a combination.

Culture type	Count × 10^7	Time of counting at high turbidity/ hrs
Str. Thermophilus	**104**	**6**
Str. + Lb. acidophilus (1 : 2)	**74**	**8**
Str. + Lb. helveticus (1 : 1)	**66**	**6**
Str. + Lb. bulgaricus (1 : 2)	**80**	**6**
Str. + Lb. acidophilus + Bifid. Bifidum (1 : 2 : 1)	**100**	**4**
Str. + Lb. helveticus + Bifid. Bifidum (1 : 1 : 2)	**104**	**6**
Str. + Lb. bulgaricus + Bifid. Bifidum (1 : 2 : 2)	**109**	**6**

Data in Table (2) indicate that the numbers of lactobacillus bulgaricus were increased when grew with *streptococcus thermophilus* from 86× 10^7 to 96 × 10^7 at the same time (8 hr). Moreover, these numbers were similar when *lactobacillus bulgaricus* were grown in a combination with streptococci and bifidobacteria but reached to this numbers in shorter time (6 hr) instead of 8 hrs when they grew in a sole culture. These results were similar to those obtained by with Champagne *et.al.*, (2009), who reported that The probiotic populations in the mixed culture were influenced by the *S. thermophilus* strain and by the time of fermentation.

Table (2): Counts of l*actobacillus bulgaricus* in sole and in combinations with other strains.

Culture type	Count × 10^7	Time of counting at high turbidity / hrs
Lb. bulgaricus	86	**8**
Str. + Lb. bulgaricus (1 : 2)	96	8
Str. + Lb. bulgaricus + Bifid. Bifidum (1 : 2 : 2)	86	**6**

Data presented in Table (3) show that the growth of *Lactobacillus helveticus* was enhanced when grown with other strains, whereas, its numbers increased from 64 × 10^7 to 88× 10^7 with decreasing the growth time from 8 hrs to 6 hrs. Moreover, this enhancement was more clear when *Lactobacillus helveticus* was grown in a combination with *streptococcus thermophilus* and *Bifidobacterium bifidum*, its numbers were increased from 64× 10^7 at 8 hrs to 120× 10^7 at 6 hrs. This might be due to the abundance of the growth factors ethicizing by both streptococci and bifidobacteria. These results were in agreement with Stéphanie Anne et al., (2009), who reported that the the probiotics *L. helveticus* and *B. spp*, grown in combination achieve good properties and the growth of *L. helveticus* was activated.

Table (3): Counts of *lactobacillus helveticus* in solo and in a combination with other strains.

Culture type	Count × 10^7	Time of counting at high turbidity / hrs
Lb. helveticus	**64**	**8**
Str. + Lb. helveticus (1 : 1)	**88**	**6**
Str. + Lb. helveticus + Bifid. Bifidum (1 : 1 : 2)	**120**	**6**

Data in Table (4) show that the growth of *Lactobacillus helveticus* was more pronounced than that of *Lactobacillus acidophilus*, where, its numbers were enhanced by its growing in a combination with *streptococcus thermophilus* and *Bifidobacterium bifidum*. Its number increased from 50 × 10^7 to 160× 10^7 in short time(4 hrs instead of 8 hrs) when it was grown in sole culture. These results might be due to abundance of growth factors ethicized by both streptococcus and bifidobacteria. These results were in agreement with Mortazavian etal., (2011) who decided that the incubation time and inoculation rate showed interactive effects on biochemical parameters and incubation time, as well as on viability of *lactobacillus acidophilus* and *Bifidobacteria subsp.* When they grown in a combination with traditional yoghurt starter.

Table (4): Counts of *lactobacillus acidophilus* in solo and in a combination with other strains.

Culture type	Count × 10^7	Time of counting at high turbidity / hrs
Lb. acidophilus	**50**	**8**
Str. + Lb. acidophilus (1 : 2)	**65**	**6**
Str. + Lb. acidophilus + Bifid. Bifidum (1 : 2 : 1)	**160**	**4**

Data in Table (5) indicate that there are different effects of lactic acid producing bacteria on the growth and viability of *Bifidobacterium bifidum*. This effect was more pronounced with *streptococcus thermophilus*, where, the bifidobacteria number was increased from 100 × 10^7 when it grew in sole culture to 112× 10^7 when it grew in a combination with *streptococcus thermophilus* during shorter time (from 8 hrs to 4 hrs) to reach to the maximum turbidity. On the other hand, there isn't intelligible effect for all lactobacilli spp., where, the bifidobacteria maximum turbidity was after 6 hrs but its number was not enhanced and this might be due to the slow production of acidity which allows more growth to *Lactobacilli spp.* more than *Bifidobacteria bifidum*. These results are in agreement with dinakar and mistry , (1994) who reported that The environment typical of many yoghurt and other cultured dairy products, including the low pH and the aerobic conditions of production and packaging, can result in decreases in the count of bifidobacteria in these dairy products to below the therapeutic minimum. Also, these data were in agreement with Mortazavian etal., (2011).

Table (5): Counts of *Bifidobacterium bifidum* in sole and in a combinations with other strains.

Culture type	Count × 10^7	Time of counting at high turbidity / hrs
Bifid. Bifidum	**100**	**8**
Str. + Lb. acidophilus + Bifid. bifidum (1 : 2 : 1)	**112**	**4**
Str. + Lb. helveticus + Bifid. bifidum (1 : 1 : 2)	**86**	**6**
Str. + Lb. bulgaricus + Bifid. Bifidum (1 : 2 : 2)	**72**	**6**

Conclusion

Lactic acid bacteria (LAB) had a positive interactive effect on the growth and viability of *Bifidobacterium bifidum*, which indicated on the increasing of the viable count on shorter time when compared with its single growth. Also, the tested combination ratios of LAB and *Bifidobacterium bifidum* enhanced the growth of probiotic LAB.

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تعزيز نمو بكتريا حامض الاكتيك من خلال تنميتها مع بكتريا البروبيوتيك

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بعض بكتريا حمض اللاكتيك ((Lactic .Acid. Bacteria تعمل كبكتريا بروبيوتيك *(Streptococcus thermophilus, lactobacillus delbrueckii subsp. bulgaricus, lactobacillus helveticus and lactobacillus acidophilus*) . و لتعزيز نمو هذه الأنواع البكتيرية تزرع بشكل منفصل أو مجتمعة وذلك لزيادة عدد النموات البكتيريه من خلال تقييم الحد الأقصى للعكارة فى البيئه. ، عند مقارنة بين نمو السلالات الفرديه مع السلالات المختلطه وجد ان خلط السلالات بنسب مختلفه ادت الى تشجيع نمو بكتريا بروبيوتيك و وصلت الى اعلى عكاره فى وقت بسيط عند مقارنتها بتنمياتها منفرده فى البيئه ، حقق هذا الخلط الوصول الى افضل نسبه تحقق الجرعه الصحيه .

قام بتحكيم البحث

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