IDENTIFICATION AND SELECTION OF LOCAL PROBIOTIC BACTERIA FOR PREPARATION OF FERMENTED MILK AND STUDY THEIR EFFECTS ON LIPID PROFILE OF RABBIT SERUM

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ABSTRACT

Two local isolates of Lactobacillus acidophilus (L-53) and Bifidobacterium (B3) was obtained from neonatal stool cultured on MRS media. These isolates was used in preparation of probiotic fermented milk products (ABT and ABY).The effect of the prepared probiotic products on rabbit serum lipid profile was studied through two experimental designs (Therapeutic: A, Prophylactic: B). Consumption of ABT and ABY products showed clear reduction in the levels of total cholesterol (TC) and low density lipid (LDL) by 4 weeks of utilization which was more reduced by 8 weeks of consumption ($P<0.01$) referring to the importance of consumption duration in reducing these levels compared to the control groups. Proportional mixing of probiotic bacteria with ordinary yogurt bacteria enhances their effect of the levels of TC and LDL. In contrast, the levels of high density lipid (HDL) and triglycerides (TG) showed no significant differences ($P>0.05$). However, the consumption of these products reduces the ratio of LDL: HDL which has a significant value in reducing the risk of acquiring atheroschlerosis. The regular consumption of probiotic products (Exp.B) showed clear prophylactic effect in maintaining lower levels of TC and LDL compared to the control group despite the utilization of cholestrol enriched diet.

INTRODUCTION

Attention was given to probiotic lactobacillus since the first observation of Tisser 1906 and Mitchnikoff 1908 when the first showed the positive effect of Bifidobacteria on the reduction of diarrhoeal diseases in children while the second suggested the elongation of age through the regular consumption of lactobacillus fermented milk[1]. The interest was grown after the use of *Lactobacillus acidophilus* isolated from human intestine by German scientist Henreburg 1926 in the preparation of reform yogurt or acidophilus milk which is considered as the first probiotic fermented milk[2], and in the last few years many nutritional and medical products provided the health benefits for the consumers called the probiotic products[3]. The bacteria *L. acidophilus* and *Bifidobacterium* are the most commonly used in the production of probiotic products because of their ability to stand the production and storage conditions and it can tolerate the strong gastric environments of low pH and high concentration of bile salts, beside their link to many health benefits such as management of lactose intolerance, diarrhoeal diseases in children and the support of immune system together with the reduction of blood cholestrol levels and gastrointestinal tract cancers[4]. These points encouraging their use in the production of many fermented milk products. Studies on *L. acidophilus* and *Bifidobacteria*[5] proved their ability in decomposition of serum cholestrol and changes bile salts in their host blood. This study aimed on identification and selection of local strains from human *L. acidophilus* and *Bifidobacterium* in order to be used in the preparation of probiotic milk products which was used in feeding rabbits to show their therapeutic and prophylactic effect on rabbit serum lipid profile.

MATERIALS AND METHODS

Stool specimens were collected from 13 healthy babies (age range 9-37 days) on breast feeding only. Specimens were introduced into Man-Rogosa-Sharp (MRS) broth (difco lab.) transport media and processed within 12 hrs of collection. Following special scheme for isolation and identification of *L. acidophilus* and *Bifidobacterium* using MRS media and MRS-NNLP (MRS-Neomycin-Nalidixic acid-Lethium-Paramycin) media (Difco Lab.) respectively[6]. Standard control microorganisms for comparison obtained from Chr-Hanson's laboratories, Denmark were included which was named as: *Lactobacillus acidophilus (La-5), Bifidobacterium longum (BB-46), B. bifidum (Bb-12), L.delbervecki, ssp bulgaricus (Lb-10442), Streptococcus thermophilus (St-143) (ordinary yogurt bacteria).* All diagnostic

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methods were done according to a standard procedure[7]. According to the selection and identification scheme, two local isolates named: *L. acidophilus (L-53)* and *Bifidobacterium (B3)* were selected and together with the reference isolates La-5 and BB-46 (reference products) as well as the ordinary yogurt bacteria were used in the preparation of 4 types of fermented milk products according to the types of bacteria introduced; ABT: inoculated with L53 and B3 isolates, *Streptococcus theromophilus*, ABY: L53, B3, *Strept. thermophilus* and *L. bulgaricus*, Y: ordinary yogurt bacteria, C: Reference bacteria La-5, BB-46 and *Strept. thermophilus*. These 4 products were incubated to reach the required bacterial count and used in the feeding of 60 male rabbits (5ml/Kg/day for each animal by oral intubation). For the study of serum lipid profile, these rabbits was grouped into two main groups A and B on the basis of cholestrol consumption; each divided into 5 groups named: C = fed with ordinary lipid free diet (formula) + cholestrol (control), S= Formula + cholesterol + skimed milk, Y= formula + cholestrol + yogurt, ABT= formula + Cholestrol + ABT product, ABY= formula + Cholestrol + ABY product, while in Exp B no cholestrol for 4 weeks, only challenged with products and the cholestrol enriched diet (500 mg/Kg of animal body weight) started by the end of the 4^{th} week and followed up to 8 weeks for the test groups. All animals were bleed by 4 and 8 weeks for the estimation of serum total cholesterol (TC), low density lipid (LDL), high density lipid (HDL) and triglyceride (TG) using special quantitative kits (bioMeriux Sa, France) according to the manufacturers instructions. All blood samples were collected from the ear marginal vein after 16 hrs fasting of animals.

For statistical analysis, the SPSS-998 model and the overall random analysis of data was used by computer.

RESULTS

The effect of fermented milk products prepared by the local isolates of L-53 and B3 of *L. acidophilus* and *Bifidobactertium* on serum lipid profile of rabbits was studied through two types of experimental design (Exp. A: to show the therapeutic and Exp. B: to show the prophylactic effects).

The levels of TC in rabbit sera were increased 7-13 times after 4 weeks of cholestrol consumption. (Table-1) shows a significant reduction ($P<0.001$) in the levels of TC in all groups of experiment A utilized the products Y, ABT, ABY which were in the levels of 535.75, 460.03 and 470 mg/100ml respectively after 4 weeks of feeding, and 1009.86, 761.51, and 747.58 mg/100ml after 8 weeks of probiotic products feeding compared to that observed among the control groups C and S which were 757.56 and 741.76 mg/100ml after 4 weeks and 1328.01 and 1249.33 mg/100ml after 8 weeks of feeding respectively. Also there were a more reduction in the levels TC in ABT and ABY products consumers compared to the ordinary yogurt (Y) consumers after 8 weeks of feeding ($P<0.05$). However, there were no significant differences in the values of HDL ($P>0.05$) by 4 weeks but there were a significant differences in these values after 8 weeks of cholestrol consumption together with probiotic products ($P<0.05$) which was 134.85, 133.61 and 137.51 mg/100ml for the products Y, ABT and ABY respectively compared to 102.61 and 105.44 mg/100ml for C and S control group. In contrast to HDL values, the consumption of Y, ABT and ABY lead to a significant reduction in the values of LDL ($P<0.01$) after 4 weeks (397.61, 314.1 and 325.65 mg/100ml) and after 8 weeks (848.81, 600.70 and 583.90 mg/100ml) of probiotic consumption respectively, compared to the control groups C and S by 4 weeks (621.65 and 609.95 mg/100ml) and 8 weeks (1196.45 and 114.85 mg/100ml) respectively. There were a significant reduction ($P<0.05$) in LDL values of those utilized the products ABT and ABY compared to the ordinary yogurt (Y product) by 8 weeks. There were no significant differences in the values of triglyceride (TG) in all groups by 4 weeks and 8 weeks of probiotic products utilization (Table-1). The ratios of LDL: HDL was significantly reduced ($P<0.05$) in the sera of rabbits consumed the products Y, ABT and ABY which was 3.54, 2.64 and 2.78 respectively by 4 weeks of feeding and 6.29, 4.49 and 4.24 by 8 weeks compared to 5.75 and 5.85 by 4 weeks and 11.66, and 10.57 by 8 weeks of feeding for the control groups C and S respectively (Figure-1).

In experiment B, the groups of rabbits were challenged with probiotic products for 4 weeks

in the presence of ordinary feeding formula. During this period no significant changes in the levels of TC, HDL, LDL and TG from the baseline before the experiment, but there were significant differences (P<0.01) in the levels of TC among the groups Y, ABT and ABY (183.78, 146.27 and 121.52 respectively) compared to the control groups C and S (310.73 and 283.90 respectively) (Table-2). After the 4 weeks, all the five groups were fed with formula enriched with cholestrol for further 4 weeks while the daily challenge with probiotic products continued to the test groups ABT and ABY together with ordinary yogurt (Y) where the levels of TC showed no significant changes from that observed before the utilization of cholestrol which was 313.36 and 309.35 mg/100ml for ABT and ABY and 523.22 mg/100ml for Y group respectively, while the levels of TC was significantly increased (P<0.01) to reach 773.61 and 717.28 mg/100ml for the control groups C and S respectively, since the reduction in the levels was 77.67%, 82.01% and 84.98% for the products Y, ABT and ABY respectively (Figure -2). Similar pattern of changes was also observed in the levels of LDL in the control groups C and S which was 623.75 and 582.93 mg/100ml respectively compared to the figures before cholestrol feeding while the LDL levels in the test groups showed no much differences from that before cholestrol uptake (471.87, 269.73 and 260.52 mg/100ml for Y, ABT and ABY respectively). However, no changes were observed in the levels of HDL and TG for all of the five groups (Table-2).

Table 1. ***The levels of TC, HDL, LDL and TG (mg/100 ml) in rabbit serum utilized the products Y, ABT, and ABY in exp. A***

Group	Initial levels				4 weeks consumption				8 Weeks consumption			
	TC	HDL	LDL	TG	TC	HDL	LDL	TG	TC	HDL	LDL	TG
C	66.28	19.26	30.81	87.22	757.56	108..01	621.65	151.15	1328.01**	102.61*	1196.45	146.43
S	69.01	20.43	32.22	78.63	741.76	104.2	609.95	140.20	1249.33	105.44	1114.85	148.28
Y	65.42	19.21	28.89	90.36	535.73	112.23	397.61	124.13	1009.86	134.85	848.81	126.38
ABT	68.56	22.84	32.47	88.38	460.03	119.05	314.10	127.06	761.51	133.61	600.70	132.51
ABY	72.71	23.42	36.98	91.16	470.0	123.71	325.65	132.68	747.58	137.51	583.90	131.73

* P < 0.05 ** P<0.01
C: Control, La-5, BB-46 and Strept thermophilus.
S: The standard bacteria La-5, BB-46.
Y: The ordinary yogurt bacteria.
ABY: A products contain L53, B3, Strept thermophilus and L. bulgaricus.
ABT: A products contain L53, B3, isolates and Strept. thermophilus.

Table 2. ***The levels of TC, HDL, LDL and TG (Mg/100ml) in rabbit sera of exp.B.***

	1-4 wks probiotic challenge				4-8 weeks probiotic challenge with cholestrol enriched diet			
	TC	HDL	LDL	TG	TC	HDL	LDL	TG
C	310.73	52.81	235.32	111.27	773.71	119.60	623.72	147.68
S	283.90	49.13	213.87	109.71	717.28	108.28	582.93	129.56
Y	183.78	37.97	125.06	98.81	523.22	127.35	471.87	120.11
ABT	146.27	41.53	83.17	107.31	313.68	118.68	269.73	122.86
ABY	121.52	39.29	61.21	102. 32	309.35	112.52	260.52	126.68

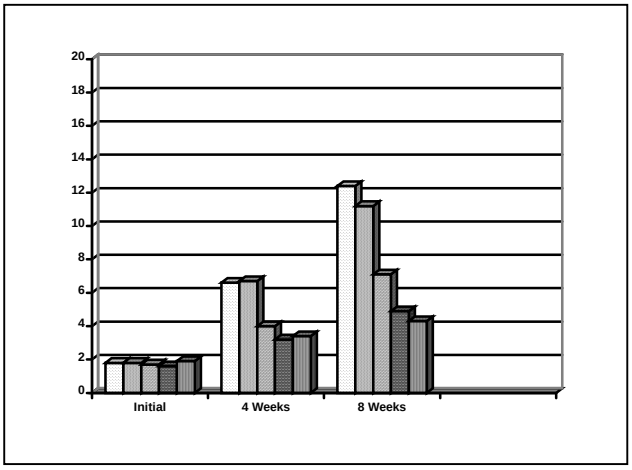

Fig 1. ***The effect of probiotic products on the LDL: HDL ratios of rabbit serum in comparison to control groups after four weeks and eight weeks consumption***

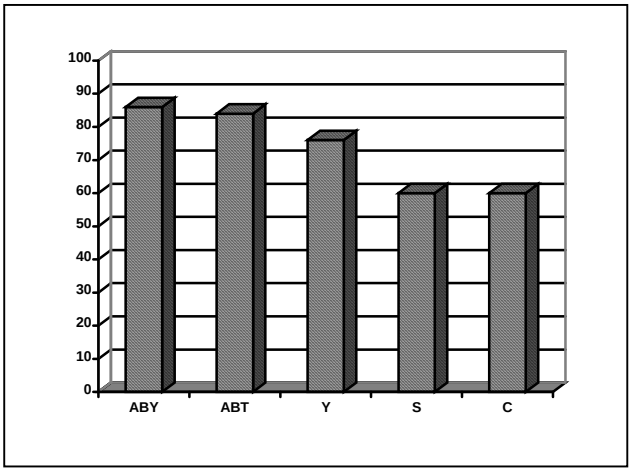

Fig 2. ***Percentage of reduction in the total cholesterol (TC) levels by four weeks of probiotic product consumption.***

ABY: A product contains L53, B3, Strept therm. and L.bulgar.
ABT: A product contains L53, B3 isolates and Strept therm.
Y: The ordinary yogurt bacteria.
S: Standard bacteria La-5, BB-46.
C: Control La-5, BB-46 and Strept thermophilus.

DISCUSSION

The results of this study showed that the consumption of probiotic milk products fermented by the local bacterial isolates L-53 and B3 (ABT and ABY) causes a clear reduction in the levels of serum TC and LDL compared to the consumption of ordinary yogurt which was fermented by L *bulgaricus* and *Strept thermophilus*. This trend of serum TC reduction by probiotic bacteria was in consistence with many other studies[9,10]. Rabbits was chosen in this study because of the high sensitivity of this animal to changes in cholestrol due to the vegetarian nature of rabbit which was proved by Xu et al[8] studies comparing the effect of cholestrol consumption on rabbits and mice. The effect of probiotic bacteria on the invivo levels of cholestrol can be attributed to 4 main reasons: 1) the action of probiotic bacteria on cholestrol enzymatic system in the liver and increase the excreted proportion in the stool, 2) due to the intervention with cholestrol absorption by conjugating cholestrol to lactobacilli bacterial cell wall, 3) induction of the released amount of bile salts after deconjugation by lactobacilli and 4) through the ability of lactobacilli to direct consumption of cholesterol[11]. Also probiotic bacteria are highly efficient in reducing serum cholestrol levels because it can tolerate the gastric high pH and intestinal bile salts, and it can be inhabitant of intestinal lumen, while the ordinary yogurt bacteria can not tolerate such environmental conditions[12]. However, a better reduction in the levels of TC and LDL associated with the consumption of probiotic products for longer period of time, an observation which was reported by other studies[9,12]. Very well known that individuals with increased LDL: HDL ratios at higher risk of acquiring atheroschlerosis and heart disease due to high consumption of cholestrol enriched diets, so the uses of probiotic bacteria fermented milk products reduces the risk of such heart diseases[13,14]. The results of this study in the first experiment showed that HDL values in rabbit serum utilized the products ABT, ABY and Y was significantly increased by 8 weeks of consumption compared to the control groups which indicate the positive effect for the long duration of probiotic products consumption because it provide a high degrees of bacterial colonization of the gut with lactobacilli[15], and due to the ability of lactobacilli to conjugate cholestrol and prevent their absorption[16]. The best reduction percentage in the TC was observed on consumption of ABY product that contain the local isolates L53 and B3 mixed with ordinary yogurt bacteria which support the therapeutic effect of these products in reducing the TC levels. This trend coincide with that reported by Pigeon et al[17], mentioning the ability of this proportional mixing of fermenting bacteria in conjugating the free bile acids and reduce the TC through the deconjugation of bile

salts to provide the free acids bound the lactobacilli cell wall making the probiotic bacteria efficient in reducing the levels of serum cholestrol. However, these mechanisms used by probiotic bacteria for reducing TC supported by the observation of Pereira and Gibson[18] which explained the reduction of serum TC on the basis of 1) the functional action of short chain fatty acids especially propionate formed as final product of milk fermentation, 2) utilization of cholestrol by bacteria, 3) binding of cholestrol to bacterial cell wall and 4) deconjugation of bile salts enzymatically. In general, the results of the two experiments (A & B) showed clearly the therapeutic benefit of probiotic bacteria fermented milk products (exp. A) in the reduction of serum cholestrol levels and the prophylactic effect (exp. B) of probiotic products consumption in maintaining serum lipids at their low levels which has an important benefits in reducing the risk of heart and coronary diseases. These observation are in consistence with Khedhkar et al[19] study which indicate that the support of ordinary yogurt with probiotic bacteria have high beneficial effect in the reduction of cholestrol. Also the consumption of probiotic products that prepared in adequate proportions of bacteria lead to the reduction in the ratios of LDL: HDL with significant effect in reducing heart diseases imposed through hypercholesterolemia[20,21]. These findings are in agreement with our results.

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