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HEALTH BENEFITS OF SOME VIABLE AND NONVIABLE MICROORGANISMS

EL-Dieb, Samia M.; Fawzia H. R. Abd Rabo; Sanaa M. Badran; A. M. Abd El- Fattah and F. M. F. Elshaghabee
Dairy Dept., Fac. Of Agric. Cairo Univ.

ABSTRACT

In Vitro Cholesterol reducing ability, β- galactosidase, antioxidant, antitumor and antibacterial activity of *L. acidophilus* La-5,*L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 were investigated. Results obtained showed that all strains were able to reduce cholesterol and had β-galactosidase activity especially yeast strains and the viable form (free and microencapsulated) of all microbial stains had higher cholesterol reducing ability and β- galactosidase activity than nonviable form. However the nonviable form of all tested microorganisms had the highest antioxidant power compared with the others. The yeast strains had higher antioxidant activity than bacterial strains in the different forms. All strains in different forms exhibited antitumor activity by using potato disc assay. Antibacterial activity was observed in *lactobacilli* and *Bifidobacterium* strains against spoilage and pathogenic bacteria.

INTRODUCTION

A food can be regarded as 'functional' if its health benefit has been shown in the consumption of a normal daily dose of the final product, or an effective dose of the ingredient is used and the impact of the food matrix is known. These foods affect beneficially one or more target functions in the body, beyond adequate nutritional effects in a way that is relevant to either an improved state of health and well being and/ or reduction of risk of disease (Milner, 2000 and Roberfroid, 2000). Dairy foods can be divided into three groups: group1, basic products (milk, fermented milks, cheeses, ice cream, etc.); group2, added-value products, group3, functional dairy products with a proven health benefit from the major part of functional foods. The most common functional dairy products are those with probiotic bacteria in that group (Saxelin *et al.*, 2003).

Probiotic bacteria are live microbial strains that, when applied in adequate doses (10^6 – 10^7), beneficially affect the host animal by improving its intestinal microbial balance (Fooks *et al*.1999 and Ouwehand *et al*., 1999). The consumption of probiotic cultures positively affects the composition of gastrointestinal tract (GIT) microflora and extends a range of host benefits which so far claimed to be: pathogens interference, immune system stimulation and immunomodulation, anticarcinogenic and antimutagenic activities, alleviation of symptoms of lactose intolerance, reduction in serum cholesterol, reduction in blood pressure, decrease incidence and duration of diarrhea, prevention of vaginitis and maintenance of mucosal integrity(Fooks *et al*.,1999 and Ouwehand *et al*.,1999).

The probiotics emphasis the viability of microorganisms. However, in some cases, non-viable bacteria function equally well. These cases include: lactose tolerance lactose- deficient subjects, treatment of acute gastro-enteritis, treatment of candidiasis and binding of mutagens. The use of non-

viable instead of viable microorganisms would have economic advantages in terms of longer shelf-life and reduced requirements for refrigerated storage (Zhang and Ohta, 1991and Ouwehand *et al*.,1998). However, all health benefits reported for using non-viable preparation are often limited.

Therefore, *Lactobacillus acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *Bifidobacterium bifidum* Bb-12, *Kluveromyces lactis* NRRL Y- 8279 and *Saccharomyces cerevisiae* DSMZ 70 449 were selected for assessment of their beneficial effect .

MATERIALS AND METHODS

Lactobacillus acidophilus La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *Bifidobacterium bifidum* Bb-12 were obtained from Chr. Hansens' Laboratories, Copenhagen, Denmark. *Kluveromyces lactis* NRRL Y- 8279 was obtained from Northern Regional Research Laboratory, USA. *Saccharomyces cerevisiae* DSMZ 70 449 was obtained from Deutsche Sammlung von Mikroorganism und Zellkulturen GmbH, German. *Ent*. *aerogenes*, *Bacillus subtilis*, *Ps*. *fluorescence*, and *Staph. aureus* were obtained from Agricultural Microbiology Department, Faculty of Agriculture, Cairo University. *Agromobacterium tumefacium* was obtained from Agriculture Research center, Giza, Egypt.

All microbiological media (MRS, M17, Malt extract and Staph 110) used were obtained from Oxoid Division of Oxiod LTD, London.

Water soluble cholesterol (polyethanyl cholesteryl sebecate), L-cystein hydrochloride, lithium chloride, Sodium tauroglycocholate, lactose were obtained from Sigma Chemical Co., USA. Anaerogen shachets were obtained from Oxoid Ltd., Basingstoke, Hampshire, England. 1, 1. Diphenyl. 2. Picrythyrazyl (DPPH) was obtained from Merck Co., Germany. Sodium alginate was obtained from MIFAD Co., for food industries, Egypt. Cholesterol, glucose, and total antioxidant capacity kits were pursched from Biodiganostc Co. Egypt. Iodine and potassium iodide were obtained from Adwic Co. Egypt.

Millipore filter 0.22 µm was obtained from Whatman Co. USA.

Direct Vat Set (DVS) lactobacilli and bifidobacteria strains were transferred at rate of 2 % into MRS broth supplemented with L-cystein hydrochloride 0.05% (MRS-C) and incubated aerobically for lactobacilli or anaerobically for bifidobacteria at 37 ºC for 18 h. However, lyophilized yeast strains were subcultured two times consequently prior to use in sterile malt extract (ME) broth and incubated aerobically at 37 ºC for 18 h. Indicator microorganisms were transferred at rate of 2 % into nutrient broth for *Ent*. *aerogenes*, *Bacillus subtilius* and *Ps*. *fluorescence* or into staph 110 for *Staph. aureus* and incubated at 37 ºC for 18 h.*A. tumefacium* was transferred at rate of 2 % into nutrient broth supplemented with 0.5 % sucrose and 0.1 % yeast extract and incubated at 25 ºC for 48 h.

The activated cultures (*L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449) were centrifuged at 3000 ×g for 5min at 4˚C then

pellets were harvested (~ 10^8 cfu/g), washed twice and resuspending in normal saline (Mandal *et al*., 2006).

Microorganisms (*L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449) were encapsulated in 3% sodium alginate matrix as described by Sheu and Marshall (1993). Cells were microentrapped by mixing one part culture concentrate with four parts sodium alginate (3%). One part of the mixture was then added dropwise to 5 parts vegetable oil (250 ml in an 800 ml beaker) containing Tween 80 (0.2%), which was stirred at 200 rpm by magnetic stirring. Within 10 min, a uniformly turbid emulsion was obtained with no evidence of a free aqueous phase. Calcium chloride (~500 ml 0.05M) was added quickly but gently (20 ml/sec) down the side of the beaker until the water/oil emulsion was broken. Calcium alginate beads were formed within 10 min. The beads were collected by gentle centrifugation (350 ×g for 10min at 4ºC) and washed with sterile water (~10^8cfu/g).

Nonviable cells were prepared by autoclaving viable cultures (*L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449) at 120˚C / 15 min (Zhang and Ohta, 1991)**.**

For the preparation of the cell free filtrates , the proper media were inoculated with 2 % of the individual cultures (*L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449) and incubated at the proper conditions previously mentioned (culture preparations). The cell free filtrates were obtained by passing the cultures through sterile membrane Millipore filter 0.22 µm pore size (Abd El-Fattah, 1994).

For Examining the cholesterol ability of the examined cultures, freshly MRS and ME broth were supplemented with water soluble cholesterol to a final concentration of 200 mg/100 ml and 0.2% sodium taurocholate. The broth media were inoculated with 2% of culture in free, microencapsulated and non viable cells and incubated aerobically for all cultures except *B. bifidum* Bb-12 was incubated anaerobically at 37 ºC for 24 h; these experiments were carried out at 37 ºC to simulate the conditions of the intestine. After incubation, cells were removed by centrifugation for 7 min at calorimetrically by Bio-diagnostic kit (Allain *et al*., 1974).

To idetect the β- galactosidase activity, all tested microorganisms in free, microencapsulated, and non viable cells were inoculated (2% w/v) in peptone yeast extract broth with lactose (10 g/L) as carbon source, and incubated at 37 ºC for 24 h under aerobic condition for all strains except *B. bifidum* Bb-12 was incubated anaerobically. Cultures were centrifuged at 20.000 ×g for 30 min and the supernatant was used to determine the β. galactosidase activity by monitoring the librated glucose (Rabiu *et al*., 2001). The free amount of glucose was determined by Bio-diagnostic kit (Trinder, 1974) and β- galactosidase activity was calculated according to the equivalent of Dahlqvist (1968) as follows:

Disaccharidase activity/ ml = a.d / n. 1080

a = µl glucose liberated after 24 h incubation.

d = dilution factor for the enzyme solution.

n = number of glucose molecule per molecule of disaccharide (n = 1).

The antioxidant activity of free, microencapsulated and nonviable cells was measured by the following methods:

The ability of the free, microencapsulated and non viable cells to reduce iron (III) was assessed by the method of Oyaiza (1986). A 1 g of free, capsules, and nonviable cells was mixed with 2.5 ml of phosphate buffer (pH 6.6) and 2.5 ml of a 1% aqueous potassium hexacyanoferrate ($K_3Fe(CN)_6$) solution. After 30 min incubation at 50° C, 2.5 ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 4000 rpm for 10 min. A 2.5 ml aliquot of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1 % aquesus $FeCl_3$, and the absorbance was recorded at 700 nm using spectrophotometer type SHIMADZU. Iron (III) reducing activity was determined as ascorbic acid equivalents (mmol ascorbic acid /g).

The 1, 1. Diphenyl. 2. Picrythyrazyl (DPPH) test was carried out as described by Cuendet *et al*. (1997) 50 µg of each form of microorganisms were mixed with 5 ml of a 0.004% methanol solution of DPPH. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm.

For assaying the total antioxidant capacity, free, microencapsulated and non viable cells were homogenated on ice in 1-2 ml of cold buffer (5 mM potassium phosphate, pH 7.4 containg 0.9 % sodium chloride and 0.1 % glucose), then centrifuged at 10.000 ×g for 15 min at 4ºC. The supernatants were used immediately for assay by Bio-diagnostic kit (Koracevic *et al*., 2001).

The antitumor bioassay was carried out by using Crown gall tumorigensis on discs of potato tubers (*Solanum tuberosum* L) was proposed as an ideal system for investigating the antitumor bioassay (Ferrigni *et al*., 1982)**.** Potatoes were washed with running water for 30 min, then sterilized by immersion in sodium hypochlorite (Clorox) for 20 min, a core of the tissue was extracted from each potatoes with a sterilized 1.5 cm cork borer, 2 cm pieces are removed from each end of tuber and discarded, and the reminder of the cylinder is cut into 0.5 cm discs. The discs were transferred to 1.5% agar plates. Each plate contains 5 discs, and 3-5 petri dishes were used for each experimental sample. One gram of each tested microorganisms was mixed with 2 ml of broth culture of *A*. *tumefacium*. 0.05 ml of this mix was inoculated into potato disc. The plates were incubated at room temperature for 12 d. The tumors were counted after staining with Lugol's solution (I_2-KI). The results are expressed as + or – percentages versus the number of tumors on the control discs; inhibition is expressed as a negative percentage and stimulation expressed as a positive percentage. Significant activity is indicated when two or more independent assays give consistent negative value of ca. 20 % or greater inhibition. The mechanism of tumor induction by *A*. *tumefacium* is during the infection process, the bacterium attaches to plant cells and transfers the Ti plasmid into the plant cells genome resulting over production of plant growth regulators (auxins) which are responsible for tumor formation (Kado, 1991).

The antibacterial activity of various cell free filtrates was tested against the four indicator microorganisms (*Staph. aureus*, *B. subtilis*, *Ent. aerogenes* and *Ps. fluorescence*) using the agar well diffusion method of Varadaraj *et al.* (1993). Sterile cell free filtrates (0.1 ml) of *L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 were added to each well and the Petri plates were kept at 4 ◌̊C for 2 h to facilitate diffusion of culture filtrate into the medium. The plates were then incubated at 37◌̊C for 24 h. The diameters of inhibition zones (mm) were measured and the whole diameter was deducted

The values of all experiments are presented as the means of triplicate analysis. Statistical analysis for obtained data was carried out using the Statistical Analysis System (SAS, 1994).

RESULTS AND DISCUSSION

In this study, the health benefits of viable and nonviable *Lactobacillus acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *Bifidobacterium bifidum* Bb-12, *Kluveromyces lactis* NRRL Y- 8279 and *Saccharomyces cerevisiae* DSMZ 70 449 was investigated. As the viable probiotic bacteria are not available or difficult to handle; in some circumstances, it may be more practical to use product with nonviable microorganisms. The use of nonviable instead of viable microorganisms would have economic advantages in terms of longer shelf life and reduced requirements for refrigerated storage (Zhang and Ohta,1991 and Ouwehand *et al*., 1998).

Cholesterol reducing abilities of *L. acidophilus* La-5, *L. casei* - 01, *L. helveticus* Lh. B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 were investigated in vitro. Data obtained show that all these strains were able to reduce cholesterol in the broth system (37ºC / 24h) used in this study, among the strains *K. lactis* and *Sacch. cerevisiae* demonstrated the best cholesterol reducing ability in the broth as shown in Table (1). Cholesterol reduction as detected may be due to the copreciptation by the cells. Another possibility for cholesterol reducing is due to the attachment of cholesterol to the surface of cells. (Tahri *et al.*, 1997; Brashears *et al.*,1998).

Data obtained show that the viable free and microencapsulated form had higher ability to remove cholesterol than the nonviable form and there were no statistically significant differences between the free and microencapsulated forms. Cholesterol removal by *B. bifidum* was higher than that recorded by the other bacterial strains.

In conclusion, these results show the potential of using lactic acid bacteria, bifidobacteria and yeasts as an adjuncts to reduce serum cholesterol levels. Further in vivo study is necessary to prove the hypocholestramic effect of these strains in humans. These health promoting effects make *L. acidophilus* and other lactic acid bacteria desirable to be incorporated into dairy products or other functional foods designed to meet the demands of today's health conscious public.

Table (1) Cholesterol reduction by some microorganisms.

Strains	% Cholseterol removal		
	Free	Caps	NV(*)
***L. acidophilus* La-5**	34.56	34.49	17.28
***L. casei* - 01**	37.40	36.77	19.00
***L. helveticus* LhB02**	31.58	31.35	15.20
***B. bifidum* Bb-12**	39.43	38.89	19.72
***K. lactis* NRRL Y- 8279**	59.93	60.00	29.50
***Sacch. cerevisiae* DSMZ 70449**	59.75	59.72	29.10
$LSD_{0.05}$	1.52 (P <0.05)		

(*) Caps: microencapsulated cells; NV: Nonviable cells

These results are in agreement with those reported by Tahri *et al*. (1997) who reported that *B. longum* BB536, *B. breve* ATCC. 15700 and *B. animalis* ATCC 25527 were able to assimilate cholesterol from TPY medium and Brashears *et al.* (1998) who demonstrated that *L. acidophilus* strains (L1 and ATCC 43121) and *L. casei* strains (N19 and E5) able to assimilate cholesterol from broth MRS medium. Psmos *et al.* (2003) examined the ability of *Sacch. cerevisiae* 832, *Sacch. cerevisiae* KK1 and *Issatchenkia orintalis* KK5.Y.1 to assimilate cholesterol from their growth media. They showed that *Saccharomyces* strains were able to remove cholesterol without degradation after 24 h of growth at 37ºC.

Results in Fig. 1. show that the β- gal activity ranged from 0.22-0.33, 0.21 – 0.32 and 0.1 – 0.2 unit / ml for free, microencapsulated and nonviable forms respectively. The β- gal activity values of free form were the highest and there were significant differences between it and those of non viable form.

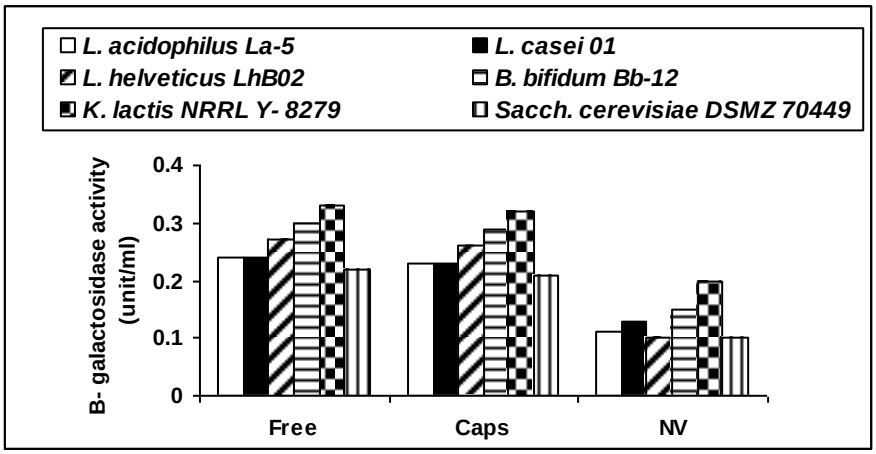

Fig. 1. β-galactosidase activity of some microorganisms. (Free: Free cells; Caps, Microencapsulated cells; NV: Nonviable cells).

The obtained results are nearly similar to those reported by Lin *et al*. (1991), they mentioned that the β-gal activity of five strains of *L. acidophilus* was less than 0.2 unit/ ml and the β-gal activity of six strains of *L. acidophilus* was lower than those obtained with yoghurt culture.

They also observed that consumption of non fermented dairy products containing *L. acidophilus* LA-1, LA-2 and NCFM and yoghurt culture (10^7 – 10^8 cfu/ml) may enhance lactose digestion in lactose maldigestion subjects. Montes *et al.* (1995) showed that milk inoculated with *L. acidophilus* NCFM and yogurt culture may enhance lactose maldigestion by production microbial β- galactosidase.

ata obtained in (Table 2) showed that the nonviable form of all tested microorganisms had the highest antioxidant power compared with the others and there were non significant differences between free and microencapsulated forms of each strain. Data in the same Table also show that the yeast strains had higher antioxidant activity than bacterial strains in the different forms. These results are in agreement with those reported by korpela *et al*. (1997**)** they showed that lactic acid bacteria are able to degrade the superoxide amino and hydrogen peroxide. In the same trend, kullisaar *et al*., (2002) showed that *Lactobacillus fermentum* E.3 and E.18 (10^7 cfu/ml) exhibited antioxidative and antitherogenic effects. Also Songisepp *et al*. (2004) showed that semi soft cheese Pikantne served as a suitable carrier of antimicrobial and antioxidative *L. fermentum* ME. 3. Saidet and Gilliland (2005) recommended that *L. delbrueckii spp. bulgaricus, L delbrueckii spp. lactis, L. acidophilus* and *L. casei* cultures can provide a source of dietary antioxidants.

Table 2. Antioxidant activity of some microorganisms.

Strains	Cell forms	TA(*)	ARP(**)	DPPH (***) scavenging
***L. acidophilus* La-5**	Free	0.15	0.90	32.12
	Caps	0.16	0.91	32.10
	NV	0.42	1.30	32.90
***L. casei* 01**	Free	0.21	1.08	34.52
	Caps	0.22	0.95	34.62
	NV	0.52	1.35	35.00
***L. helveticus* LhB02**	Free	0.21	0.95	33.00
	Caps	0.22	0.94	33.01
	NV	0.45	1.35	33.31
***B. bifidum* Bb-12**	Free	0.52	1.20	36.10
	Caps	0.51	1.11	36.00
	NV	0.75	1.52	36.40
***K. lactis* NRRL Y- 8279**	Free	0.73	1.47	37.26
	Caps	0.72	1.48	37.26
	NV	0.94	1.95	38.35
***Sacch. cerevisiae* DSMZ 70449**	Free	0.6	1.48	36.30
	Caps	0.61	1.40	36.30
	NV	0.82	1.83	37.00
$LSD_{0.05}$ (P <0.05)		0.12	0.3	0.55

(*) TA: Total antioxidant activity, (**) ARP: Anti reducing power, (***) DPPH scavenging: Diphenyl. 2. Picrythyrazyl scavenging.

As can be observed from the data presented in Figs (2, 3 & 4) the *L. acidophilus* La-5, *L. casei* -01, *helveticus* Lh. B O2, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 and *Sacch. cerevisiae* DSMZ 70 449 in free, microencapsulated and nonviable forms had antitumor activity where potato discs had blue color after stained by I_2KI. In this respect, .Hosono *et al.* (1990) found that from 40 strains of LAB examined, *Leuconostoc paramesenteroids*, *S. lactis*, *S. cremoris*, *Lactobacillus spp.* and *Bifidobacterium spp*. had antimutagenic activity toward chemical mutagens. Also, Tavan *et al.* (2002**)** showed that some LAB had antimutagenic activity against food mutagens. As for bacterial cells either killed thermally or nonthermally Orrhage *et al.* (1994) found that the nonviable cells bind mutagens with equal efficiency to viable cells and the efficiency to viable cells and the efficiency of binding may depend on the chemical structure of the cell wall. Another report by Sreekumar and Hosono (1998) show that polysaccharides components of the cell walls are essential for binding, more so than the peptidoglycans. Ouwehand and Salminen (1998) reported that viable and nonviable probiotics may enhance the immune system especially tumour necrosis factors (TNF) which play an important role to suppress tumour cells.

The potential control of intestinal pathogens by *Lactobacillus*, *Bifidobacterium* and two yeast strains with probiotic properties is a valuable feature for considering their application in functional food development. Some strains have been shown to be both preventative and therapeutic in controlling intestinal infections.

In the present study, the inhibitory activity of cell free filtrate of some LAB, *Bifidobacterium* and yeast strains toward some spoilage and pathogenic bacteria are presented in Table (3). The antibacterial activities of all strains were variable. *L. casei* – 01 showed the highest antibacterial activity towards *B. subtilis* and *Ent. aerogenes*, while *L. acidophilus* showed the highest antibacterial towards *Staph. aureus* and *Ps. fluorescence*. On other hand, results obtained indicated that, *L. helveticus* had low antimicrobial activity toward the indicator microorganisms especially *Staph. aureus*, *Ent. aerogenes* and *Ps. fluorescence*. Also as shown in Table (3) no inhibition zone was detected using culture filtrate of yeast strains.

Table 3. Antibacterial activity of cell free filtrates of some microorganisms.

Strains	Indicator organisms Diameter of inhibition Zone (mm)(*)			
	Staph. aureus	***B. subtilis***	***Ent. aerogenes***	***Ps. fluorescence***
***L. acidophilus* La-5**	13	13.5	12	13
***L. casei* – 01**	12	14	15	12
***L. helveticus* Lh. B 02**	11	12	11	11
***B. bifidum* Bb-12**	11	14	12	11
***K. lactis* NRRL Y- 8279**	0	0	0	0
***Sacch.cerevisiae* DSMZ70 449**	0	0	0	0
$LSD_{0.05}$	0.64 (P <0.05)			

(*) Initial zone diameter is 5 mm

Fig تصـــــــــــــــــغير فـــــــــــــــــى المطبعـــــــــــــــــة

These results are in agreement with those obtained by Gilliland and Speck (1977) who found that *L*. *acidophilus* exerted antagonistic actions on growth of *Staph*. *aureus* and enteropathogenic *E*. *coli*. Misra and Kulla (1991) reported that *Bifidobacterium spp*. exhibited antibacterial activities against pathogenic bacteria. Kimoto *et al*. (2000) reported that LAB had inhibitory effect, they produce lactic acid, hydrogen peroxide and bacteriocins or combination of two or more of these factors. Abd El- Salam *et al*. (2004) showed the ability of cell free filtrate of nine strains of lactobacilli to suppress the growth of some harmful bacteria (*E*. *coli*, *Staph*. *aureus*, *Ps. aeurogenosa* and *B*. *cereus*), they indicated that *L. johnsonii* ATCC 33200 and *L. acidophilus* TISTR 450 were able to suppress all the above mentioned indicator bacteria.

Conclusion

It could be concluded that the viable form of *L. casei* -01 and *B. bifidum* Bb-12 and nonviable form of *K. lactis* NRRL Y- 8279 had highest beneficial effects between all investigated microorganisms so they were selected for preparation of bio-yoghurt in next study.

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الفوائد الصحية لبعض الميكروبات الحية والغيرحية.

سامية محمود الديب، فوزية حسن رجب عبدربه, سناء محمد بدران , علاء محمد عبد الفتاح و فؤاد محمود فؤاد الشغبي.

قسم علوم و تكنولوجيا الالبان- كلية الزراعة- جامعة القاهرة.

يهدف هذا البحث إلي دراسة الفوائد الصحية لكل من *L. acidophilus* La-5, *L. casei* 01, *L. helveticus* Lh . B 02, *B. bifidum* Bb-12, *K. lactis* NRRL Y- 8279 , *Sacch. cerevisiae* DSMZ 70 449 و قد شمل ذلك دراسة قدرة هذة الميكروبات علي خفض الكوليسترول و نشاط \انزيم البيتا جلاكنوسيدايز و النشاط المضاد للأكسدة و تكوين الأورام و المضاد للبكتريا و ذلك بأستخدام أختبارات معملية و كانت أهم النتائج المتحصل عليها مايلي:

كان لكل السلالات المختبرة القدرة على خفض الكوليسترول وخاصة الخميرة. وجد أن نشاط إنزيم بيتاجلاكتوسيدايز كان أعلي في الصورة الحية عن الصورة الغير الحية لكل السلالات. كان للصورة الغير حية لكل الميكروبات المختبرة نشاطاً مضاد للأكسدة أعلى من الصورة الحية. أظهرت كل السلالات المختبرة في الصور المختلفة نشاطاً مضاداً لتكوين الأورام. أظهرت بكتريا *Lactobacilli,Bifidobacterium* نشاطاً مضاداً لبكتريا الفساد والبكتريا الممرضة.