British Microbiology Research Journal
4(6): 678-697, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Possibility of Using Fibers as a Prebiotic in Making of Probiotic Based On-Some Dairy Products

Gehan H. Bisar[1], Kh. El-Saadany[2], A. Khattab[2] and Wedad M. El Kholy[1*]

[1]Dairy Research Department, Food Technology Research Institute, A.R.C., Egypt.
[2]Dairy Science and Technology Department, Faculty of Agriculture, El–Shatby, Alexandria University, Egypt.

Authors' contributions

All authors read and approved the final manuscript.

Original Research Article

Received 22nd December 2013
Accepted 20th February 2014
Published 12th March 2014

ABSTRACT

Probiotic microorganisms were found to affect the host health beneficially when found in an a certain count not less than 106 CFU/g (Colony-forming unit / gram), and they have some benefits as protection from cancer, relief of lactose intolerance, reduce the risk from diarrhea, normalize the bowel movement, and enhance the immune functions, reduce cholesterol level and reduce the risk of eczema. This study was carried out to examine some of fibers and polysaccharide for their assimilation by some lactic acid bacterial strain specially known for their probiotic effect. It was concluded from the present study the following: studying the capability of *Esherichia coli* (*E. coli*), bifidobacteria and 9 strains related to lactic acid bacteria included in assimilating 7 different (polysaccharides, fibers and other materials) included (Polydextrose, Maltodextrine, inulin, prolia, resistant starch, wheat fiber and gumarabic) when substituted with dextrose inde Man, Rogosa and Sharpe (MRS) broth and incubated at their optimal temperature. The results revealed that the examined culture were varied in their assimilation of the 2% polysaccharides tested, furthermore maltodextrin, showed a good assimilation by *Bifidobacterium longum* ATCC 15707 (*B. longum*) and *Lactobacillus acidophilus* NRRLB1910 (*L. acidophilus*). The effect of certain concentrations (2, 3and 4%) of the selected (polysaccharides, fibers and other materials) on the growth activity of the lactic acid bacterial cultures tested in addition to *E. coli* (as a representative for coliform bacteria). The results revealed that upon increasing

**Corresponding author: Email: wedad_elkholy1970@yahoo.com;*

the concentration of the selected polysaccharides there was a remarkable decrease in pH compared to *E. coli* which showed contrast outcomes on which its pH were significantly higher than the tested bifidobacteria and lactic acid bacteria. Studying the effect of incubation duration and it's relation on the selected (polysaccharides, fibers and other materials) assimilation by the tested lactic acid bacterial cultures. Results revealed that there is a direct proportion relation between long incubation timing and polysaccharide assimilation (indicated by decrease in pH). This decrease was very clear at 24 hours of incubation at the optimum temperature for each strain. Upon studying the antagonistic effect between *E. coli* with *B. longum* ATCC 15707, *L. acidophilus* NRRLB 1910 and *Lactobacillus reuteri* B 14171 (*L. reuteri)* grown on modified MRS with 3% of each polysaccharide (polydextrose, maltodextrin and inulin). The change in the growth of these cultures combinations were determined by counting on MRS and Violet red bile agar (VRBA). It was shown that these (polysaccharides/fibers) challenged the growth of the probiotic bacteria and the count of *E. coli* (wild) E.W was lowered significantly due to the inhabitation effect of the used probiotic bacteria. It was concluded that good results was shown from using the three polysaccharides/fibers (maltodextrin, inulin andpolydextrose) that was elected to base the rest of work on.

Keywords: Probiotic; lactic acid bacterial; fibers; polysaccharide.

ABBREVIATIONS

B. longum	*Bifidobacterium longum*
E. coli	*Esherichia coli*
L. acidophilus	*Lactobacillus acidophilus*
L. plantarum	*Lactobacillus plantarum*
L. rhamnosus	*Lactobacillus rhamnosus*
L.paracasei	*Lactobacillus paracasei*
L. reuteri	*Lactobacillus reuteri*
L. lactissubsp. lactis	*Lactococcus lactissubsp lactis*
S. thermophilus	*Streptococcus thermophilus*
CFU/ml	*Colony-forming unit / milliliter*
CFU/g	*Colony-forming unit /gram*
DP	*Degree of polymerization*
NDOs	*Non-digestible oligosaccharides*
VRBA	*Violet red bile agar*
MRS	*de Man, Rogosa and Sharpe*
FOS	*Fructo oligosaccharides*
GOS	*Galacto oligosaccharides*
TOS	*Trans galacto oligosaccharides*
cP	*Centipoise*
°C	*Degree Celsius*

1. INTRODUCTION

The word probiotic comes from the Greek word "for life" and is defined as "a live microbial food supplement that is beneficial to host health" [1]. The definition of probiotics has evolved over the years but the consensus designates probiotics as "nonpathogenic, live microbial, mono- or mixed-culture preparations, which, when applied to humans or animals in high

enough doses, beneficially affect the host by improving the intestinal microbial balance and its properties" [2].

The most widely used bacteria as probiotics are the lactobacilli and bifidobacteria that have been applied in traditional fermented food products such as yoghurt, sauerkraut and kefir. The recent explosion of probiotic-containing food stuffs incorporating a wide variety of different strains [3].

On the other hand prebiotics are food additives whose favorable effect on the organism is associated with the stimulation of growth and activity of some strains of the native micro-flora or micro-flora introduced with the ingested food [4]. A prebiotic is defined as «a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon» [14]. Prebiotics can be fermented by certain micro-organisms e.g. *Bifidobacterium* and *Lactobacillus* belong to the most common species used as probiotics in the human diet [5].

Oligosaccharids and polysaccharides are a group of short chain non-digestible polysaccharides that occur naturally in some foods. They are typically defined as glycosides that contain between 3 to 10 sugar moieties and are characterized by the type and sequence of the monosaccharide moieties present. They may be linear or branched [6]. Initially, oligosaccharides were developed as sucrose substitutes and for use as bulking agents in foods [6]. Later, it was determined that certain oligosaccharides had the potential to increase bifidobacteria in the colon without being utilized by other intestinal bacteria [7,8]. Because of their prebiotic properties, oligosaccharides have received much recent attention as functional food ingredients [9]. In addition, research has been promoted into the ability of oligosaccharides to provide beneficial changes in the composition and metabolism of the colonic micro-flora [10].

Currently, there are several types of oligosaccharides commercially produced, all claimed to be bifidogenic by the manufacturers [6]. Fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and trans-galacto-oligosaccharides, TOS) and soybean oligosaccharides (main functional components being raffinose and stachyose) have been most extensively studied and may provide the best evidence of prebiotic effects in humans [9,6]. Inulin is used primarily as a fat substitute [11]. Similar to other oligosaccharides, inulin is not hydrolyzed or absorbed in the small intestine and has been shown to reach the colon mostly intact and is thought to act as a prebiotic [12,13,14]. Inulin is heterogeneous with respect to polymer chain length. Its degree of polymerization (DP) ranges from 3 to 60 but it primarily consists of DP 20-25 [15].

Several food additives are used in preparation of certain kinds of dairy products as to improve texture, fat feeling, lowering syneresis as polydextrose, arabic gum, resistant starch, soy bean fiber, maltodextrin and inulin [16,17,18] beside their synbiotic effect. Aim of study stimulation of some (polysaccharides, fibers and tested materials) by bifidobacteria and some strains related to lactic acid bacteria including those that have a probiotic effect in comparison with *Echerichia coli* as a preservative for coliform bacteria.

2. MATERIALS AND METHODS

2.1 Tested Cultures

Streptococcus thermophiles (Texl5842) (*S. thermophiles*), *Lactobacillus plantarum* (B813) (*L. plantarum*), *Lactobacillus reuteri (*B 14171) (*L. reuteri*), *Lactococcus lactis* subsp. *Lactis* (ATCC 53214) (*L. lactis* subsp. *lactis)*, *Bifidobacteriumlongum* (ATCC 15707) (*B. longum*), *Lactobacillus rhamnasus* (NRRLB 1934) (*L. rhamnosus)*, *Lactobacillus casei* (NRRLB 1922) (*L. casei*), *Lactobacillus acidophilus (*NRRLB 2092) (*L. acidophilus*), *Lactobacillus paracase i*(NRRLB 4562) (*L. paracasei)*, *Lactobacillus acidophilus (*NRRLB 1910), *Esherichia coli* (wild E.W). They were obtained from cultures store of Dairy Science and Technology department, Faculty of Agriculture, Alexandria University.

2.2 Polysaccharides and Fibers

2.2.1 Polydextrose

Polydextrose is a randomly bonded condensation polymer of D-glucose with some bound sorbitol and a suitable acid with a molecular weight of 2200 was obtained from MENGZHOU TAILIJIE CO., LTD in town China. It has a white to cream white color, odorless and slightly sweet, soluble in water pH range from 3.5-7.

2.2.2 Maltodextrine

Maltodextrine C*Dry MD 01915was obtained from Cargill company, town France. It is a Spray-dried product obtained by enzymatic conversion of starch, with 6% moisture, DE 16, soluble in water, bulk density 440 g/l, pH 3.5 and Granulometry< 63 µm 35% .

2.2.3 Inulin

Long chain inulin was obtained from Fenchem Biotek LTD town China. It's botanical source Jerusalem Artichoke, used part is the root, having a white color, carbohydrate 99.6% min density 500 g/l, ash 0.5%,water 5%, good solubility in water and pH range from 5-7.

2.2.4 Soy flour

Prolia 68237 (Soy flour) was obtained from Cargill company in town France. With a 52% protein, fat 0.7%, moisture 5%, fiber 3.5%, having a white yellow color.

2.2.5 Wheat fibers

Wheat fibers (Sancel wheat 200) was obtained from CFF Company, town Germany. (Sancel wheat 200) is a dietary fiber obtained from fiber-rich parts of the wheat plant. The vegetable parts are cleaned, purified and milled in several steps. The final product appears as a white powder and is neutral in taste and odor. It's Fiber content (AOAC) more than 96%, pH range from 5-8,bulk density higher than 65 g/l, water binding capacity more than 7,slightly soluble in water, oil absorption more than 5and screen analysis bigger than 32 µm is maximum 85%

2.2.6 Gum arabic

Gum Arabic (Fiber gum P) was obtained from CNI Company, town Germany. Fiber gum is a natural product obtained from selected acacia gum with no chemical or enzymatic conversion, it has a 10% moisture, white colored, ash 4%, pH 4.1-5, viscosity 100 mPa.s, mesh size through a 75 µm 15% it show a good solubility in water .

2.2.7 Resistantstarch

Resistant starch (C*Tex 06210) was obtained from Cargill Company, town France. It is a resistant acetylated distarch adipate, with a 5% moisture, pH 5.5,ash 0.2%, bulk density 800 g/l , soluble upon heating at 40°C in water but when heating it forms a gel and a having a white color.

2.3 Lactic Acid Bacteria Count

De Man Rogosa and Sharpe agar medium (MRS) [19] was used for enumeration of lactic acid cultures, the plates were incubated at 37°C for 48 hours and then the cfu/ml (Colony-forming unit / milliliter) were counted.

MRS agar ingredients per liter final pH 6.2+/0.2 at 25°C	
Components of MRS media	**Amount in gm/ml**
Peptone	10 gm
Beef extract	10 gm
Yeast extract	5 gm
Dextrose	20 gm
Tween 80	1 ml
Di potassium hydrogen phosphate	2 gm
Sodium acetate .3 H_2O	5 gm
Di ammonium citrate	2 gm
Magnisimsulphate .7 H_2O	0.1 gm
Manganese sulphate .4 H_2O	0.05 gm
Agar	15 gm

2.4 *Esherichia coli* Count

Violet red bile agar (VRBA) was used for enumerating coliform bacteria as described by [20] the plates were incubated at 37°C for 24 hours then the $\log^{10}$cfu/ml were determined.

2.5 Examining the Tested Culture for their Assimilation of Polysaccharides in Comparing with Dextrose

Modified MRS broth was prepared by substituting each type of polysaccharides instead of dextrose, pH was adjusted to 6.2 and filled in test tubes then autoclaved at 121°C for 15 min. Each kind of (polysaccharides, fiber and other tested material) were inoculated in duplicate by each of the tested culture then incubated at the optimum temperature of each 32°C for lactobacillus strains, 37°C for *E. coli, B. longum* and *S. thermophilus*. The changes in pH was measured using pH meter 3310 Jenway, Germany, after 24 hours of incubation.

2.6 Studying the Effect of Different Concentration of the Polydextrose, Inulin and Maltodextrinon the Growth and Activity of the Tested Cultures

Lactic acid bacterial cultures were inoculated in modified MRS broth with 2,3 or 4% concentrations of (polydextrose, inulin ormaltodextrin), instead of dextrose then incubated at 37°C for 24 hours. The changes in pH were determined as indicator for assimilation and growth activity of the tested culture.

2.7 Studying the Effect of Several incubation Time on the Assimilation of the Selected Polysaccharides by Bifido Bacteria and some Strains Related to Lactic Acid Cultures

Lactic acid culture were inoculated in modified MRS broth with 3% of (polydextrose, inulin and maltodextrin) and incubated at 37°C. The pH was determined at different intervals (zero, 2, 4, 8, 24 and 48) hours.

2.8 Studying the Antagonistic Effect between the Lactic Acid Cultures and *Esherichia coli* in the Presence of 3% Concentration of Selected Polysaccharide and Bifido Bacteria

Modified MRS broth was prepared by substituting the selected polysaccharide instead of using dextrose, then distributed in test tubes and inoculated individually by lactic acid cultures with or without *E. coli* the changes in the growth were determined by plate counting on MRS and VRBA.

2.9 Statistical Analysis

The experimental design was a factorial experiment in a completely randomized design-test, with two replications and analysis of variance of treatments difference was performed according to [21]. Statistical analysis was done by, ANOVA, F-test and L.S.D procedures available within the SAS software package **(**version 9.13, 2008).

3. RESULTS AND DISCUSSION

3.1 Testing the Lactic Acid Cultures for their Assimilation of Polysaccharides in Comparing with Dextrose

The outcomes in Table 1 and Fig. 1 showed that no significant differences between the assimilation of maltodextrin and dextrose by the tested strains except for *L. lactis* subsp. *Lactis* ATCC 53214 and *L. Rhmnosus* NRRLB 1934 where the pH values of the MRS broth for maltodextrin was ranged between 5.30-4.26, while the corresponding pH values of dextrose ranged between 5.21-4.35.

Table 1. Changes in pH in modified MRS media substituted with certain polysaccharides instead of dextrose inoculated with lactobacillus strains and *Bifido bacterium longum* bacteria for 24 hours

Culture	Dextrose	Maltodextrin	Inulin	Starch	Soy flour (proli)	Gum arabic	Wheat Fiber	Polydextrose	Mean
L. reuteri B 14171	5.21^{e§} ±0.13	5.25^{e}± 0.08	5.66^{de}±0.01	6.61^{a}±0.02	6.10^{ab}±0.06	6.49^{ab}±0.09	6.20^{ab}±0.09	5.73^{de}±0.11	5.90^{a}
L. plantarum B813	4.84^{c}±0.03	4.85^{c}± 0.05	5.02^{c} ±0.14	6.10^{a}±0.19	5.82^{ab}±0.19	5.31^{b}±0.18	6.27^{a}±0.07	5.04^{c}±0.10	5.40^{b}
B. longumATCC 15707	4.46^{c}±0.12	4.59^{c}± 0.15	5.30^{b} ±0.13	5.48^{ab}±0.06	5.57^{ab}±0.06	5.25^{b}±0.06	5.23^{b} ±0.06	5.16^{b}±0.06	5.13^{d}
L. lactis subsp. *lactis ATCC 53214*	4.35^{e}±0.09	5.30^{cd}± 0.09	5.26^{cd} ±0.13	5.83^{ab}±0.04	5.71^{ab}±0.04	5.83^{ab}±0.04	6.21^{a} ±0.07	5.14^{d}±0.04	5.45^{b}
L. acidophilus NRRLB 2092	4.76^{e}±0.04	4.85^{de}± 0.04	5.04^{cde}±0.09	6.55^{a}±0.10	5.54^{b}±0.15	5.32^{b}±0.07	6.05^{a} ±0.10	5.26^{cde}±0.07	5.42^{b}
L. acidophilus NRRLB 1910	4.64^{de}±0.09	4.58^{e}± 0.09	5.27^{c} ±0.11	6.11^{a}±0.09	5.56^{ab}±0.16	5.23^{c} ±0.07	5.51^{ab}±0.16	5.25^{c}±0.07	5.26^{c}
L. rhamnosus NRRLB 1934	4.96^{b}±0.09	4.26^{c}± 0.09	5.00^{b} ±0.09	5.96^{a}±0.11	6.13^{a} ±0.13	5.22 ^{b}±0.04	6.065^{a}±0.13	5.02^{b}±0.09	5.32^{b} c
L. paracasei NRRLB 4562	4.70^{cd}±0.09	4.42^{d}± 0.09	5.04^{c} ±0.09	6.21^{a}±0.10	5.19^{b}±0.05	5.70^{ab}±0.10	6.20^{a}±0.09	5.10^{c}±0.05	5.32^{b} c
L. casei NRRLB 1922	4.75^{de}±0.12	4.44^{e}± 0.12	5.32^{cd} ±0.16	6.04^{ab}±0.06	5.58^{b}± 0.15	6.38^{a}±0.06	5.45^{b}±0.15	5.25^{cd}±0.03	5.40^{b}
Mean	4.74^{e}	4.72^{e}	5.21^{d}	6.09^{a}	5.68^{c}	5.63^{c}	5.91^{b}	5.22^{d}	

*$^{\$}$Means (± standard deviation) followed by the same letter(s) are not significant, but different letters are significant according to LSD procedure, at0.01 level of probability. Means followed by the same upper case letter(s) are not significant, but different letters are significant according to LSD procedure where α = 0.01 , the LSD for culture *fiber = 0.7137, LSD for fiber = 0.17 and LSD for culture = 0.135 levels of producer.*

§comparison between different fibers/media within the same bacterial culture

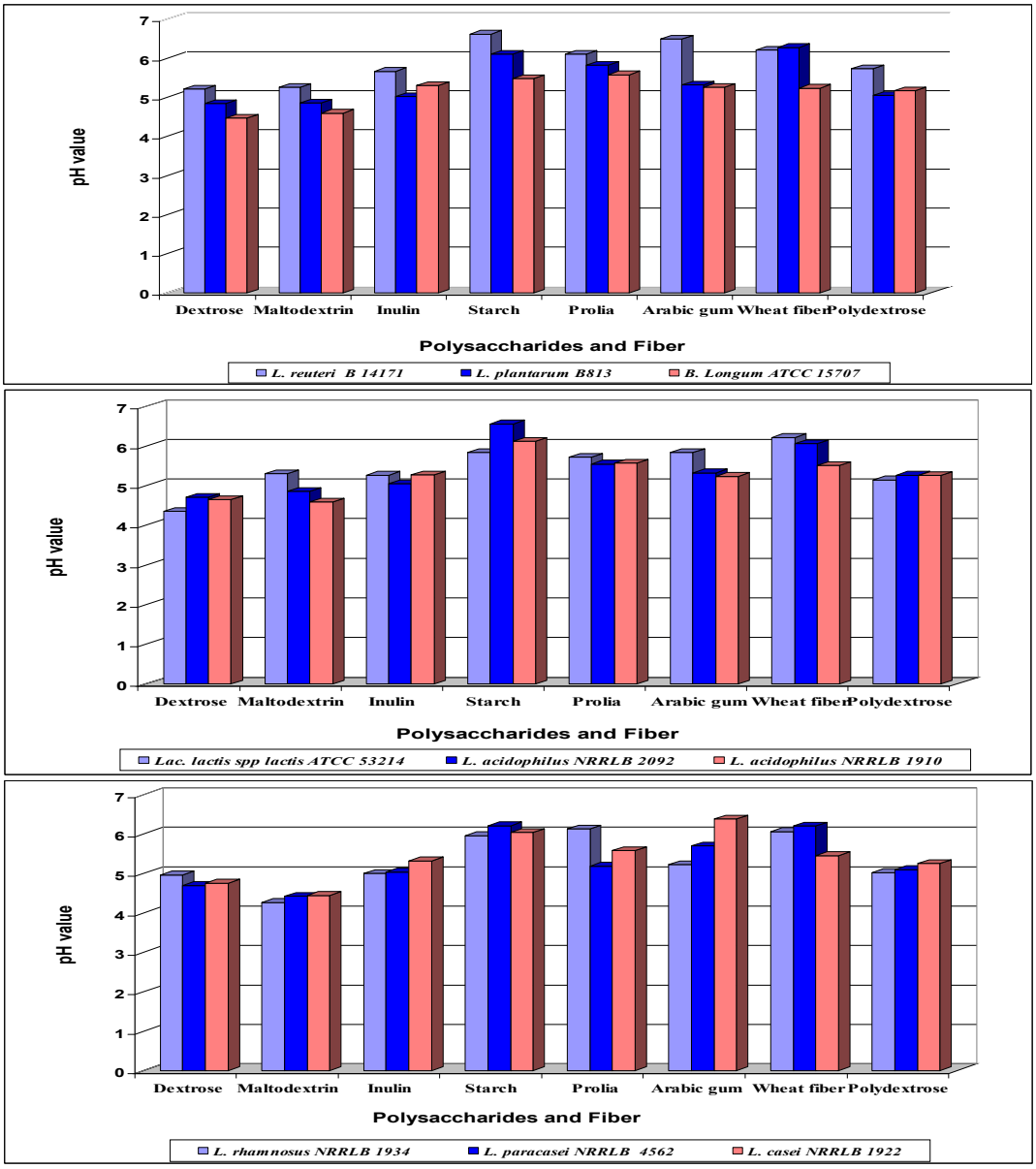

Fig. 1. Changes in pH in modified MRS medium substituted with certain polysaccharides instead of dextrose inoculated with the tested strains of lactic acid and bifidobacteria for 24 hr

On the other hand the results reveal an insignificant difference between inulin and polydextrose, those polysaccharides are significantly lower than the maltodextrin and higher than the rest of polysaccharides in being assimilated by lactic acid cultures where it's pH values after 24 hours was ranging from (5.66- 5.02) and for polydextrose it was ranged from (5.02-5.73).

Furthermore it was noticed that the assimilation of gum arabic and prolia (soy flour) whereas they are significantly lower in their assimilation than the dextrose (measured in pH), maltodextrin, inulin and polydextrose and significantly higher than the (resistant starch and wheat fiber), on which a low assimilation activity was noticed for gum arabic by *L. reuteri B* 14171, *L. lactis* subsp. *lactis* ATCC53214, *L. paracasei* NRRLB4562 and *L. casei*NRRLB1922, for soy flour low assimilation was noticed in *L. reutri* B14171, *L. acidophilus* B4495, *B. longum*ATCC15707, *L. lactis* subsp*. lactis* ATCC53214 and *L. plantarum* B*813*. The pH for both (gum arabic and prolia (soy flour)) was ranged between (6.49-5.22) for the former and (6.13-5.19) for the latter ones. Concerning the wheat fiber and resistant starch they were shown to be hardly assimilated by the lactic acid cultures with a mean 5.91 and 6.09 respectively.

According to the previous results, it would be concluded that the maltodextrin was highly assimilated by the lactic acid cultures same as dextrose on which no significant difference between both following to it inulin and polydextrose that were having the same assimilation rate as both were showing no significant difference between themselves.

On the other hand, the polysaccharides, fibers and other tested materials, were varied in assimilation by lactic cultures specially maltodextrin, polydextrose and inulin while *B. longum* was shown to assimilate these polysaccharides and that's why it was selected to continue our work. Similar finding were reported by [22] who concluded that polydextrose lowered the pH. More than the control samples in fermented milk. Also same results of inulin were highlighted by [23] who mentioned that the rate of pH decrease of fermented milk products was increased by addition of inulin. [24]as well reported that maltodextrinhad better resistance when compared with inulin as examined in the oat gruel environment (simple stomach based model system) with a 4% concentration, [25] reported that Soybean oligosaccharides were fermented to a far greater degree by bifidobacteria than other organisms tested. Also, [26] studied that gum Arabic establishes prebiotic functionality they report significant increase in bifidobacteria and lactobacilli sp on which it bears a prebiotic efficiency like the inulin as established via quantitative development of bacteria in stool samples.

3.2 The Effect of Different Concentration of Polydextrose, Inulin and Maltodextrinon the Growth Activity of the Tested Culture Cultivated in MRS media

The purpose of this study was to investigate whether or not the tested polysaccharides have positive effect on the growth of lactic acid cultures when substituted in MRS broth instead of dextrose in comparison with *E. coli* as a representative for coliform bacteria. Taking in account the data in Table 2 illustrated in Fig. 2, the results revealed that increasing the concentration of the polysaccharide in the modified MRS broth resulted in a significant decrease in the pH of the broth media of all the tested lactic acid cultures due to enhancing the growth of the cultures.

Table 2. Effect of different concentration of polysaccharides in modified MRS broth tested on the progress of pH values of MRS after 24 hours of incubation at optimum temperature of each strain

		pH value of modified MRS with polysaccharides at different concentrations			Mean	
		2%	**3%**	**4%**	**Culture**	**Fiber**
E. coli (wild) E.W	Maltodextrin	5.80±0.007	5.62±0.021	5.64±0.056	6.04[a]	5.68[c]
	Inulin	6.57±0.007	5.90±0.028	5.86±0.035		6.11[b]
	Polydextrose	6.50±0.007	6.22±0.007	6.25±0.042		6.32[a]
L. reuteri B 14171	Maltodextrin	5.66±0.042	4.91±0.007	4.97±0.014	5.17[b]	5.18[c]
	Inulin	6.40±0.014	5.79±0.007	5.62±0.035		5.93[b]
	Polydextrose	6.23±0.021	5.89±0.007	5.84±0.035		5.98[a]
L. plantarum B813	Maltodextrin	4.66±0.007	4.32±0.014	4.03±0.049	4.83[e]	4.33[c]
	Inulin	5.23±0.021	4.86±0.070	4.74±0.028		4.94[b]
	Polydextrose	5.37±0.007	5.15±0.070	5.05±0.070		5.19[a]
*L.lactis*subsp.*lactis*ATCC 53214	Maltodextrin	5.20±0.028	4.73±0.042	4.47±0.021	5.00[c]	4.80[c]
	Inulin	5.12±0.014	4.90±0.007	4.63±0.212		4.88[b]
	Polydextrose	5.31±0.021	5.33±0.049	5.14±0.056		5.26[a]
L. rhamnosus NRRLB 1934	Maltodextrin	5.43±0.007	4.95±0.007	4.71±0.014	5.04[c]	5.03[b]
	Inulin	5.12±0.000	4.85±0.042	4.60±0.007		4.85[c]
	Polydextrose	5.45±0.021	5.05±0.070	5.23±0.063		5.24[a]
L. acidophilus NRRLB 2092	Maltodextrin	4.51±0.021	4.55±0.007	4.43±0.028	4.91[d]	4.49[c]
	Inulin	5.16±0.056	4.82±0.035	4.61±0.007		4.86[b]
	Polydextrose	5.45±0.021	5.33±0.049	5.16±0.028		5.31[a]
L. paracasei NRRLB 4562	Maltodextrin	4.71±0.014	4.50±0.007	4.53±0.021	5.00[c]	4.58[c]
	Inulin	5.05±0.070	4.85±0.007	4.65±0.028		4.85[b]
	Polydextrose	5.31±0.021	5.72±0.035	5.58±0.021		5.53[a]
L. casei NRRLB 1922	Maltodextrin	4.56±0.120	4.30±0.007	4.32±0.028	4.21[g]	4.39[c]
	Inulin	5.12±0.000	4.73±0.028	4.62±0.014		4.82[b]
	Polydextrose	5.26±0.021	4.95±0.070	4.88±0.014		5.03[a]
L. acidophilus NRRLB 1910	Maltodextrin	4.67±0.028	4.22±0.014	4.10±0.007	4.90[d]	4.33[c]
	Inulin	5.32±0.035	5.10±0.007	4.73±0.049		5.05[b]
	Polydextrose	5.53±0.028	5.15±0.070	5.12±0.021		5.26[a]
*B. longum*ATCC 15707	Maltodextrin	4.52±0.035	4.23±0.014	4.12±0.007	4.77[f]	4.29[c]
	Inulin	5.13±0.007	4.73±0.042	4.55±0.042		4.80[b]
	Polydextrose	5.41±0.014	5.11±0.007	5.21±0.014		5.24[a]
Mean		5.37[a]	5.01[b]	4.95[c]		

*Means (± standard deviation) followed by the same upper case letter(s) are not significant, but different letters are significant according to LSD procedure where α = 0.01, the LSD for fiber = 0.016 and the LSD for culture = 0.045 levels of producer LSD interaction (fiber * concentration * culture)= 0.125, LSD for concentration = 0.016*

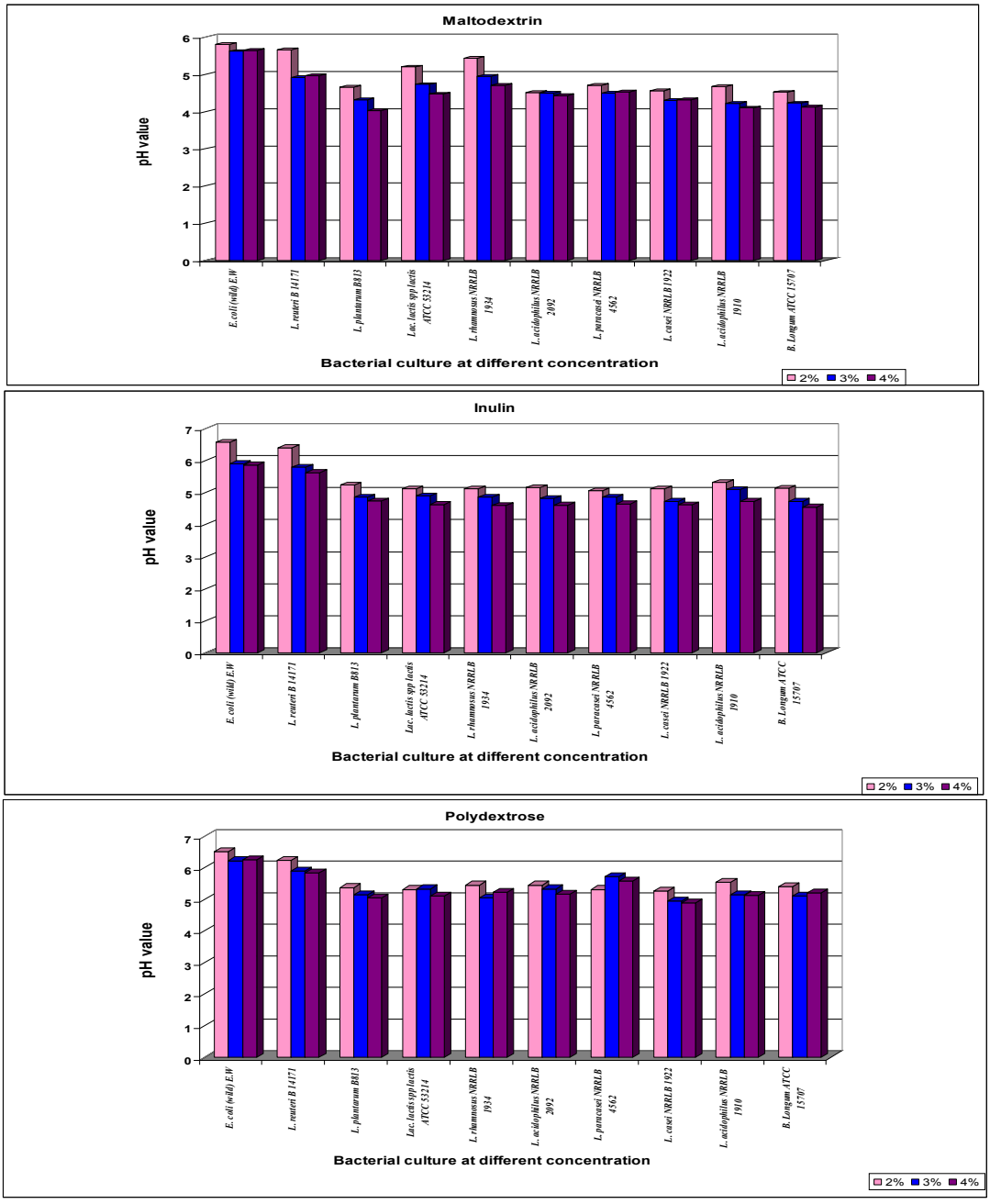

Fig. 2. Effect of different concentration of polysaccharides in modified MRS media tested incubation on the progress of pH values after 24 hrs on the examined bacterial strains

The results revealed that, the assimilation of the 3 polysaccharides tested by the lactic acid cultures was significantly showing a higher assimilation rate than *E. coli* (wild) E. Wstrain, which revealed that adding the polysaccharide has a priority on the growth of these culture if

it is present in the colon, further more increasing the percent of the polysaccharide resulted in an increase in growth as indicated by a significant decrease in the pH of the modified MRS as illustrated in Fig. 3.

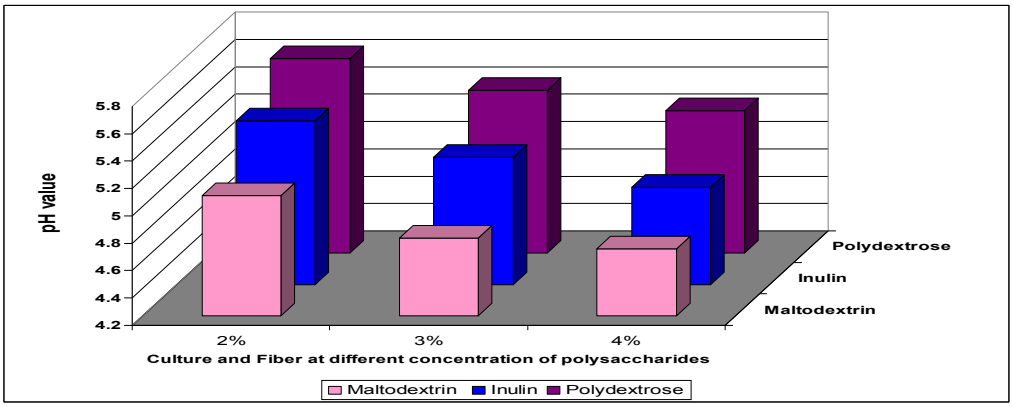

Fig. 3. Effect of different concentration of Inulin, Maltodextrin and Polydextrose on the progress of pH values of modified MRS

On the other hand *B. longum* ATCC 15707, *L. plantarum*B813, *L. acidophilus* NRRLB 1910 strains showed a significant assimilation activity for the three polysaccharides tested in comparison with the other tested bacterial strains.

The selected polysaccharides were such hardly in being fermented by *E. coli* (wild). That can be concluded from being low assimilated by the tested polysaccharides in comparing it with other lactic acid cultures examined and that is a criterion to select these polysaccharides as a prebiotics.

On the other hand maltodextrin exhibited more effect on lowering the pH value followed by inulin and polydextrose.

A 3% concentration were selected to continue the study with, although the 4 % concentration showed a more declination in pH values, especially after handling the sensory experiment it was noticed that the 4% concentration exhibited a noticeable after taste for 3 polysaccharide used beside 3% can show a significant difference in lowering the pH than 2% concentration. On contrary [27,28] reported that growth of *L. acidophilus* and *L. rhamnosus*was enhanced with 2% inulin and they consider it the best low fat yogurt.

Same findings to our results are reported by [29], they stated that the pH values of functional white soft cheeses made with 3 probiotic Lactobacillus strains in the presence of 3% of different prebiotics (dextrin and polydextrose) were significantly lower than the control white soft cheese made with the traditional starter (*S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) without adding prebiotics.

Also, [30,31] supplemented fermented milk with 4% (w/w) maltodextrin and polydextrose, they concluded that maltodextrin led to the highest amounts of conjugated linoleic acid and polydextrose addition led to the highest post-acidification.

On the other hand, [32] stated that increased bifidobacterial viability was observed in a dose ranging from 1 to 5%. Also [33] found that the count of *Lactobacillus* was on the level 10^7-10^8cfu/g in yoghurts with the 2-5% inulin addition.

3.3 Effect of Supplementation of MRS Broth Media with 3% of Maltodextrin, Inulin and Polydextrose Instead of Dextrose on the Growth of the Tested Bacterial Strain during Several Incubation Time Intervals

The purpose of this part was to evaluate the *B. longum* ATCC 15707 and related lactic acid culture beside *E. coli* (wild) E.W for their assimilation of maltodextrin, inulin and polydextrose during several incubation time intervals at certain incubation temperatures. Results in Table 3 and Fig. 4 revealed that the pH was decreased as incubation time increased. At the beginning of 8 hours of incubation the pH was declined slightly but after 24 hours of incubation a clear decrease in the pH was noticed. The tested bacterial strains showed a highly significant decrease in pH value compared to *E. coli* (wild) E.W. However increasing the incubation time to 48 hours showed the same trend of the tested culture for their assimilation of the examined polysaccharide. *B. longum* ATCC 15707 which recommended as a probiotic culture showed a high assimilation activity for the polysaccharide tested.

According to the assimilation activity of the tested 3 polysaccharides by lactic acid bacteria, maltodextrin showed a higher significant increase in their assimilations comparing to inulin and polydextrose. While the pH value for *B. longum* ATCC 15707 cultured on MRS broth with maltodextrin, inulin and polydextrose were 4.78, 5.28 and 5.42 after 24 hours of incubation respectively.

As shown in Table 3, E. coli(wild) E.W has been hardly assimilate the 3 sugars tested where, the pH value for MRS broth containing maltodextrin, inulin and polydextrose were 5.6 , 6.15 and 5.89 after 24 hours respectively. Similar findings were reported by [34]. They tested bifido bacteria or lactobacilli in the presence of enteropathogens such as *E. coli*, *Campylobacter jejuni* and *S. enteritidis*. Their results showed inhibition of the growth of these pathogens when inulin was added to the medium. The combination of *B. longumor L. plantarum* with inulin was very effective, causing a 6-log decrease in the numbers of *E. coli* and compromising the growth of *C. jejuni* and *S. enteritidis* to undetectable levels. Also [34], who shows the inhibition of enteropathogens by *L. plantarum* in culture experiments in the presence of starch [35] illustrated that a significant reduction (P, 0•05) in the E. coli numbers associated with the intestinal piglet tissue v. the controls upon feeding the animal on inulin .Also it was reported that adding inulin (3%) in the diet significantly increased the level of bifidobacteria and this increase was associated with a significant decrease (P, 0•05) in pathogen numbers (*E. coli*, *C. perfringens*, *C. difficile* and *C. ramosum*) v. the control diet containing lactose [36].

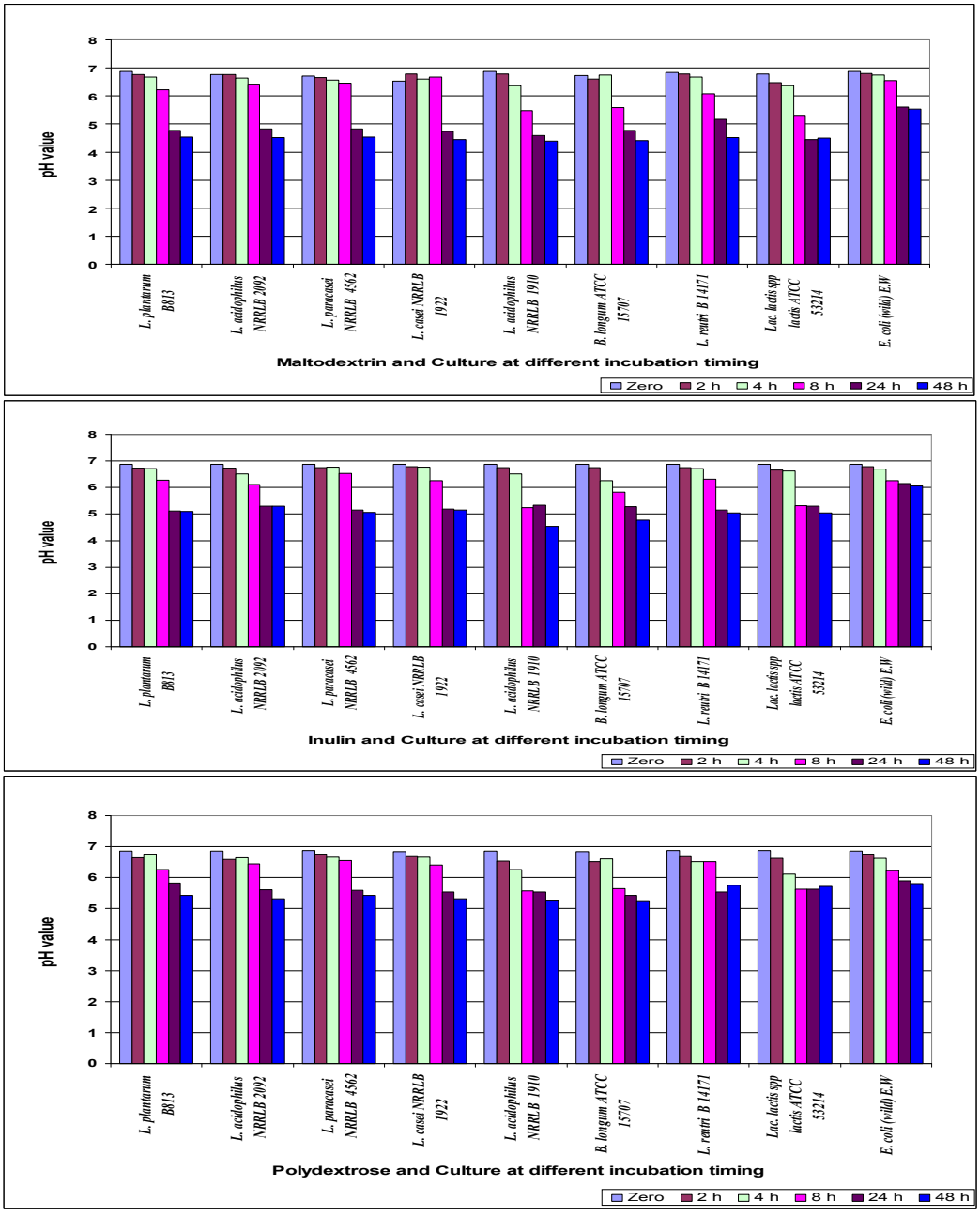

Fig. 4. Changes in pH of MRS with 3% concentration of maltodextrin, inulin and polydextrose instead of dextrose during different incubation time

Table 3. Changes in pH of modified MRS broth media with 3% concentration of maltodextrin, inulin and polydextrose instead of dextrose and inoculated with the tested bacterial strains during several incubation time intervals at the recommended temperature of each strain

		Time						Mean	
		Zero	**2 h**	**4 h**	**8 h**	**24 h**	**48 h**	**Fiber**	**Culture**
Maltodextrin	*L. plantarum B813*	6.88±0.007	6.76±0.042	6.67±0.014	6.23±0.021	4.78±0.028	4.53 ±0.007		5.97^{b}
	L. acidophilus NRRLB 2092	6.76±0.120	6.76±0.042	6.64±0.007	6.42±0.028	4.82±0.035	4.51 ±0.007		5.98^{b}
	L. paracasei NRRLB 4562	6.71±0.014	6.66±0.070	6.57±0.056	6.45±0.014	4.83±0.000	4.53 ±0.007		5.95^{b}
	L. casei NRRLB 1922	6.53±0.000	6.79±0.021	6.61±0.035	6.67±0.007	4.73±0.000	4.45 ±0.021		5.9 ^{b}
	L. acidophilus NRRLB 1910	6.88±0.0070	6.78±0.007	6.36±0.028	5.48±0.014	4.59±0.021	4.39 ±0.021	5.95^{c§}	5.74^{cd}
	B. longum ATCC 15707	6.73±0.007	6.60±0.007	6.74±0.007	5.58±0.042	4.78±0.007	4.40 ±0.021		5.80^{c}
	L. reuteri B 14171	6.83±0.007	6.78±0.007	6.67±0.007	6.07±0.007	5.17±0.007	4.51 ±0.021		6.00^{b}
	*L.lactis*subsp.*lactisATCC53214*	6.78±0.014	6.47±0.0141	6.37±0.049	5.27±0.028	4.45±0.007	4.50 ±0.021		5.64^{d}
	E. coli (wild) E.W	6.88±0.0353	6.81±0.007	6.75±0.042	6.55±0.028	5.60±0.007	5.54 ±0.007		6.35^{a}
Inulin	*L. plantarum B813*	6.87±0.007	6.73±0.141	6.72±0.042	6.28±0.007	5.11±.0353	5.10 ±0.049		6.13^{bc}
	L. acidophilus NRRLB 2092	6.87±0.070	6.73±0.212	6.52±0.007	6.11±0.056	5.30±0.014	5.30 ±0.021		6.13^{bc}
	L. paracasei NRRLB 4562	6.87±0.049	6.75±0.212	6.77±0.212	6.53±0.049	5.16±0.021	5.07 ±0.014		6.19^{b}
	L. casei NRRLB 1922	6.87±0.028	6.79±0.021	6.76±0.049	6.25±0.007	5.18±0.021	5.15 ±0.028		6.16^{bc}
	L. acidophilus NRRLB 1910	6.87±0.028	6.74±0.042	6.52±0.035	5.24±0.212	5.33±0.007	4.54 ±0.007		5.87^{de}
	B. longum ATCC 15707	6.87±0.028	6.74±0.042	6.25±0.014	5.82±0.007	5.28±0.028	4.77 ±0.007		5.95^{d}
	L. reuteri B 14171	6.87±0.028	6.75±0.042	6.72±0.042	6.31±0.014	5.15±0.028	5.05 ±0.049		6.14^{bc}
	L. lactis subsp. *lactis ATCC 53214*	6.87±0.028	6.66±0.042	6.63±0.042	5.30±0.014	5.30±0.014	5.05 ±0.014		5.96^{d}
	E. coli (wild) E.W	6.87±0.028	6.79±0.042	6.7±0.042	5.16±0.021	5.16±0.021	6.05 ±0.014		6.40^{a}
Polydextrose	*L. plantarum B813*	6.86±0.028	6.64±0.028	6.73±0.035	6.26±0.063	5.83±0.007	5.43 ±0.021		6.29^{ab}
	L. acidophilus NRRLB 2092	6.85±0.028	6.58±0.042	6.64±0.028	6.44±0.028	5.61±0.007	5.31 ±0.028		6.23^{b}
	L. paracasei NRRLB 4562	6.88±0.028	6.73±0.042	6.66±0.028	6.54±0.028	5.59±0.007	5.42 ±0.028		6.30^{ab}
	L. casei NRRLB 1922	6.84±0.028	6.67±0.042	6.65±0.028	6.41±0.028	5.54±0.007	5.32 ±0.028		6.23^{b}
	L. acidophilus NRRLB 1910	6.86±0.028	6.53±0.063	6.26± 0.063	5.57±0.021	5.54±0.007	5.25 ±0.028	6.23^{a}	6.00^{c}
	B. longum ATCC 15707	6.83±0.028	6.52±0.063	6.60±0.028	5.64±0.007	5.42±0.021	5.22 0.028		6.20^{b}
	L. reuteri B 14171	6.87±0.028	6.67±0.056	6.52±0.063	6.52±0.028	5.54±0.007	5.75 ±0.021		6.31^{ab}
	L. lactis subsp. *lactis ATCC 53214*	6.87±0.028	6.63±0.042	6.12±0.042	5.62±0.007	5.62±0.007	5.71 ±0.007		6.09^{c}
	E. coli (wild) E.W	6.85±0.028	6.73±0.063	6.62±0.028	6.23±0.042	5.89±0.007	5.81 ±0.007		6.35^{a}
Mean		6.84^{a}	6.70^{ab}	6.59^{b}	6.12^{c}	5.31^{d}	5.07^{e}		

*Means (± standard deviation) followed by the same upper case letter(s) are not significant, but different letters are significant according to LSD procedure where α = 0.01 , the LSD for fiber = 0.086 , the LSD for the culture= 0.105 , the LSD for time duration = 0.149 , the LSD for duration *fiber * culture = 0.258 levels of producer*

3.4 Effect of Using Selected Polysaccharides on the Growth of Probiotic Cultures in Combination with *Escherichia coli*

This study was carried out to investigate the antagonistic effect of some probiotic cultures (*B. longum* ATCC 15707, *L. reuteri* B 14171, *L. acidophilus* NRRLB 1910) in combination with *E. coli*(wild) E.W. as shown in Table 4 .The log cfu/ml of *E. coli* (wild) E.W. cultured on MRS both modified with inulin, polydextrose, maltodextrin was 8.08, 8.25 and 8.98 and was 7.9,8.1 and 8.3 when cultured on VRBA respectively. The tested culture (*B. longum* ATCC 15707, *L. reuteri* B 14171, *L. acidophilus* NRRLB1910) didn't exhibit any growth when cultured on VRBA.

L. reuteri B 14171, *L. acidophilus* NRRLB 1910 and *B. longum* ATCC 15707 were slightly varied on their growth on MRS in the presence of the selected tested polysaccharides, *L. reuteri* B 14171 when grown on a MRS both containing inulin show a slight higher log cfu/g when compared to its growth on polydextrose and maltodextrin, there were no significant difference between the three polysaccharides. Also, *L. acidophilus* NRRLB 1910 was highly grown on MRS containing maltodextrin following to it, polydextrose then inulin their log cfu/g were 8.72, 8.36 and 8.28 respectively although no significant difference was shown in the growth of the three of them. Finally for the *B. longum* ATCC 15707 was highly grown on MRS containing polydextrose, it's log cfu/g was 8.855 following the inulin 8.04 log cfu/g finally maltodextrin 7.96 cfu/g.

On other hand [34] explained that lactic acid-producing bacteria have antibacterial activity against pathogens. The production of organic acids (acetic acid and lactic acid) and antimicrobial substances that are active against virulent micro-organisms might be two of the underlying inhibitory mechanisms. The presence of increasing concentrations of organic acids acidifies the medium. The lower pH is of benefit for their growth, since lactic acid-producing bacteria are very acid-tolerant and, hence, their growth is less affected. This characteristic provides bifidobacteria and lactobacilli with the advantage to survive acidic environments that inhibit the growth of certain noxious bacteria. The fermentation of inulin by bifidobacteria led to an increase in the concentration of acetate and lactate in the medium, which lowered the pH of the culture. At a pH of 4.5–5.0, the numbers of *E. coli* (wild) E.W. andClostridium fell to zero whereas the growth of bifidobacteria was unaffected [37]. Subsequent culture subsequent tests carried out by the same researchers [12] showed that in the presence of oligofructose, several species of bifidobacteria are able to excrete antimicrobial substances with a broad spectrum of activity. This antagonistic activity of bifidobacteria against the growth of *E. coli* (wild) E.W was confirmed recently by [38], according to these authors, the antagonistic activity of bifidobacteria against pathogenic Gram-negative bacteria appears to be widespread.

Upon combining *L. reuteri* B 14171 with *E. coli* (wild) E.W. it was shown that the log cfu/g was increased on MRS media containing polydextrose, maltodextrin and inulin which were 8.90, 8.73 and 8.34 respectively. While the log 10 cfu/g of *E. coli* (wild) E.W. on VRBA decreased to 6.19, 6.30 and 6.47 in the presence of the polysaccharides mentioned previously which may be due to the antagonistic effect of *L. reuteri* B 14171 on inhibiting the growth of *E. coli* (wild) E.W. Also combining *E. coli* (wild) E.W. with *B. longum* ATCC 15707 where the log10 cfu/g of *E. coli* (wild) E.W. on VRBA decreased to 6.13, 6.10 and 6.88 in the presence of polydextrose, maltodextrin and inulin .Similar resultswere shown by [37] who reported that the growth of *B. longum* ATCC 15707 had an inhibitory effect towards Esherichia coli .While the corresponding results of *E. coli* (wild) E.W. with *L. acidophilus* NRRLB 1910 showed the same trend were 6.45,6.28 and 7.31 on VRBA respectively.

Table 4. Effect of using selected polysaccharides on the growth of probiotic ltures in combination with *Esherichia coli* after 24 hours of incubation at 37°C

Culture	Log $_{10}$ cfu/gon selected culture media					
	MRS			VRBA		
	Inulin	polydextrose	Maltodextrin	inulin	polydextrose	maltodextrin
E. coli (wild) E.W.	8.08^{abc}	8.25^{abc}	8.98^{a}	7.90^{bcd}	8.10^{abc}	8.30^{abc}
E. coli (wild) E.W. + *L. reuteri B 14171*	8.90^{a}	8.73^{abc}	8.34^{abc}	6.47^{ef}	6.19^{f}	6.30^{f}
E. coli (wild) E.W.**+***B. longum*ATCC 15707	8.51^{abc}	7.92^{bcd}	7.86^{cd}	6.88^{ef}	6.13^{f}	6.10^{f}
E. coli (wild) E.W. + *L. acidophilus* NRRLB 1910	8.37^{abc}	8.28^{abc}	8.60^{abc}	7.31^{de}	6.45^{ef}	6.28^{f}
*L. reuteri*B 14171	8.58^{abc}	7.90^{bcd}	7.90^{bcd}	-	-	-
L. acidophilus NRRLB 1910	8.28^{abc}	8.36^{abc}	8.72^{abc}	-	-	-
*B. longum*ATCC 15707	8.04^{abc}	8.855^{ab}	7.96^{abc}	-	-	-

Means followed by the same upper case letter(s) are not significant, but different letters are significant according to LSD procedure where α = 0.01, the LSD for culture and fiber/media = 0.965 levels of producer

§comparison between different fibers/media within the same bacterial culture

4. CONCLUSION

Maltodextrin was found to have good results in stimulation the probiotic bacterial count and enhancing the sensory properties of fermented milk compared with inulin, and that is recommended by our work to use it in industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Fuller R. Probiotics in man and animals. J Appl Bacteriol.1989;66:365–78.
2. FAO/WHO 2002. Guidelines for the evaluation of probiotics in food. London, Ontario, Canada.
3. Holzapfel WH, Schillinger U. Introduction to pre- and probiotics. Food Res Int. 2002;35:109-116.
4. De Vuyst L, Schrijvers V, Paramithiotis S, Hoste B, Vancanneyt M, Swings J, Kalantzopoulos G, Tsakalidou E, Messens W. The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. Appl Environ Microbiol. 2002;68:6059–6069.
5. Fooks LJ, Fuller R, Gibson GR. Prebiotics-probiotics and human gut microbiology. Int Dairy J. 1999;9:53-61.
6. Crittenden RG. Prebiotics. In: Probiotics: A Critical Review. G.W. Tannock, ed. Horizon Scientific Press, Wymondham. 1999;141-156.
7. Ballongue J. Technical problems related to in vitro study of colon flora. Scand J Gastroenterol .1997;32:14–16.
8. Bouhnik Y, Flourie BL, D'Agay-Abensour P, Pochart G, Gramet M, Durand M, Rambaud JC. Administration of transgalactooligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J Nutr. 1997;127:444-448.
9. Crittenden RG, Playne MJ. Production, properties and applications of food-grade oligosaccharides. Trends Food Sci Technol. 1996;7:353-360.
10. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. J Nutr. 1995;125:1401–1412.
11. Gorski D. Fat replacement technologies. Dairy Foods. 1995;1:38-39.
12. Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructoseand inulin. Gastroenterology. 1995;108:975–982.
13. Kleessen B, Sykura B, Zunft HJ, Blaut M. Effects of inulin and lactose on fecal microflora, microbial activity and bowel habit in elderly constipated persons. Am J ClinNutr. 1997;65:1397-1402.
14. Roberfroid MB, Van LJ, Gibson GR. The bifidogenic nature of chicory inulin and its hydrolysis products. J Nutr. 1998;128:11-19.
15. Van LJ, Coussement P, De Leenheer L, Hoebregs H , Smits G. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit Rev Food Sci Nutr. 1995;35:525-552.
16. Aryana KJ, McCrew P .Quality attributes of yogurt with *Lactobacillus casei* and various prebiotics. LWT -Food Sci Technol. 2007;40:1808-1814.

17. Donkor ON, Nilmini SLI, Stolic P, VasiljevicT, Shah NP. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. Int Dairy J. 2007;17:657-665.
18. Helland M H, Wicklund T , Narvhus J A. Growth and metabolism of selected strains of probiotic bacteria in milk- and water-based cereal puddings. Int Dairy J. 2004;14:957-965.
19. DeMan JD, Rogosa M, Sharpe ME. A Medium for the Cultivation of lactobacilli. J ApplBacteriol. 1960;23:130-135.
20. Marshall R T. Standard methods for the examination of dairy products. 16th ed. American Public Health Association. Washington, D.C.; 1993.
21. Steel RGD, Torrie JH. Principles and Procedures of Statistics. A biometrical approach. 2nd Ed. McGraw Hill Inter. Book Co. Tokyo, Japan; 1980.
22. Srisuvor N, Ninnart C, Cheunjit P , Suwanna S. Effects of inulin and polydextrose on physicochemical and sensory properties of low-fat set yoghurt with probiotic-cultured banana purée. LWT -Food Sci Technol. 2013;51:30-36.
23. Raju NP, Pal D. Effect of bulking agents on the quality of artificially sweetened mistidahi (caramel colored sweetened yoghurt) prepared from reduced fat buffalo milk. LWT -Food Sci Technol. 2011; 44: 1835-1843.
24. Bekeris M, Grūbe M, Upite D, Kaminska E, Linde R, Pelce E, Daņiļevičs A , Guavare M. The resistance of some prebiotics and probiotic bacteria in the stomach model environment. LLU Raksti. 2010;24:55-64.
25. Hayakawa K, Mizutani J, Wada K, Masai T, Yoshihara I , Mitsuoka T. Effects of soybean oligosaccharides on human fecal flora. Microb Ecol Health D. 1990;3:293-303.
26. Calame W, Weseler AR, Viebke C, Flynn C , Siemensma AD. Gum arabic establishes prebiotic functionality in healthy human volunteers in a dose-dependent manner. Br J Nutr. 2008;100:1269-75.
27. Sadek ZI, El-ShafeiK, Murad HA. Utilization of xanthan gum and inulin as prebiotics for lactic acid bacteria. In: Proceedings of 9thEgyptian Conference for Dairy Science and Technology, Cairo, Egypt. 2004;25:269–283.
28. Boeni S, Pourahmad R. Use of inulin and probiotic lactobacilli in synbiotic yogurt production. Ann Biol Res. 2012;3(7):3486-3491.
29. Baher AME, Ahmed MMM, Zainab IS, GehanAMH, Mohamed NIM. Production of novel functional white soft cheese. JMBFS. 2012;1(5):1259-1278.
30. Coussement PAA. Inulin and oligofructose: safe intakes and legal status. J Nutr. 1999;129(7 suppl):1412S–1417S.
31. Oliveira RPS, Perego P, Converti A, Oliveira MN. The effect of inulin as a prebiotic on the production of probiotic fiber-enriched fermented milk. Int J Dairy Technol. 2009;62(2):195-203.
32. Shin HS, Lee JH, Pestka JJ, Ustunol Z. Growth and viability of commercial *Bifidobacterium* spp in skim milk containing oligosaccharides and inulin. J Food Sci. 2000;65(5):884–887.
33. Juhkam K, Elias P, Roasto M, Tamme T. Viability of *Lactobacillus acidophilus* in yoghurt containing inulin or oligofructose during refrigerated storage. Milchwissenschaft. 2007;62(1):52-54.
34. FooksL, Gibson G. Probiotics as modulator of the gut flora. Br. J of Nutr. 2002;88Suppl 1:S39-S49.
35. Naughton PJ, Mikkelsen LL, Jensen BB. Effects of non digestible oligosaccharideson *Salmonella entericaser* ovar Typhimurium and non pathogenic *Escherichia coli* in the pig small intestine. Appl Environ Microbiol. 2001;67(8):3391–3395.

36. Catala I, Butel MJ, Bensaada M, Popot F, Tessedre AC, Rimbault A, Szylit O. Oligofructose contributes to the protective role of bifidobacteria in experimental necrotisingenterocolitis in quails. Journal of Medical Microbiology.1999;48:89–94.
37. Wang X, Gibson GR. Effects of the in vivo fermentation of oligofructose and inulin by bacteria growing in the human large intestine. J Appl Bacteriol. 1993;75(4):373–380.
38. Makras E , De Vuyst L. The *in vitro* inhibition of Gram-negative pathogenic bacteria by bifidobacteria is caused by the production of organic acids. Int Dairy J. 2006;16:1049-1057.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=432&id=8&aid=3948