Biomedical & Pharmacology Journal Vol. **9**(2), 451-462 (2016)

Investigation of the Growth Ability of Probiotic (*Lactobacillus* and *Bifidobacterium*) in Infant's Milk under Different Environmental Conditions

HUSSAH SHAFI AL-OTAIBI[1], RUKAIA MOHAMMED GASHGARI[1], AFRAH ELTAYEB MOHAMMED[2], SHADEN ABDULMOHSEN ALMOJEL[3], MUDAWI MUKHTAR ELOBEID[4] and JEHAN SAUD AL ABRAHAIM[2]

[1]Department of Biology, Faculty of Science, King Abdul Aziz Univerisity, Jeddah - Saudi Arabia
[2]Department of Biology, Faculty of Science, Princess Nourah Bent Abdul-Rahman University, Riyadh - Saudi Arabia
[3]Department of Medicin, Faculty of Medicine, Princess Nourah Bent Abdulrahman University, Riyadh - Saudi Arabia
[4]Department of Silviculture, Faculty of Forestry, University of Khartoum, Khartoum north, Shambat – Sudan.
*Corresponding author E-mail: jsaa242@hotmail.com

http://dx.doi.org/10.13005/bpj/958

(Received: May 19, 2016; accepted: June 25, 2016)

ABSTRACT

In the present investigation five species of probiotic bacteria, which belong to Lactobacillus (*L. reautari*, *L. rhamnosus*, *L. acidophilus*), Bifidobacterium (*L. casei*, *L.* helveticus, *B. bifidum*) were evaluated for their growth in dried infant's milk. Three kinds of milk which is used for healthy infants and two other kinds which are widely spread and commonly used for unhealthy breastfeeding infants (vomiting and diarrhea).The study has clearly indicated that all the probiotics employed in this investigation have grown in all milk types to which the nutrient MRS medium was added as well as for the tested milk types without any additives. Findings showed that the optimal time period for adequate growth of probiotic ranges from 12 to 24 hours. Furthermore, it was found that during incubation period of the tested probiotics the pH was in the range of 7.20 to 7.87 for all the milk types tested. It was generally observed that changes in pH were meager during the first 8 hours after incubation and then pH started to decrease reaching a value of d" 4 with the increase in growth until the end of incubation period (i-e, 72 hours). The current results also showed that the optimal temperature for adequate growth of probiotics investigated is 37 ºC whereas the least temperature is 5 ºC. Variation in pH had a limiting effect on bacterial growth. Results showed that the best probiotic growth was detected at pH 6 and the least growth was at pH 11. Furthermore, the maximum growth levels at 55% relative humidity, except *L. helveticus* bacteria for which the highest growth rate was recorded at 75% RH and growth of the tested probiotics was decreased at RH of 35% and 100%.

Key words: Probiotic, pH, *Bifidobacterium, Lactobacillus,* temperature, infant milk

INTRODUCTION

The mother's milk contains approximately 87% water, 4.9% carbohydrates, 3.5% fats and 3.5% protein and minerals. These percentages are not fixed and they differ with variation in breastfeeding phase and also depending on the infant's need for nutrients elements[1]. Most of the important changes for both proteins and minerals take place during the first two weeks from delivery, at the same time the amount of milk that flows from the mother varies widely. The mother's milk increases since the first day of delivery to an average of 500 cm^3 in the first week. These changes has increased the questions

addressed by pediatrics and nutrition scientists in their attempts to evaluate the nutritional substances taken by the healthy infant during breastfeeding from its mother's milk.The first drops of milk breastfed by the infant are called "*Liba*", which is a unique liquid with respect to its nutritional value. The formation of *Liba* begins in the second phase of pregnancy period and continues until the fifth day after delivery. *Liba* offers the infant what it needs in this very short period for the best start of a healthy life. *Liba* differs radically in its composition from the normal mother's milk; it contains more quantities of water, vitamins and minerals, but with lower fats than the milk that forms later on. *Liba* is quite vital and unique for newly born infant due to its high content of a special type of proteins called Immunizing Globulin. Such protein constitutes protection against germs to which both a child and its mother are subjected to everyday.The anti-germs are transferred from a mother to the infant via milk in the form of protein, hence this high amount of proteins give the infant's body the ability to resist the pathogenic germs. When breastfeeding the infant from a mother's milk, only some of the useful bacteria (Bifidobacteria) remain in the intestine to offer immunity as the intestine is the a safe place for its growth[2]. The significance of intestinal probiotics is mainly in its potential role in maintaining the immunity system, which helps in avoiding suffering from diarrhea and mitigate the probable exposure to sensitivity, limiting the growth of pathogenic bacteria, enhancement of absorption process and digestion of nutritional constituents, help in protection of the child against sensitivity-causing substances that might be transported by various sources of protein. Recently, new fermented milk products have been produced and developed and such products contain these probiotics either singly or combined together. During the last two decades many studies were carried out and almost all findings strongly indicated that feeding with milk products, which contain the bacterial type (Probiotic bacteria) has an important healthy role and quite beneficial to the consumer. Such bacteria are known as Lactobacilli Probiotic bacteria and these microorganisms potentially help in the process of microbial balance inside the intestine. Some of the major genera known as probiotic bacteria include *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and the milk products containing these probiotics has numerous health and nutritional advantages involving conservation of the natural flora in human intestine particularly for children and the old people such as improving the efficiency of lactose digestion and fermented milky products, getting rid of some cancer tumors and reduction of cholesterol in blood in addition to improving the nutritional value, for instance by releasing the free amino acids[3,4]. Probiotic are defined as extracts or bio-products containing microorganisms in sufficient amounts leading to a change in its culture in the intestine of a host organism and their presence in the intestine lead to health effects on the microflora[5,6]. The most commonly studied probiotic bacteria include the genera: *Saccharomyces*, *Bifedobacterium, Lactobacillus*, *Enterococcous* and some *Streptococcus*[7,8]. The probiotic has the ability for protein hydrolysis in different ways. For instance, *L. casei* 279 and L26 *L. paracasei* have exhibited the highest hydrolysis rates for the casine where they have produced high concentrations of free amino acids depite the fact that *Bifidobacterium* was characterized by low hydrolysis, however the concentrations of free amino acids were high. Several probiotic bacteria excrete inhibiting substance for *in-vitro* exoeriments. Previous studies have shown the capability of probiotics bacteria for resistance against the tested microorganisms (*Salmonella enteritidis, Escherichia coli, Listeria monocytogen, Bacillus cereus*). It was observed that the two species (*Bifidobacteria breve* and *Streptococcus faecalis*) were characterized by high anti-activity whereas the ant-activity was less for the species *Bifidobacteria infants* C1*5*. This anti effect was attributed to the fermentation products and anti-biotic substances which are excreted by probiotics and replace the pathogenic bacteria and thus decreasing the production of toxic substances that affect the body negatively[9,10,11,12].

MATERIALS AND METHODS

Bacterial isolates

Two bacterial isolates (One isolate belongs to the genus *Lactobacillus* whereas the other belongs to the genus *Bifidobacteria*) were obtained from the feaces of a newly-born child (9-days old) in Riyadh city, Kingdom of Saudi Arabia using dilutions method, these isolates are:

1. *Lactobacillus* sp

2. *Bifidobacterium* sp

It was not possible the identification for these isolates at the species level. Therefore, five species of *Lactobacillus* which are identified in international centres were used and these are as follows:

1. *L. reautari* (B 14171 from NRRL, Illionois, USA).
2. *L. rhamnosus* (B – 445 from NRRL, Illionois, USA).
3. *L. acidophilus* (from Char. Hansen's Lab. Denmark).
4. *L. helveticus* (from Char. Hansen's Lab. Denmark).
5. *L. casei* (FMCC 1093).

In addition to one species of *Bifidobacterium, B.bifidium* (from Char. Hansen's Lab. Denmark).

Milk samples used: The artificial milk is divided into two

1. The artificial milk special for healthy breastfeeders starting from delivery until the age of one or more than one year.
   In the present study three types of this milk as follows:

a) Milk, Cemelac – Advanced from delivery until the age of 6 months.
b) Milk, Cemelac – Ghain from the age of 6 months and above.
c) Milk, Progress – Gold from the age of one year to three years.

2. The artificial milk special for unhealthy breastfeeders (under age and under weight since delivery time and lactose deficiency for the cases of diarrhea and in-tolerance of cow protein).
   In the present study two types of milk were used:

a) Milk – S 26 LF: is a milk powder for infants, lactose-free, special for infant breastfeeders extremely sensitive to lactose. The sensitivity to lactose is often related with nutrition problems such as diarrhea, gases, vomiting and fast irritation.
b) Milk – S 26 IR: is a milk powder for infant breast feeders suffering from vomiting (slight to moderate). It is possible to use this special composition for children since delivery time to help in limiting involuntary spitting or vomiting. It is also easy to use and Glutene-free.

Growth media

The nutrition medium (M.R.S Broth): A ready medium was purchased from Sigma, characteristics are indicated below:

Fifty-two grams were dissolved in one litre distilled water and well mixed, then sterilized in an autoclave at 121ºC for 15 min. and the solid ready (M.R.S) nutrition medium was used which are comprised from the same constituents to which 10 grams Agar Agar was added. The anaerobic ajar was used for growth and the anaerobic gas generating kits (Oxoid Hampshire Company, England) were employed for anaerobic incubation of the bacteria.

METHODS

Isolate storage

For safe storage of the probiotic bacteria at 5ºC, ten ml of 50% glycerol were added for each liter.

Preparation of inoculum

The inoculum was prepared by growing bacteria on the MRS Agar nutrition medium to which 5% Lactose was added (Hughes and Hooves 1995). The incubation was performed anaerobically at 37ºC for 24 hours, then activated by transferring 9 ml of the inoculation from the MRS broth nutrition medium, and subsequently incubated at 37ºC for 24 hours, then measured with the aid of colour spectrum (Spectro 23, Labomed Inc. England) spectrophotometer so that the reading was in the range 0.05 0.08, i.e., the number of living cells approximately 10^4 living cell/ml.

Growth curve of probiotics in MRS nutrition medium with milk types

In this study the growth curve was evaluated for the tested probiotics which were pre-activated individually as described in the above section, preparation of inoculum. The milk samples mentioned before in accordance to the instructions provided in each can (a certain amount of milk was

added to the MRS broth growth medium). Thereafter, the nutrition medium was inoculated individually by the previous inoculum with the rate of 1% bacteria, which is then anaerobically incubated at 37ºC for 24 - 48 hours. Three replicated were performed for each period. The number of bacteria was adjusted for each species to obtain approximately equal number (10^4 cell/ml), then the bacteria was measured/evaluated at different growth periods (0, 4, 8, 12, 16, 20, 24, 48, 72 hours) using an appropriate equipment for measuring the color spectrum (Spectro 23, labomed, Inc.) Spectrophotometer at an absorption wave length of 620 nm. On each occasion for taking the sample for microbial count the gas containers were substituted with new ones to ensure continuity and consistency of the anaerobic incubation conditions.

Growth curve of the tested probiotics only on different milk types

In the present study, the growth curve of probiotics which were individually activated, then only the milk samples were inoculated with a rate of 1% bacteria individually. Subsequently, bacteria were anaerobically incubated at 37ºC for 24 - 48 hours. The growth was evaluated for the same periods time with the same method described above.

Effect of the temperature variation on microbial growth

Typical nutrition media previously described (MRS broth medium + different milk types) were prepared. One ml of the inoculum was taken and placed in tubes containg 9 ml nutrition medium, then incubated at different temperature (4, 25, 37, 55ºC). At the end of incubation period growth was measured by measuring the color spectrum (Spectro 23, labomed, Inc.) Spectrophotometer at an absorption wave length of 620 nm.

Effect of the pH on microbial growth

The pH in the nutrition medium (MRS + milk) was adjusted using HCl and NaOH with a concentration of 1% with the aid of a pH meter (MP220 pH Meter , Mettr Toledo Switzerland). The degree of acidity and alkalinity used were (2, 4, 6, 9, 11), then the inoculum was added individually with adjusting the number of cells around 10^4 living cell/ ml and incubated at 37ºC, then evaluating the growth by means of measuring the color spectrum (Spectro 23, labomed, Inc.) spectrophotometer at an absorption wave length of 620 nm.

Effect of humidity variation on microbial growth

In the present study the method adopted by Solomon *et al*. (1951) by placing a test tube containing water in addition to sulphuric acid, H_2SO_4 so that the humidity would be 35%, 55%, 75%, 95%, 100%. The tube was placed in a container for anaerobic growth so that the required humidity can be provided. At the end of incubation period the effect of humidity variation on the microbial growth was measured the color spectrum (Spectro 23, labomed, Inc.) spectrophotometer at an absorption wave length of 620 nm.

Statistical analysis

Results were statistically analysed using SPSS programme. After obtaining the experimental results, data was treated and statistically analysed with the computer employing SPSS, Statistical Package for Social Sciences using two-way ANOVA.

RESULTS

It has been scientifically and medically quite evident that natural breast feeding is of great importance and greatly advantageous for both the infant and the breast feeding mother. When comparing breast feeding with bottle feeding in milk types used and these are:Cemelac Advanced, Cemelac Progress Gold, 26 LF and S – 26 AR. Results indicated in table (2) showed that the mother's milk contains all the essential nutritional substances required for a child growth in a consistent manner like bottle milk, where it was found that the percentage of all constituents in the mother's milk is considered relatively little and of limited quantity compared with Cemelac Advanced, Cemelac Ghain and Progress Gold, respectively and approaching S 26 – LF and S 26 – AR, which are utilized for special pathogenic cases, however they are lacking some important substances such Lactose in the former. Cemelac Advanced is characterized by high calories and increased percentage of fats. The milk from the age of 6 months and above contains greater quantity of proteins due

to the increasing demand of the infant. The milk from the age of one to three years is characterized by increased percentages of vitamins, iron and mineral elements.

The source of information about the bottled milk types is the data documented in the can (milk container). An explanation for the increased weight in some children who feed on bottled milk compared with children who are naturally breast fed is the presence of some components in the milk which the child does not need for its growth.

Table 1: Components (g/L) of the nutrition medium (M.R.S. Broth)

Peptone	10
Yeast extract	4
Glucose	20
Tween 80	1ml
Di-potassium hydrogen phosphate	20
Sodium acetate 3H2O	5
Tri-ammonium citrate	2
Magnesium sulphate 7H2O	2
Manganese sulphate 4H2O	0.05
pH	6.2

Growth curve of probiotics grown in MRS growth medium with added milk types

The aim of this experiment was to study the effect of different incubation periods on the growth of the tested probiotic previously activated individually. The milk types mentioned before were prepared according to the information provided with each container (by adding the required weight of the milk to the MRS broth nutrition medium), than inoculating the nutrition medium with milk by the activated inoculum from the bacteria, the adjusting the number of bacteria for each type to obtain approximately equal number (10^4 inoculated cell/ml) each separately, and incubated anaerobically using anaerobic jar at 37ºC for 24 – 48 hours, then growth was measured at different time period (0, 4, 8, 12, 16, 20, 24, 48, 72 hours) using the color spectrum (Spectrophotometer) at the absorption wave length of 620 nm. On each occasion of taking the sample, the gas containers were substituted

Table 2: Comparison between mother's milk and the other milk types used in this study (100 mg/ 100 ml)

Milk type	Mother's milk	Cemelac Advanced	Cemelac Ghain	Progress Gold	S 26 – AR	S26 – ALF
Calories	70 – 72	52.6	49.5	20	67	67
Protein (Ghain)	1.0 – 1,3	11.8	18.8	14.5	1.6	1.5
Carbohydrates (Ghain)	6.8 – 7.4	54.6	50.3	64.1	7	7.2
Fats (Ghain)	4.2 – 4.5	28.9	24.3	15.1	3.6	3.6
Vitamin A (Univ. unit)	190	1577	1765	1666	250	250
Vitamin D (Univ. unit)	2.2	315	335	500	43	43
Vitamin E (Univ. unit)	0.27	16	13	13	1.1	1.1
Vitamin K (µg)	1.5	42	41	50	6.7	6.7
Vitamin G (mg)	4.3	47	73	120	9	9
Vitamin B6 (µg)	10	0.32	0.94	1	60	60
Vitamin B12 (µg)	0.03	1.31	2	2.1	0.2	0.2
Thiamine (µg)	16	-	-	-	100	100
Riboflavin (µg)	36	-	-	-	150	150
Folic acid (µg)	0.5	79	88	50	8	8
Ca (mg)	34	410	771	1000	56	55
P (mg)	14	221	444	800	44	37
Na (mg)	16.1	126	244	470	22	16
K (mg)	50.7	552	858	1800	80	70
Cl (mg)	38.5	342	565	1100	55	43
Fe (mg)	0.05	4.7	8	13	0.8	1.2

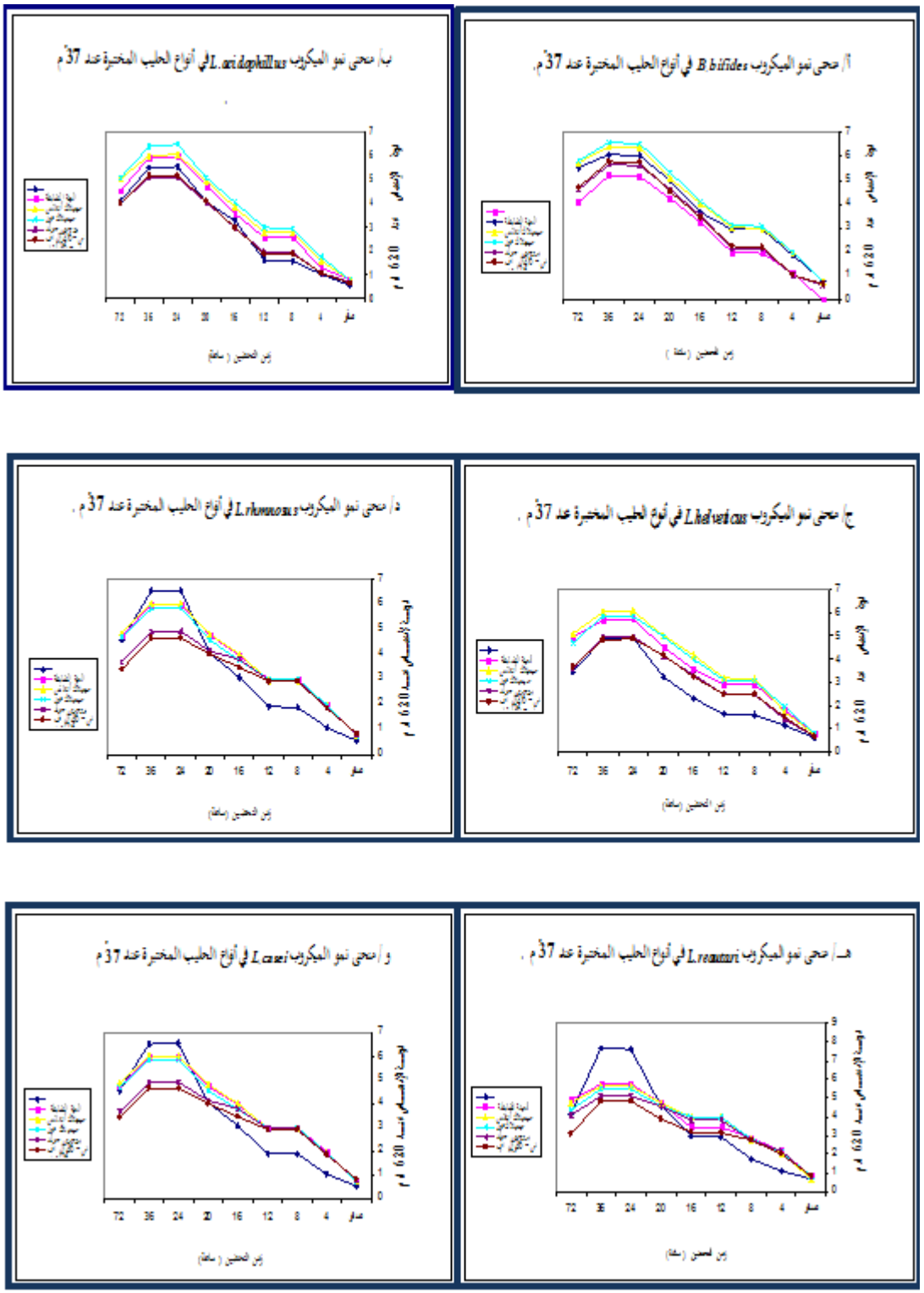

Fig. 1: Growth curves of the tested microbes in the different milk types at 37 ºC

Fig. 2: Microbial growth in the tested milk types at 37 ºC in relation to low pH

with new ones to ensure consistent anaerobic conditions for incubation.

The results illustrated in tables (2) and Fig. (1) that all the probiotics used have successfully grown in the nutrition medium to which the tested milk types were added. Growth of probiotics tested was characterized by the appearance of logphase starting from 8 hours after incubation and the growth was increased at 12 hours the 24 hours then was constant at 48 hours, thereafter a decline started, which indicated that the optimal time for growth of the tested probiotics is from 12 – 24 hours. It was found that in all the bacterial species (*L. helveticus, L. acidophilus, L. rhamnosus, L. casei, B. bifidum and L. reautari*) the maximum growth rate was observed at 24 hours, then kept constant at 48 hours in the two milk types (Cemelac ghain followed by Progress Gold) and the growth was higher in the tested milk types compared with the control. The minimum growth at the same time period was observed in the milk types (Milk S 26 – LF and Milk S – AR) and was lower than (8 – 9)% compared with the control.

Growth curve of probiotics gown in the tested milk types

The aim of this experiment was to study the growth of tested probiotics only on the tested milk types. Same steps of the previous experiment was used without addition of the nutrition medium and the growth was measured at the same time period using the color spectrum (Spectrophotometer) at the absorption wave length of 620 nm, and measuring the extent of variation in pH concentration using the pH- meter. Results shown in Fig. (2) indicated that the probiotics used have grown in all tested milk types and the Logphase also appeared starting from 8 hours after incubation, then the growth continued steadily until 12 then 24 hours then become constant at 48 hours, after that it declined. The least growth for the tested probiotics was observed at 4 hours. In all the bacterial species (*L. helveticus, L. acidophyllus, L. casei and B. bifidum*) the highest growth rate was observed at 24 hours and constant at 48 hours in Cemelac – Ghain followed by Progress Gold and Cemelac Advanced, where the growth was 1% higher in the tested samples compared with the control. The minimum growth at the same time period was observed in Milk S – 26 LF and Milk S – AR and were lower than that of the control samples with 7% and 9%, recectively. Reductions were found in (*L. reautari* and *L. rhamnosus*) with the percentages of 0.6% and 0.7% in the tested milk types, respectively compared with the control. Results also indicated the extent of variation in the concentration of pH during the incubation period of the tested probiotics, where the pH before incubation ranged from 7.2 – 7.87 in all the tested milk types and the changes were slight during the first 8 hours of incubation. Thereafter, the pH started to decline with the increase in growth till the end of the

Effect of temperature variation on microbial growth

The aim of this experiment was to study the effect of temperature variation on growth of the tested probiotics to determine the optimal temperature for growth. The best time period for the growth of each microbe individually was determined based on the results of the growth curve and the nutrition medium (MRS + the tested milk types) was prepared. The amount of 1 ml of the inoculum was used and inoculated in the nutrition medium, then incubated at different temperatures (4, 25, 37, 45, 55ºC). three replicates were used for each temperature treatment, then growth was measured by the color spectrum spectrophotometer at the absorbance wave length of 620 nm. The results illustrsted in Fig. (3) indicated that the optimum the growth of the tested probiotics is 37ºC then started to decrease with the increase in temperature. The least growth of the tested probiotics was observed at 5ºC all all results were significant.

At the temperature of 37ºC the highest growth level for the two probiotics (*L. reautari* and *L. helveticus*) in the milk type (Cemelac Advanced) where the growth reached 11.31 and 6.64, which represent 48% and 35%. The probiotics (*L. acidophilus, L. rhamnosus*, *L. casei*, and *B. bifidum*) displayed the highest growth rates in the milk type (Cemelac Ghain) where the growth rates were 7.56, 8.52, 7.88, and 5.92, respectively representing 37%, 30%, 50%, and 40%. At the same temperature, the probiotics (L. helveticus, B. bifidum and L reautari) displayed the least growth rate in the milk type (S

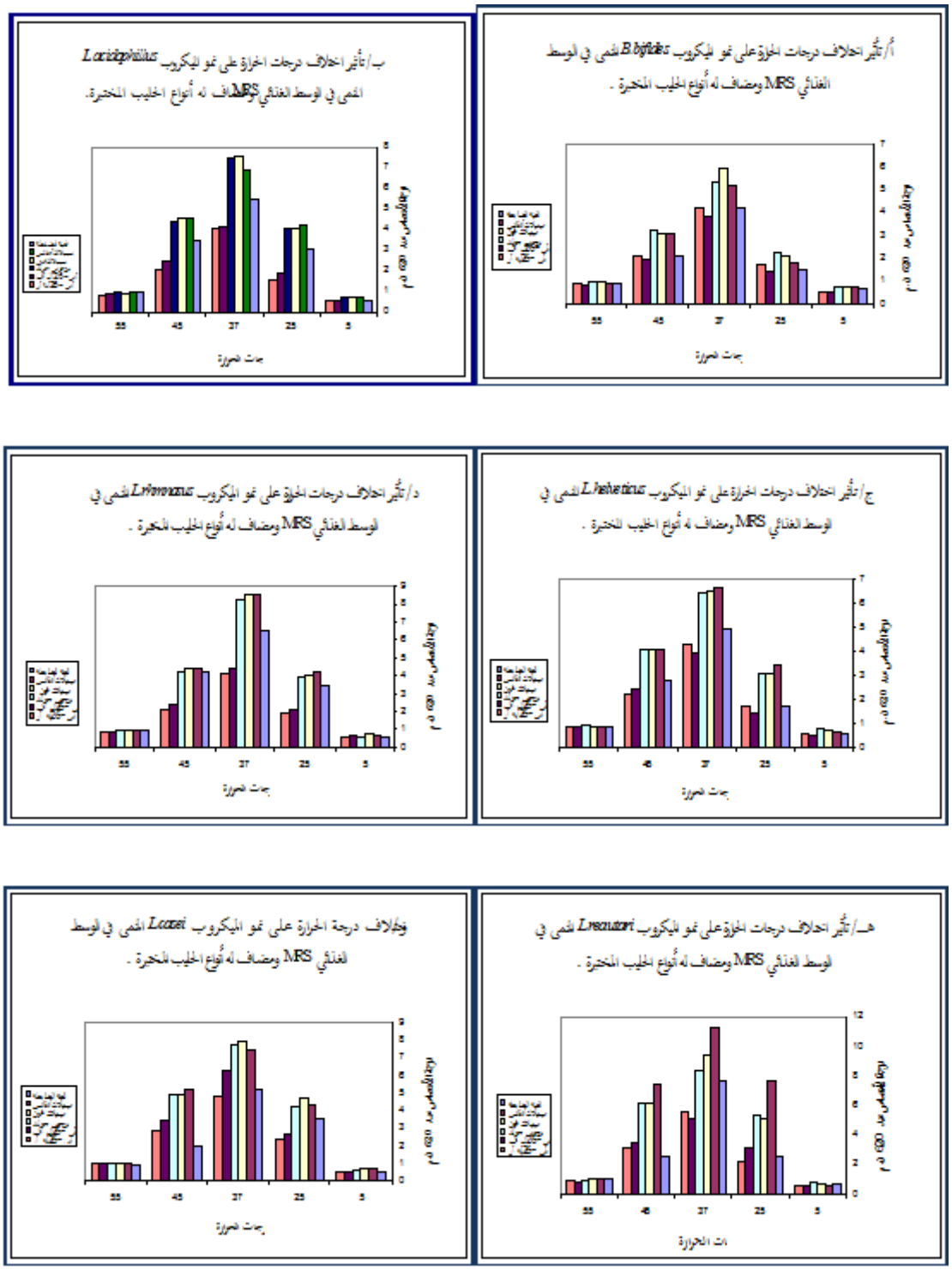

Fig. 3: The effect of the different temperature on the growth of the tested microbes

26 - LF) where it has reached 3.97, 3.82 and 5.1, respectively representing 81, 9, and 33%. However, the probiotics (*L. acidophilus*, *L. rhamnosus* and *L. casei*) have recorded the least growth rate in the milk type (S 26 - AR) where the growth rates were 4.1, 4.1, and 4.81, respectively representing 74%, 62%, and 92%

Effect of pH variation on microbial growth

The aim of this experiment was to study the effect of pH variation on the growth of the tested probiotics. The concentration of the pH of the nutrition medium (MRS + the tested milk types) was adjusted and the pH was (2, 4, 6, 9, 11). The microbe inoculum was added and then incubated at 37ºC. the microbial growth was measured by the color spectrum spectrophotometer at 620 nm absorbance wave length. Results indicated that the best growth was observed when the pH was 6 while the least growth was observed when the pH was 11.

DISCUSSION

The probiotic bacteria are defined as microorganisms which help in the microbial balance in the intestine. The two genera: *Lactobacillus* and *Bifidobacterium* are among the major genera, which are well known as probiotics generally characterized asrod-shaped Gram-positive. The probiotics have numerous health and nutritional advantages, including conservation for the natural flora in the human intestine, particularly in children [3,4]. In the present investigation, the microbes (*L. reautari*, *L. rhamnosus*, *L. acidophilus*, *L. casei*, *L. helveticus* and *B. bifidum*) were grown on different types of milk for healthy children. The milk type (Cemelac Advanced) for newly born infants, (Cemelac Ghain) for children at 6 months old and above and (Progress Gold) for children at the age of 1 – 3 years. Two kinds of milk were employed according to the pathogenic condition most spread among breast feeding children (vomiting and diarrhea) and these are: Milk S – 26 LF which is nutritionally improved with iron and free from lactose and this type of milk is normally used for infants since the delivery time. The second king of milk: Milk S – 26 AR, a milk powder which is used for breast feeding children who are suffering from vomiting and also used since the delivery time. When comparing the chemical composition for the mother's milk (natural breast feeding) with the artificial milk feeding (bottled milk feeding) for the milk types used it was found that the mother's milk contains all the essential nutritional substances required for growth of a child in a consistent fashion similar to that of the bottled milk where it was found that all the nutritional components in the mother's milk are considered relatively limited amounts and give rise to the ideal children growth compared with the bottled milk which is characterized by greater nutritional components which are commonly used in special pathogenic cases, however important constituents are lacking such as lactose. It was found that 90% of the beneficial bacteria is in intestine of the children who are depending on natural breast feeding whereas, the percentage of such bacteria is decreased to 30% in children depending on bottled feeding. One of the reasons might be the presence of some special sugar substances (carbohydrates) in mother's milk which lead to increasing the growth of probiotics which are working on developing the infant immune system and reducing the occurrence of sensitivity and inflammatory diseases. These sugar substances do not exist in the bottled milk and instead some carbohydrates and fatty substances which cause increment or overweight because these substances are present in great amount the child doesn't need for growth. In recent years, the components of children bottled milk were changed and the probiotics were added to increase the ability of probiotic bacteria and providing the special food for the probiotics which helps in multiplication and called Prebiotics, which are working on the growth of probiotics which ultimately leads to enforcement of the immune system in a natural way for the children[13]. All the tested probiotic (*L. reautari*, *L. acidophilus*, *L. casei, B. difidum, L. helveticus and L. rhamnosus*) were grown in the children milk types chosen for this investigation. The growth of all microbes was characterized by the appearance of logphase after 12 hours in all the milk types tested, which shows that the optimal time period for growth of the tested isolates is ranging between 12 – 48 hours and this means provision of enzymes for analyzing Lactose and protein in all the tested isolates. It was also found that these was a similarity in the products from protein analysis for the types of milk used. These results were not in agreement

with respect to the time period where the Log phase has reached has attained the maximum growth after 12 and 24 hours. This can be attributed to the types of probiotic used and the milk types, where the milk type is influencing the growth of probiotic and from the present findings it was found that the least growth rate s were found in Milk S – 26 LF and Milk S – AR compared to other milk types used. These results were confirmed by [14] where it was found that four types of bifidobacteria used (*B. bifidum* ATCC 15696, *B. longum* ATCC 15708, *B. infantis* ATCC 15697 and *B. breve* 15700) the Logphase for all types when grown in three types of breast feeding children at a time period of 8 – 12 and differ in its reaching to the Log phase according to its type and particularly the milk types used. It was found that the variation in pH were slight during the first 8 hours of incubation from 7.20 to 7.87 in all the tested milk types and thereafter the pH started to decline with the increase in growth till the end of incubation period (72 hours) where the pH was d" at the end of incubation period and this was attributed to the regulatory capacity for the milk types used, which in return is associated with the percentage and composition of protein and some mineral salts and there is a proportional relationship between the growth rate and acid production [15,16,17,18.] Regarding the effect of temperature the current results have indicated that the best temperature for optimal probiotics was 37ºC and this is the temperature at which the number of cells capable of growth is quite stable and this observation is in accordance with some researchers [6,18,19,20]. In contrast, the temperature of 37ºC has caused a reduction to the total number of probiotics[21] and in contrary it was reported that storage of dried camel milk at this temperature has led to decreasing the humidity until the 3rd month, and the minimum temperature for growth of the tested probiotic is 5ºC and at this temperature the microbial activity is decreased until it reached a point where the activity is totally ceased. The microbe doesn't die, but it assumes little activity on milk property during the storage period. In contrast, some researcher [21,22,23]. With respect to the effect of pH it was found that all isolates have displayed the highest growth at pH 6 and this is the suitable acidity level for growth of the bacterial isolates in milk and this is in conformity with studies and researches on probiotics. The probiotics has recorded the least growth under extreme acid (pH = 2) and alkaline (pH = 11) media. It was also observed that during the incubation period at 37ºC for 72 hours the pH was decreased in the nutrition medium reaching a value of 4.2 and is was due to the ability of microbes to produce acetic acid and milk acid in addition to a limited amount of Formic acid when metabolizing carbohydrates in the body and production of these acids lowers the pH which leads to the growth of pathogenic microorganisms and this is in agreement with [24].

REFERENCES

1. Margaret C. Benefits of breastfeeding for maternal and child health: an essay on the scientific evidence] *Pediatr Clin North Am* 2001; 48 (1): 13-34.
2. Hytten, F. Is breast feeding best. *Am J Clin Nutr* 1959; **7**(3): 259-63.
3. Hughes D, Hoover D. Viability and enzymatic activity of *bifidobacteria* in milk *J. Dairy Sci.* 1995; **78**(2): 268-76.
4. Abu- Taraboush H, AL- Dagal M, AL- Royli M. Growth, Viability and Proteolytic activity of Bifidobacteria in whole camel milk. *J Dairy Sci* 1998; **81**: 354-361.
5. Schrezenrneir J. de Vrese M. Probiotics, Prebiotics and synbiotics- approaching a definition. *Am J Clin Nutr* 2001; **73**: 361S-364S.
6. Helland M, Wicklund T, Narvhus J. Effect of ger-mination time on alpha amylase production and viscosity of maize porridge. *Food Res Int* 2002; **35**: 315-321.
7. Mcfarland L, Surawicz C, Greenberg R, Fekety R, Elmer, G, Moyer K, Melcher S, Bowen K, Cox J, Noorani Z, Harrington G, Rubin M, Greenwald D. Arandomized placed – controlled trial of *Saccharomyces boulardii* in combination with standard antibiotics for *Clostridium difficile* disease. *J Am Med Asso* 1994; **271**(24): 1913-1918.
8. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G,

Stolte M. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining resmission of ulcerative colitis. *Alimen Pharmacol Ther* 1997; **11**: 853-858.

9. Anand S, Srinivasan R, Rao L. Antibacterial activity associated with Bifidobacterium bifidum *Cult Dairy Pro J* 1985; **2**; 21-23.
10.. Scardovi V. The genus *Bifidobacterium.* Page 1418 in Bergey's Manual of systematic Bacteriology. J.G. Holt, ed.Wiliams and Wilkins Co., Baltimore, MD. 1986.
11. Mantere-Alhonen S, Nero K, Sippola L. Preliminary Studies on the antibacterial effect of some *bifidobacterium* ssp. and *Lactobacillus* ssp. *Meijeritiet Alkak* 1989; **47**(1): 19
12. Yazid A, Salina A, Shuhaimi M, Osman H, Normah J. Inhibitory effects of probiotics bacteria against selected food- borne pathogens. *Pakistan J. Biochemistry. Sci* 1999; **2**(3): 660- 663.
13. Moreno V J M. Probiotics in infant formulae. Could we modify the immune response ? *An Pediatr (Barc)* 2008; **68**(3): 286- 394.
14. Bubey UK and Mistry VV. Effect of bifidogenic factors on growth characteristics of bifidobacteria in infant formulas *J Dairy Sci* 1996; **79**(7):1156-63.
15. Sawaya W, Khalil J, Al-Shalhat A, Al-Mohammed H. Chemical composition and nutrition quality of camel milk *J. Food Sci* 1984; **49**: 744.
16. Desjardins M, Roy D, Goulet J. Growth of *bifidobacteria* and their enzyme profiles. *J. Dairy Sci*. 1990; **73**: 299.
17. Al- Saleh A, Hammad Y. Buffering capacity of camel milk. *Egyption J. Food Sci.* 1992; **29**(1): 85- 97.
18. Ostlie H, Helland M, Narvhus J. Growth and metabolism of selected strains of probiotic bacteria in milk. *Int. J. Food Microbio* 2003; **87**(12): 17-27.
19. Ballongue J. Bifidobacteria and probiotic action .pp. 357-428 in Lactic Acid Bacteria. Salminen S. and Von Wright A., eds. Marcel Dekker, New York, NY. 1993.
20. Rasic J, Kurmann J. *Bifidobacteria* and their role. *Experientia* 1983; **39**: 3.
21. Al-Salaeh A. Bifidus camel milk powder: Sensory evaluation and viability of Bifidobacteria. *J. King Saud Univ. Agric. Sci*. 2001a; **13**(1): 45-35.
22. El- Shafei S, Sharaf O, Abbas H, Ibrahim G. Properties of yoghurt- like product (proghurt manufactured by using propionica bacteria and yoghurt startes. *Annals of Agric. Sci. Moshtohor* 1995; **33**(3): 1073- 1082.
23. Kailasapathy K. Survival of free and encapsulated probiotic bacteria and their effect on the sensory properties of yoghurt. *LWT- food Sci Technol* 2006; **39**(10): 1221-1227.
24. Van de Guchte M, Ehrich S, Maguin E. Production of growth- inhibiting factors by *Lactobacillus delbrueckii. J. Appl. Microbiol* 2001; **91**(1): 147- 153.