Effect of Bile Salts and Acidity on the Viability of Some Lactic Acid Bacteria

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ABSTRACT

Probiotic microorganismis were found to affect beneficially the host by improving intestinal microbial balance. They have also many benefits such as reducing the risk of diarrhea, normalize bowel movements, enhancing immune functions, reducing cholesterol levels, reducing the risk of eczema, protection from cancer and relief of lactose intolerance symptoms.Some probiotic properties of identified strains of lactic acid bacteria (21 strains) which isolated from healthy breast-feeding infants were studied in the presence of different concentrations of bile salts (0.2, 0.3 and 0.4%) and different values of pH (4, 3 and 2). The results as compared to control revealed that 11 strains of *Lb. fermentum* and all strains of *Lb. salivarius* exhibited variant growth at all concentrations of bile salts. On the other hand, all strains of *Lb. fermentum* and 8 strains of *Lb. salivarius* were tolerant to the changing in pH. They kept their viability at pH 3.0 and 4.0.

Keywords: probiotics, bile salts, acid tolerance, Lb. salivarius, Lb. fermentum, LAB.

INTRODUCTION

During last years, numerous studies have been undertaken to obtain scientific evidences for the beneficial effects of fermented foods containing probiotic bacteria (Renault, 2002, Rafter, 2002). At the present, a large number of dairy products are presented on the market and are being promoted with health claims based on several characteristics of selected strains belonging to the genera *Lactobacillus* and *Bifidobacterium* (Shah, 2000).

To provide health benefits, the suggested concentration for probiotic bacteria is 10^6 CFU/g of a product (Robinson, 1987). Viability and survivability of probiotic bacteria are the most important parameters in order to provide therapeutic functions. Several factors have been claimed to affect the viability of probiotic bacteria in fermented dairy foods, i.e. yoghurt and fermented milks. Moreover, the tolerance to human gastric transit constitutes an important selection criterion for probiotic bacteria (Goldin & Gorbach, 1989, Ouwehand *et al*., 1999). Probiotic related microorganisms ingested with food begin subjected to successive stress factors that influence their survival (Marteau *et al*., 1993) during their transit through the gastrointestinal tract the time reported from entrance to release from the stomach is about 90 min (Berrada *et al*., 1991), but further digestive processes have longer residence times. Bacterial stress starts in the stomach juice, at pH as 1.5 (Lankaputhra & Shah, 1995). Bile salts secreted in the small intestine reduce the survival of bacteria by impairment their cell membranes, whose major components are lipids and fatty acids and these modifications may affect not only the cell permeability and viability, but also the interactions between the membrane and the environment (Gilliland *et al*., 1984, Gilliland, 1987). Therefore before a probiotic can benefit human health it fulfills several criteria such as the ability to tolerate acid and bile salts as well as to grow in the lower intestinal tract (Hirayama & Rafter, 2000).

So, the first tool in the selection of probiotic strains is represented by *in vitro* methods aiming to ascertain the ability to survive passage through the upper gastrointestinal tract and arrive alive at its site of action (Saarela *et al*., 2000). The present research was tailored to study the ability of *Lb. fermentum* and *Lb. salivarius* to grow at different bile salts concentrations and at different pH levels.

MATERIALS AND METHODS

Test organisms

Twenty one isolates were obtained from healthy, breast-feeding Egyptian infants (3-6 m old) and identified as follows: (12 strains) *Lactobacillus fer-*

mentum, (9 strains) *Lactobacillus salivarius*. The strains were grown in MRS broth at 37°C. Organisms were subcultured every 18 hr or 14 hr twice before experimental use. Bacterial stock cultures were stored at – 80°C in 15% (v/v) glycerol.

Bile tolerance

All strains were evaluated for rapidity of growth in MRS (Biolife, Milano, Italy) broth with and without bile salts at 37°C. Overnight cultures were inoculated 10% (v/v) into MRS broth and MRS broth containing 0.2, 0.3 and 0.4% (w/v) bile salts and incubated at 37°C.

Development of bacterial growth was followed for 6 hr by measuring the absorbance at 650 nm (A_{650nm}) using a spectrophotometer at one hour interiors. Comparison of cultures was based on their growth rates in each broth. The experiments were conducted twice in duplicates.

Acid tolerance

Overnight cultures were previously prepared by inoculated (0.1% v/v) into MRS broth at 37°C for 16 hr to until logarithmic phase. Cultures were inoculated (10% v/v) into MRS broth previously adjusted to pH (2.0, 3.0 and 4.0±0.1) with HCl. The mixtures were incubated at 37°C and growth development was followed for 6 hr by measuring the absorbance at 650 nm (A_{650nm}) using a spectrophotometer (Pharmacia LKB NOVASPEC II). Comparison of cultures was based on their growth development in each broth and compared with MRS broth (pH 6.6) inoculated with the previous strains. The experiments were repeated two times in duplicate.

RESULTS AND DISCUSSION

Bile salts tolerance

Figures (1 and 2) show the effect of different bile salt concentrations (0.2, 0.3 and 0.4% w/v). Generally, bile salt concentration at 0.4%, exhibited the most suppression to all *Lb. fermentum* and *Lb. salivarius* strains as compared to control values at 6 hrs. It is noted that all strains were able to grow in the presence of 0.2% bile salts. Data in Fig. (1) show that *Lb. fermentum* (5) was less bile tolerant than the other strains tested. It was clear that this strain was able to grow in the precence of bile salts but with little decrease in the growth as compared with the control. Also, the results revealed that *Lb. fermentum* (1, 2, 3, 4, 6, 7, 8, 9, 10, 11 and 12) showed less difference in their growth in the media with bile salts. It was clear that the growth rate in the presence of bile salts was almost similar to the absence of bile salts.

Previous work done by Charteris *et al*. (1998) and Pereira & Gibson (2002) reported that, the small intestinal transit tolerance of bile salts-resistant lactobacilli was found to be strain-dependent. The majority of strains was intrinsically resistant to simulated pancreatic juice and showed no reduction in viability for up to 4 hr. However, minorities of strains were sensitive and could be divided into tow groups: The first was characterized by complete or progressive loss of viability during exposure and was represented by strain *B. adolescentis* 15703T. The second was characterized by a rapid reduction in viability on exposure followed by recovery of viability during prolonged exposure and was represented by *Lb. fermintum* KLD.

The results in Fig. (2) show that *Lb. salivarius* (1, 2, 3, 4, 5, 6, 7, 8 and 9) were more resistant than the other strains tested. It was obvious that these strains were able to grow in the presence of bile salts with little decrease in the growth as compared with control values.

Resistance to bile salts is generally considered as an essential property for probiotic strains to survive the conditions in the small intestine. The presence of bile salts in the environment of bacteria cultures is much more detrimental than effect of pH 3.0. The choice of the bile concentration selected for our screening (0.4% Oxgall solution) was based on its being equivalent to the physiological concentration in the duodenum or the human bile juice (Brashears *et al*. 2003). Many authors investigated the effect of bile on survival of LAB. Kim *et al*. (1999) examined the effect of bile concentration in the range of 0-0.4% on the *Lb. lactis* survival and they reported inhibiting effect of bile at concentration over 0.04%. They detected that all bacterial cells were killed at 0.2% and higher (Olejnik *et al*. 2005). Comparing to this study, our experiments showed much more resistance to detrimental actions of bile salts where the viability of strains of *Lb. plantarum* and *Lb. fermentum* seemed to improve when exposed to high levels of oxgall (0.4%).

Acid tolerance

The effects of acidity on the growth of the identified strains are presented in Figs. (3 and 4). The results in Fig. (3) indicate that *Lb. fermentum*

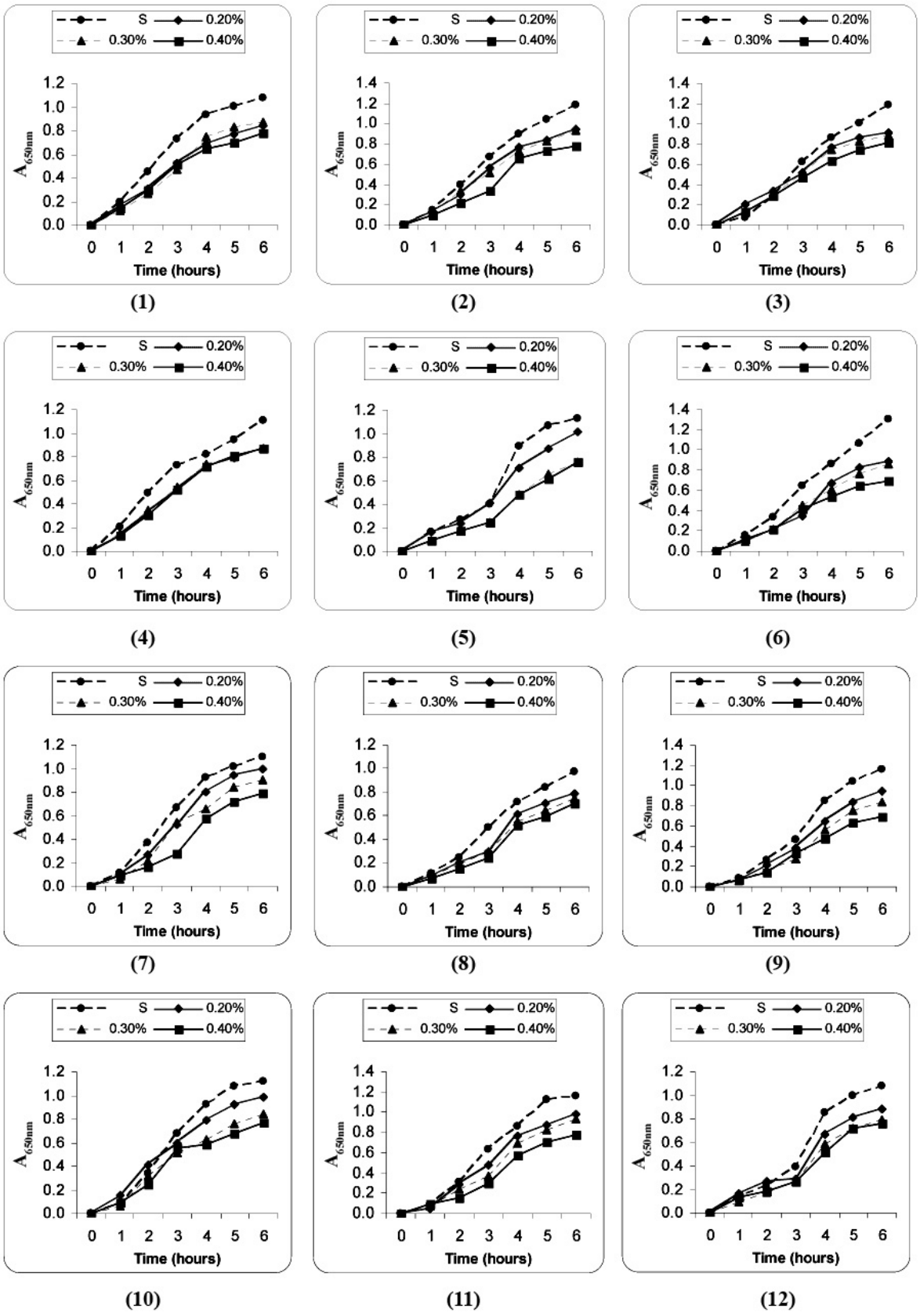

Fig. 1: Growth development of *Lb. fermentum* (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) in standard and supplemented MRS broth with different bile salts concentrations for 6 hrs at 37°C

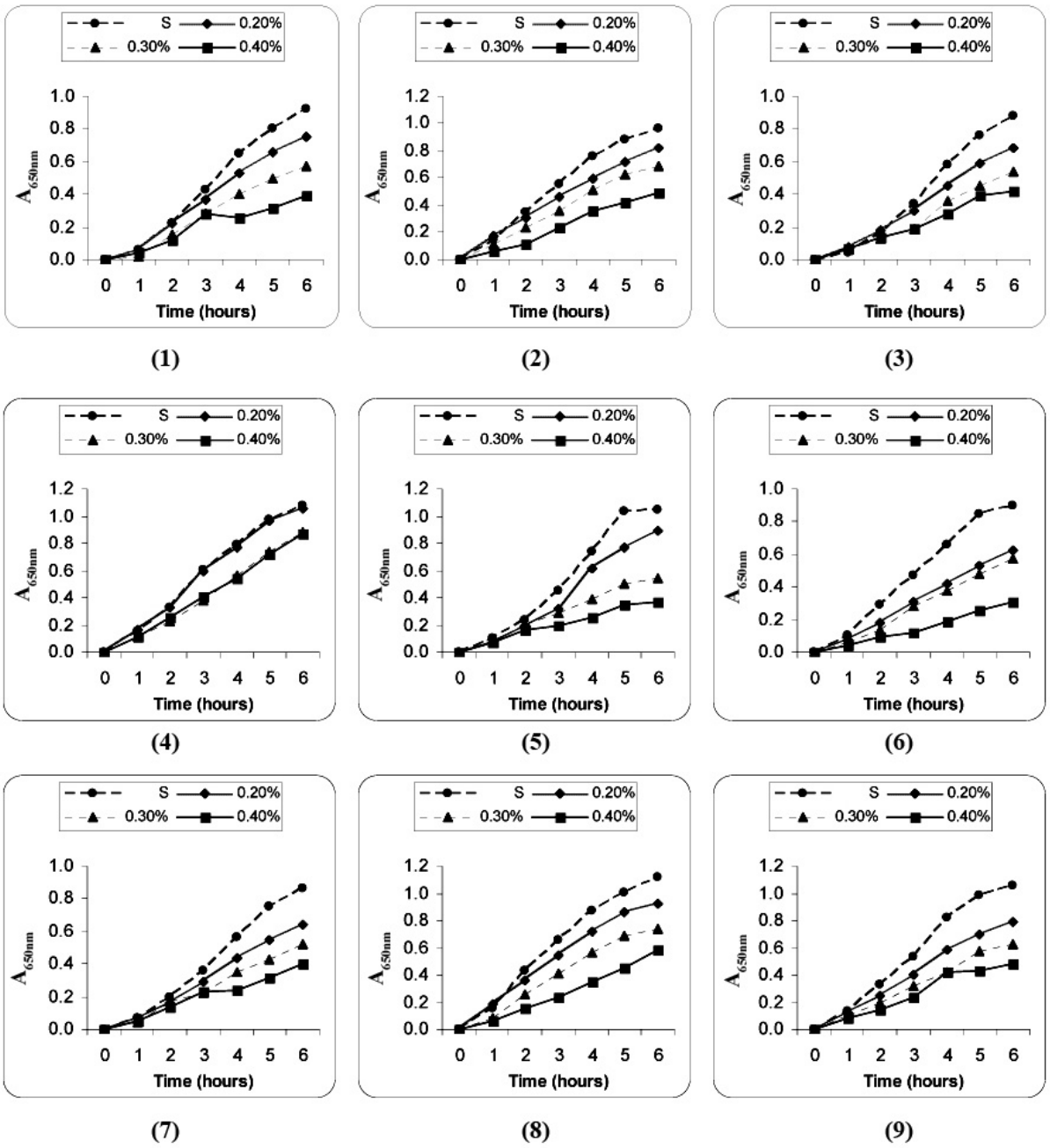

Fig. 2: Growth development of *Lb. salivarius* (1, 2, 3, 4, 5, 6, 7, 8 and 9) in standard and supplemented MRS broth with different bile salts concentrations for 6 hrs at 37°C

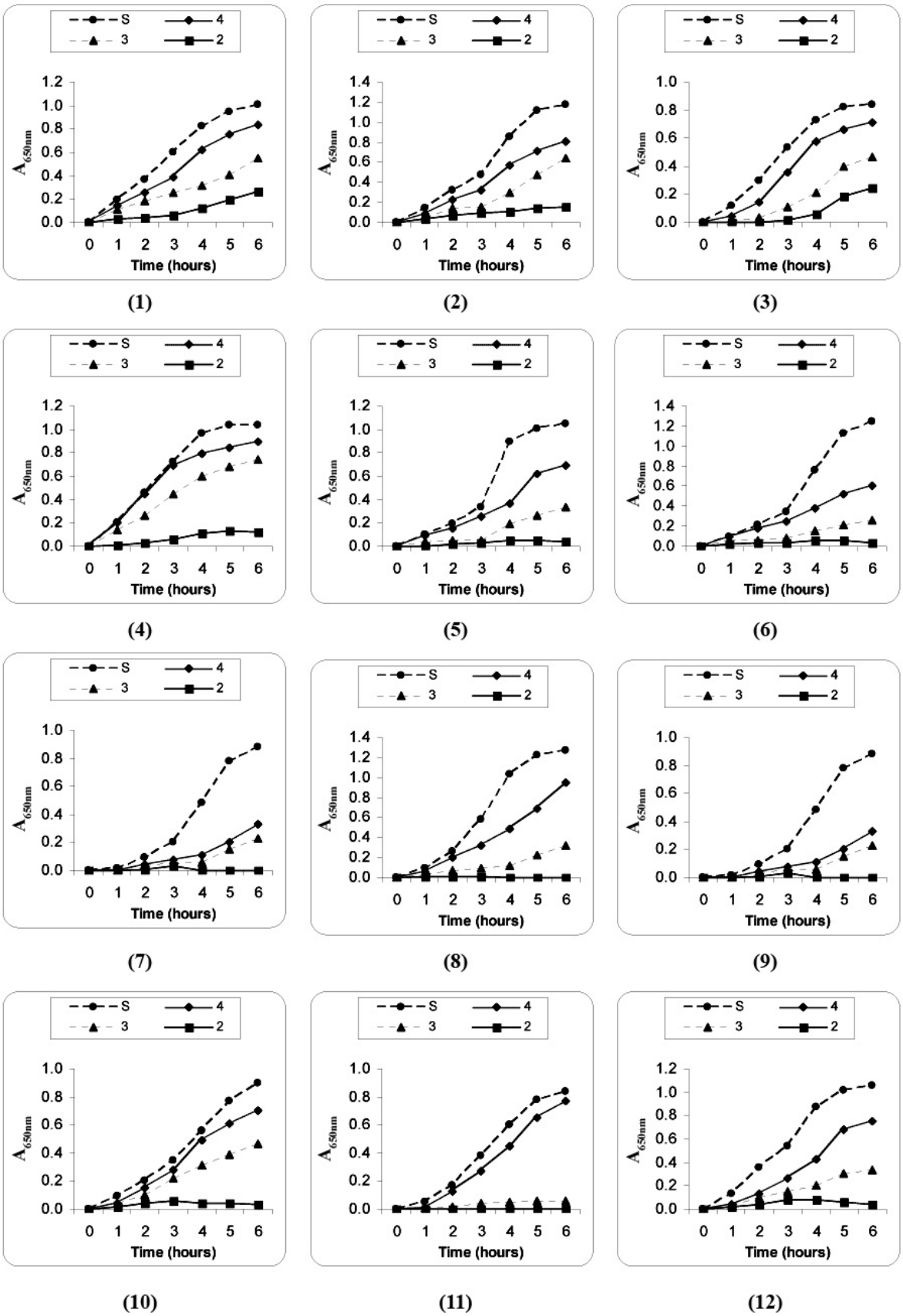

Fig. 3: Growth development of *Lb. fermentum* (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) in standard and acidified MRS broth for 6 hrs at 37°C

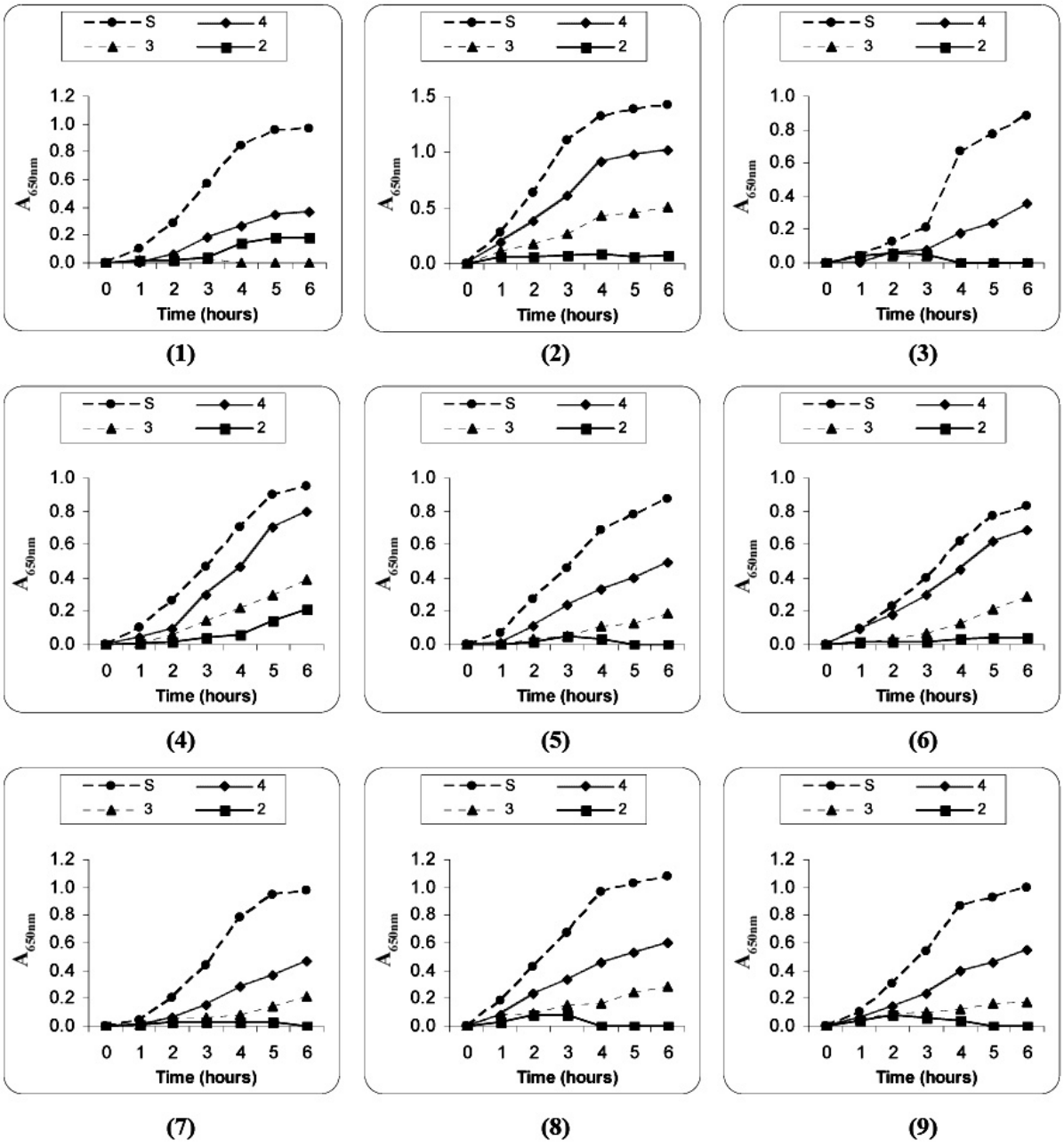

Fig. 4: Growth development of *Lb. salivarius* (1, 2, 3, 4, 5, 6, 7, 8 and 9) in standard and acidified MRS broth for 6 hrs at 37°C

(2, 3, 5, 6, 7, 9, 10, 11 and 12) were more tolerant to the changing in pH than the previous group. Viability could be measured at pH 4.0 and 3.0. *Lb. fermentum* (1, 4 and 8) were the most tolerant strains to changes of pH values. These results are in agreement with those reported by Pereira & Gibson (2002) who reported that human isolates of *Lb. fermentum* were able to maintain viability at pH 2.

The results in Fig. (4) demonstrate that *Lb. salivarius* (3) was less sensitive strain to low pH, the culture could not grow at pH 3.0 and 2.0 at 37°C. While, *Lb. salivarius* (1, 2, 5, 6, 7, 8 and 9) were more tolerant to the changing pH. Viability could be measured at pH 4.0 and 3.0. *Lb. salivarius* (4) was the most tolerant strain to changes of pH values. This result was in agreement with Rönkä *et al*. (2003).

In conclusion, Lactobacilli of intestinal origin are considered intrinsically resistant to acid environments and are often employed in fermented foods as probiotics (Cocoran *et al*. 2005). Probiotic tests conducted on *Lb. fermentum* indicated its potential as good probiotic candidate tolerate the gastrointestinal tract acid and bile conditions. These results are in accordance with those previously reported by Pereira & Gibson (2002) and Pereira *et al*. (2003). Moreover, they found that no undesirable microbial-metabolic characteristics have been caused which could hamper its use as a probiotic for human consumption.

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تأثير أملاح الصفراء والحموضه على حيوية بعض بكتريا حامض اللاكتيك

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وجد أن بكتريا البروبيوتك التي يمكنها أن تتواجد بالقناة الهضمية لها دور مهم في الحفاظ على التوازن البكتيري بالقناة الهضمية علاوة على فوائد أخرى عديدة مثل تقليل خطر الإصابة بالإسهال، تحسين الجهاز المناعي، و تقليل حدوث الإصابة بالحساسية و الحماية من السرطان كما إنها أيضاً تقلل من أعراض الحساسية التي يسببها سكر اللاكتوز باللبن نتيجة نقص إنزيم اللاكتيز والحفاظ على حركة الأمعاء بصورة طبيعية.

تمت دراسة بعض الصفات الخاصة ببكتريا البروبيوتك لبعض السلالات (٢١سلالة)، والتي عزلت من أطفال أصحاء – حديثي الولادة (٣–٦ أشهر). والتي تم التعرف عليها كما يلي: ١٢ سلالة تابعة لبكتريا *Lb. fermentum* و٩ سلالات تابعة لبكتريا *Lb. salivarius*. حيث تم دراسة مقدرة السلالات على تحمل تركيزات مختلفة من أملاح الصفراء (٠,٢ , ٠,٣ ، ٠,٤٪) ومقدرتها على البقاء حية عند قيم مختلفة من الـ pH (٢، ٣، ٤) وكانت النتائج عند مقارنتها بالكنترول كما يلى:

استطاعت ١١ سلالة تابعة لبكتريا *Lb. fermentum* وجميع السلالات التابعة لبكتريا *Lb. salivarius* النمو على تركيزات مختلفة من أملاح الصفراء بدرجات متفاوتة، بينما تمكنت كل السلالات التابعة لبكتريا *Lb. fermentum* و٨ سلالات من بكتريا *Lb. salivarius* من مقاومة الحموضة حيث استطاعت هذه السلالات الاحتفاظ بحيويتها على رقمى pH ٣، ٤ .