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Influence of Temperature on Growth Pattern of *Lactococcus lactis*, *Streptococcus cremoris* and *Lactobacillus acidophilus* Isolated from Camel Milk

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Abstract: The various strains of lactic acid producing bacteria such as *S. cremoris* and *L. lactis* and *L. acidophilus* isolated from the camel milk, were examined for their optimum growth temperatures, generation time, pH and acidity. All these strains grew rapidly at their respective optimum temperatures; however their generation time and pH varied. *L. acidophilus* strains showed greater generation time varied from 123.01 to 135.08/min and showed 0.85-0.96% acidity and 4.40-4.81 pH values, respectively while for *L. lactis* and *S. cremoris* generation time varied from 60.17 to 87.16/min and 64.9-80.0/min, respectively. *S. cremoris* and *L. lactis* produced 0.51-0.55% and 0.56-0.58% acidity and showed 4.7-4.79 and 4.80-4.89 pH values at their respective optimum temperatures. The optimum growth temperature for *L. lactis* and *L. acidophilus* was found to be 37 to 40°C while for *S. cremoris* was 37°C. There was inverse relationship between acidity, pH and generation time. It was observed that there was a correlation between the acidity, pH and generation time and there was also an inverse relationship between total titratable acidity and pH values of each strain.

Key words: *S. cremoris*, *L. lactis*, *L. acidophilus*, generation time, camel milk

INTRODUCTION

Optimum temperature is the basic characteristic of all lactic acid bacteria which helps to differentiate them from each other because temperature controls the growth of bacteria. Temperature affects the generation time of bacteria according to the phase of growth as each species has a unique optimum growth. *L. lactis* produces lactic acid quickly and reduces manufacturing time. *S. cremoris* grows more slowly and produces well-flavored cheese. Therefore, a starter may consist of suitably paired strains of *L. lactis* and *S. cremoris*. Mixed mesophilic starters produce more acid than the individual bacteria and largely inhibit *E. coli* and *Staphylococcus aureus*. Mesophilic bacteria grow rapidly and high populations develop within hours at temperature between 20-30°C. Lactic acid produced by lactic acid producing bacteria is a good method for the production of acid. Acid production is slow or absent at temperature less than 20°C. Temperature growth range is between 10-42°C, growth is inhibited at temperature greater than 39°C and optimum growth range is between 20-35°C. *Lactococcus lactis* subspecies *cremoris* is more salt sensitive (<4%) and more temperature sensitive (40°C) than *Lactococcus lactis* subspecies *Lactis* (Ellenton, 1999).

Optimum growth temperature is 30°C for the members of the genus *Lactococcus* and they can grow at temperatures as low as 10°C but not at 45°C. (Batt, 1999). The optimum growth temperature for lactobacilli lies between 30 and 40°C but they can grow at temperatures ranging from as low as 5°C to an upper limit of 53°C, depending on the species (http://www.positiveaction.co.uk). During milk fermentation processes, lactic acid bacteria are exposed to various environmental stress conditions, such as temperature fluctuations, acid, pH, high osmotic pressure and absence of available nutrients. Many of these conditions will often coincide. Like other bacteria, lactic acid bacteria have evolved intricate stress response systems enabling them to adapt to adverse conditions in order to survive. The stress responses of the industrially important species *Lactococcus lactis* have gained increased interest in recent years, reports include studies of responses to heat and cold shock, low pH, UV light, salts, starvation, oxidation and DNA damage chloride (Sanders *et al*., 1999; Van de Guchte *et al*., 2002).

Ronald (2000) also reported that these bacteria are mesophilic in nature. Marr and Ingraham (1962) reported that cultures produced at low temperature show higher survival rate during freezing. The optimum growth temperature for culture propagation is the basic requirement for obtaining the cell concentrate. Champagne *et al*. (1991) reported that the pH must be controlled at the two levels, i.e., during the cell growth and in the suspension medium. The pH of the medium

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should be adjusted so as to fall in the range of 5.5-7.0. Growth of *S. cremoris* under controlled pH not only resulted in higher biomass yield but also increased survival after freezing. Breheny *et al.* (1975) also reported that the growth of *S. cremoris* was inhibited by high temperature.

The main objective of the present study was to determine the optimum growth temperature and generation time of different strains of lactic acid producing bacteria isolated from camel milk, as growth pattern or generation time depends on optimum growth temperature of bacteria. It is more important in fermented milk products like yogurt, Swiss and various Italian cheeses, in which starter culture of lactic acid producing bacteria has been used for many years.

MATERIALS AND METHODS

Sample collection: Present study was conducted in 2002 at Dairy Technology Research Laboratory (DTRL) of Animal Sciences Institute, NARC, Islamabad. Twelve camel milk samples were collected from Barani Livestock Production Research Institute (BLPRI), Kherimurat, Fateh Jang, District Attock. Milk samples were collected in sterile test tubes, put into the ice box and brought to the Dairy Technology Research Lab for microbiological study and evaluation for different biochemical properties.

Cultures: Bacterial strains were isolated and identified from camel milk in our previous study. Cultures were maintained in reconstituted nonfat dry milk (11% solid wt/vol) plus 0.075% Bacto Litmus (Difco).

Optimum growth temperature determination: The method of Kanasaki *et al.* (1975) was used to determine optimum growth temperature of all bacterial strains in milk. This method allowed for turbidometric monitoring of bacterial growth in milk.

pH and total titratable acidity determination: The pH values of milk were determined by the method as given in Anonymous (1990). Electronic digital type pH meter (model Beckman # 44) was used for pH determination of the above mentioned tubes containing milk cultures. pH was recorded at hourly interval uptil 7 h of incubation period. Total titratable acidity expressed as % lactic acid was measured by the method of Atherton and Newlander (1977).

RESULTS AND DISCUSSION

The various strains of lactic acid bacteria isolated from the camel milk were examined for their optimum growth temperatures, generation time, pH and acidity. The results of all these experiments are presented in graphs. It is clear from the results that except three out of four strains of *S. lactis* and all the three strains of *S. cremoris* and three strains of *L. acidophilus* had an optimum growth temperature at 37°C, while the exceptions out of four *Streptococci* and *Lactobacilli* strains, strain no 23 of *L. lactis* and strain no 22 of *L. acidophilus* had an optimum growth temperature at 40°C as shown in Fig. 1 and 7 respectively.

It was observed in this study that there is a correlation between the acidity, pH and generation time (Fig. 2 and 3). The strains of *L. lactis* 18, 20 and 22, had 68.16, 65.68 and 60.17 min generation time and produced 0.58, 0.56 and 0.57% acid and had 4.88, 4.89 and 4.82 pH values at 37°C, however strain 23 deviates from others strains as it had 87.16 min generations time and produced 0.56% acid and 4.80 corresponding pH at 40°C. The three tested strains of *S. cremoris* showed similar correlation at 37°C.The strains 18, 20 and 22 had 80.0, 64.90 and 69.30 min generation times and produced 0.51, 0.52 and 0.55% acid and 4.79, 4.77 and 4.71 pH, respectively.

Four strains of *L. acidophilus* were tested. The 18, 20 and 23 strains tested had 135.08, 123.01 and 126.05 min generation times and produced 0.94, 0.95 and 0.96% acid, respectively and their corresponding pH values were 4.42, 4.41 and 4.40 at their optimum growth temperatures 37°C, where as strain 22 had 125.54 min generation time and produced 0.85% acid and 4.81 pH at 40°C. It remarks that there is a correlation between acidity, pH and generation time.

Growth patterns at optimum temperatures: The results presented showed the pattern of growth of tested strains at their respective optimum temperatures were 37 and 40°C. It is clear that all these strains in lag phase, in first two hours, showed less growth where after they showed rapid growth. It was observed from Fig. 1 that *L. lactis* 18, 20 and 22 strains showed rapid growth at 37°C and slow growth at 40°C and 43°C except strain 23 that showed maximum growth at 40°C as shown in Fig. 1.

The results for *S. cremoris* that strains 18, 20 and 22 grow rapidly at 37°C while slow growth was observed at 40 and 43°C as shown in Fig. 4. The Fig. 7 indicates the results for *L. acidophilus* 18, 20 and 23 that they showed rapid growth at 37°C, while at 40 and 43°C their growth was slow except strain 22 that showed rapid growth at 40°C as shown in Fig. 7.

Patterns of acid production: The results of the experiments related with the rate of acid production by the studied microbes are presented in graphs along with the corresponding pH values. These experiments were

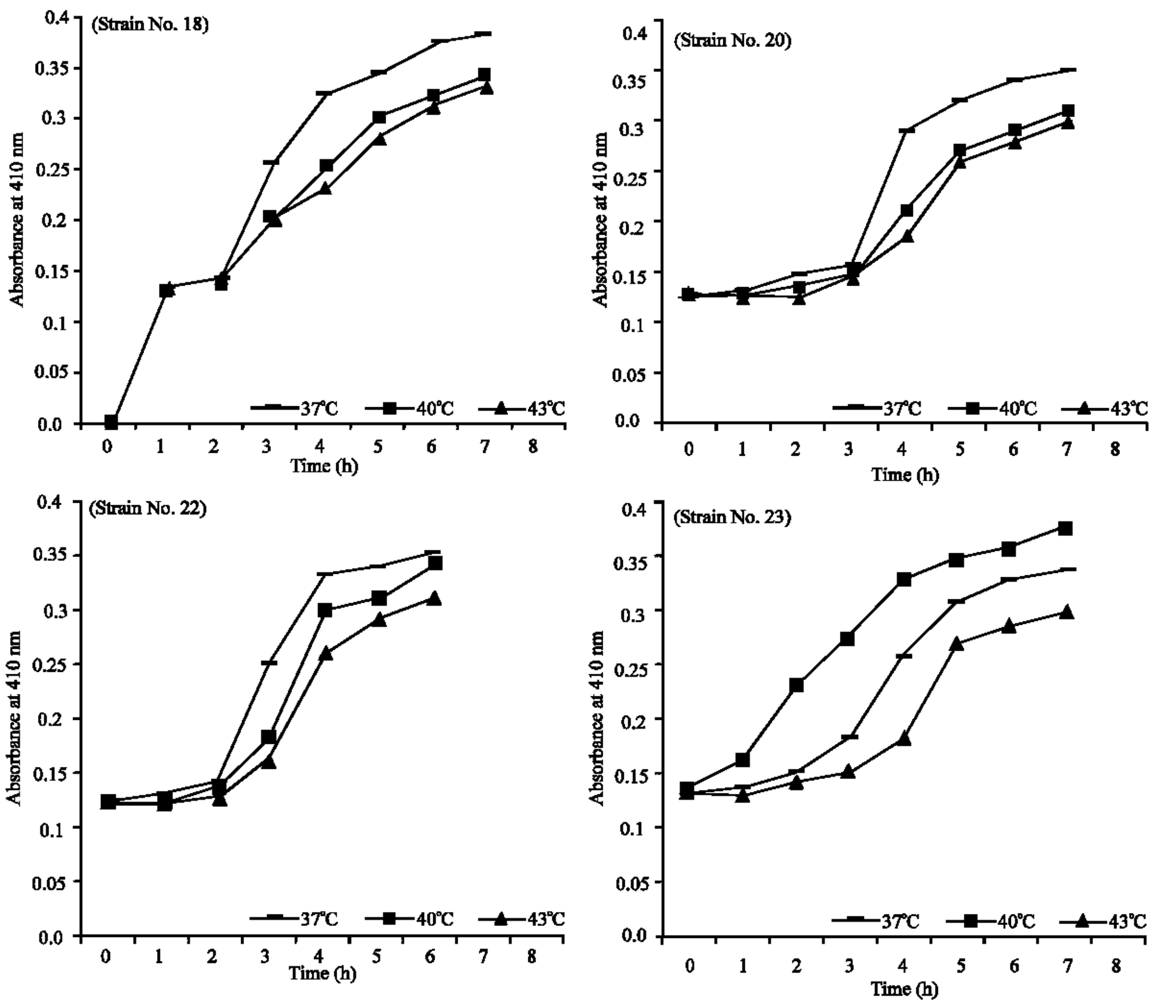

Fig. 1: Growth of *L. Lactis* at different temperatures

performed at the respective optimum growth temperatures and the estimations were made at hourly intervals. The results indicate that there was an inverse relationship between total titratable acidity and pH values of each strain. It appeared from graphs that strains 18, 20 and 22 of *S. cremoris* produced maximum acidity and lowest pH values at 37°C as shown in Fig. 5 and 6.

The results of graphs showed that *L. lactis* strains 18, 22, 20, produced maximum acidity and lowest pH values at 37°C whereas 23 produced maximum acidity and lowest pH values at 40°C as shown in Fig. 2 and 3. It was further observed from graphs that *L. acidophilus* strains 18, 20 and 23 produced maximum acidity and lowest pH values at 37°C whereas strain 22 produced maximum acidity and lowest pH values at 40°C as Shown in Fig. 8 and 9.

The results obtained from the present study related with optimum growth temperature indicated that there is a correlation between generation time, acidity and both these factors depend upon the optimum growth temperature. The strains that have more generation time produce more acid at their respective optimum temperature. It was further observed that there was a difference in generation time between the species of *Streptococcoi*. The *L. lactis* has higher generation time than *S. cremoris*. The difference in generation time is also observed among the *Streptococci* and *Lactobacilli* genera. The generation time of *L. acidophilus* strains is higher than *S. cremoris* and *L. lactis*. It was also observed that *L. acidophilus* coagulate milk earlier at same optimum temperature and *L. acidophilus* produce more acid than *S. cremoris* and *L. lactis*. All these strains produce maximum acid at optimum growth temperature. These observations are supported by Sampolinski *et al.* (1978) he found that optimum growth temperature is one of the unique characteristics of bacteria. This trait would be expected to influence the growth compatibility of

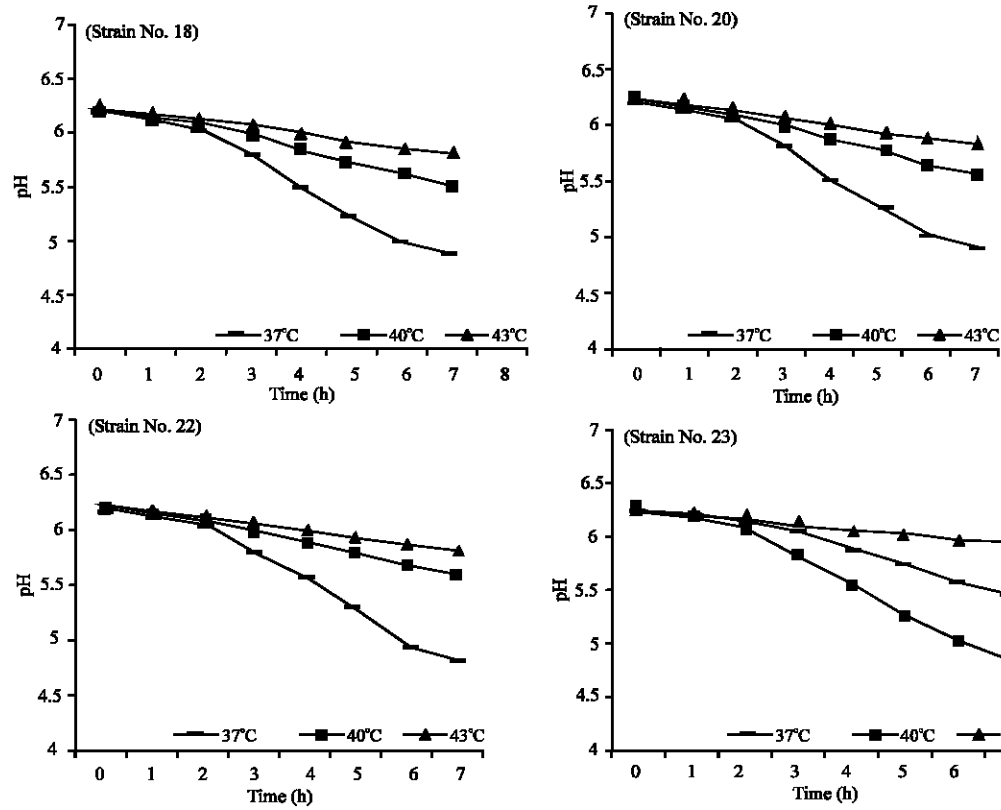

Fig. 2: Change in pH of *L. Lactis* at different temperatures

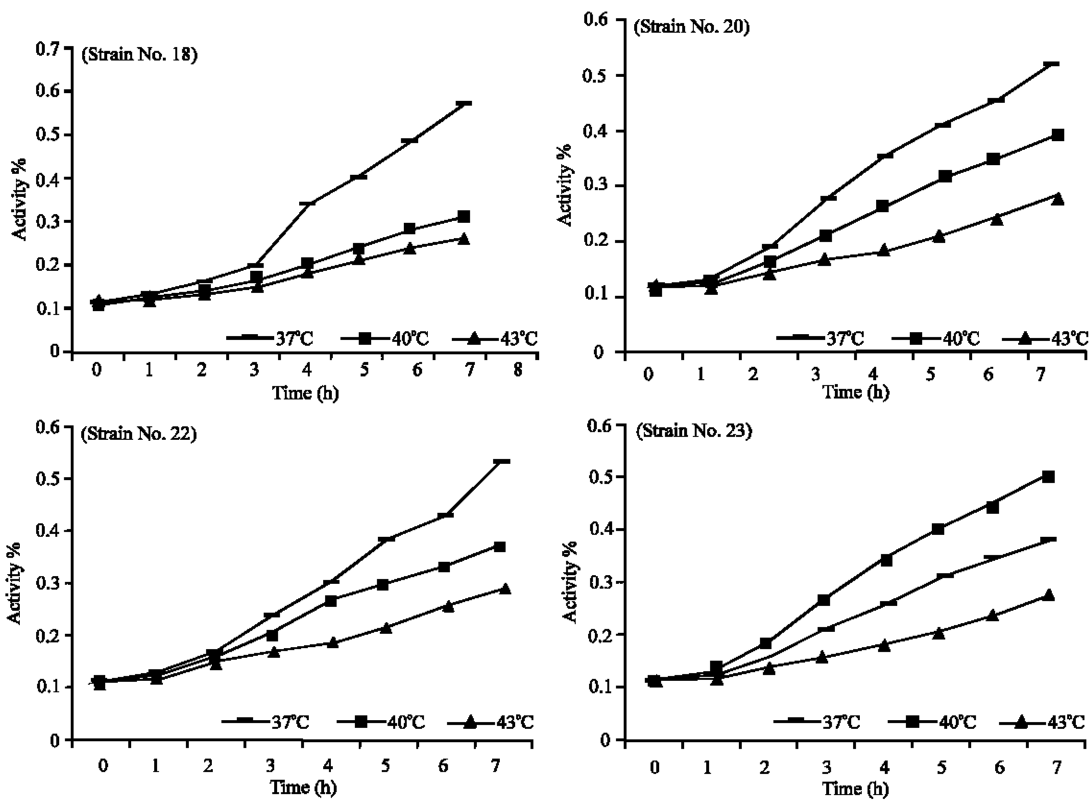

Fig. 3: Change in acidity of *L. lactis* at different temperatures

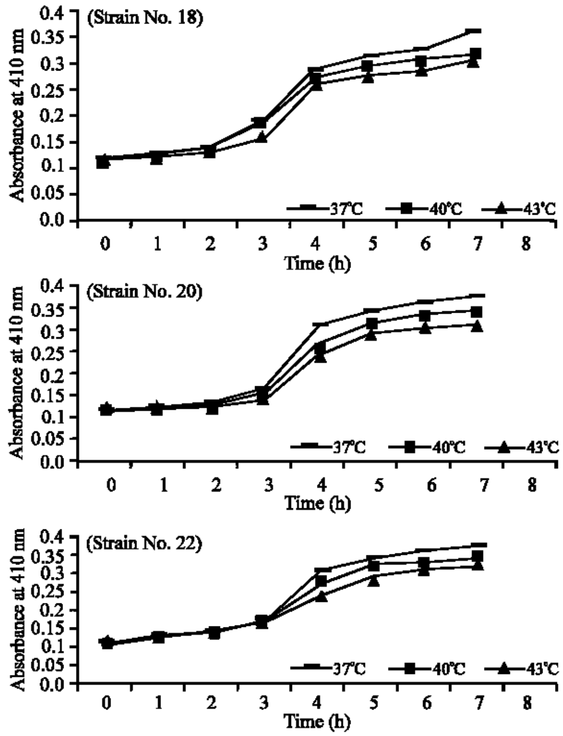

Fig. 4: Growth of *S. cremoris* at different temperatures

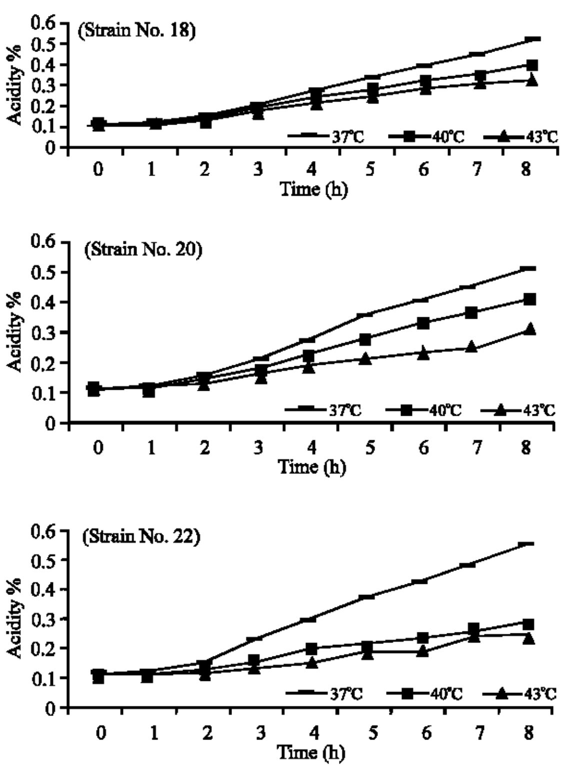

Fig. 5: Changes in acidity of *S. cremoris* at different temperatures

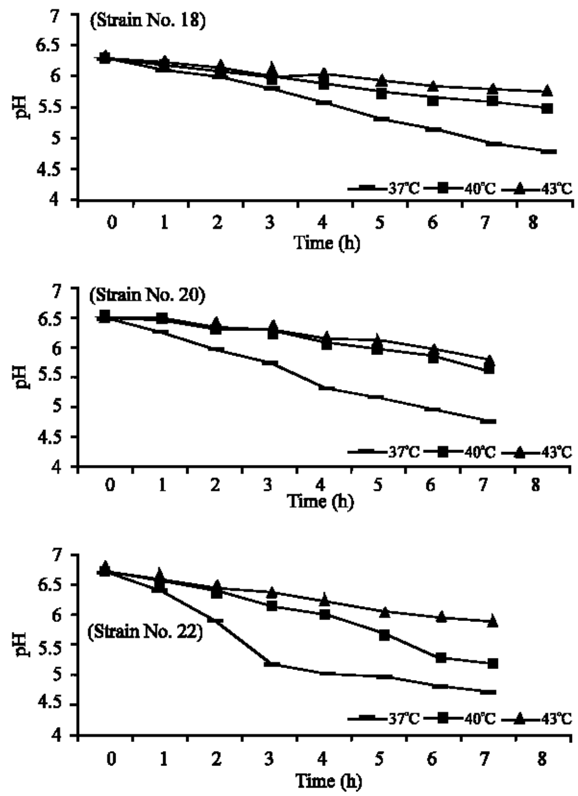

Fig. 6: Changes in pH of *S. cremoris* at different temperatures

strains especially those with different optimum growth temperature.

These strains of *L. lactis* showed maximum growth at 37°C and thus they have greater generation time at this temperature, as they are mesophilic in nature. Only one strain showed maximum growth at 40°C. Same is the case, in *L. acidophilus* that these strains have optimum growth at 37°C and one strain showed maximum growth at 40°C. The variation of optimum temperature among the *L. lactis* and the *L. acidophilus* strains may be due to difference in their genetic make up. Jeffery (1985) reported that some of the properties essential for successful milk fermentation are enclosed by genes located on plasmid DNA. Furthermore, it was observed by Yu *et al.* (1983) that if the plasmid profile of the two organisms is similar or even identical, there may be differences in their nucleotide composition, nucleotide sequence or even both. The *S. cremoris* strains are also mesophilic in nature and have maximum growth and acidity at 37°C. The present study also showed that there was an inverse relationship between titrateable acidity and pH values of each strain at different time intervals. Growth temperature is correlated with the

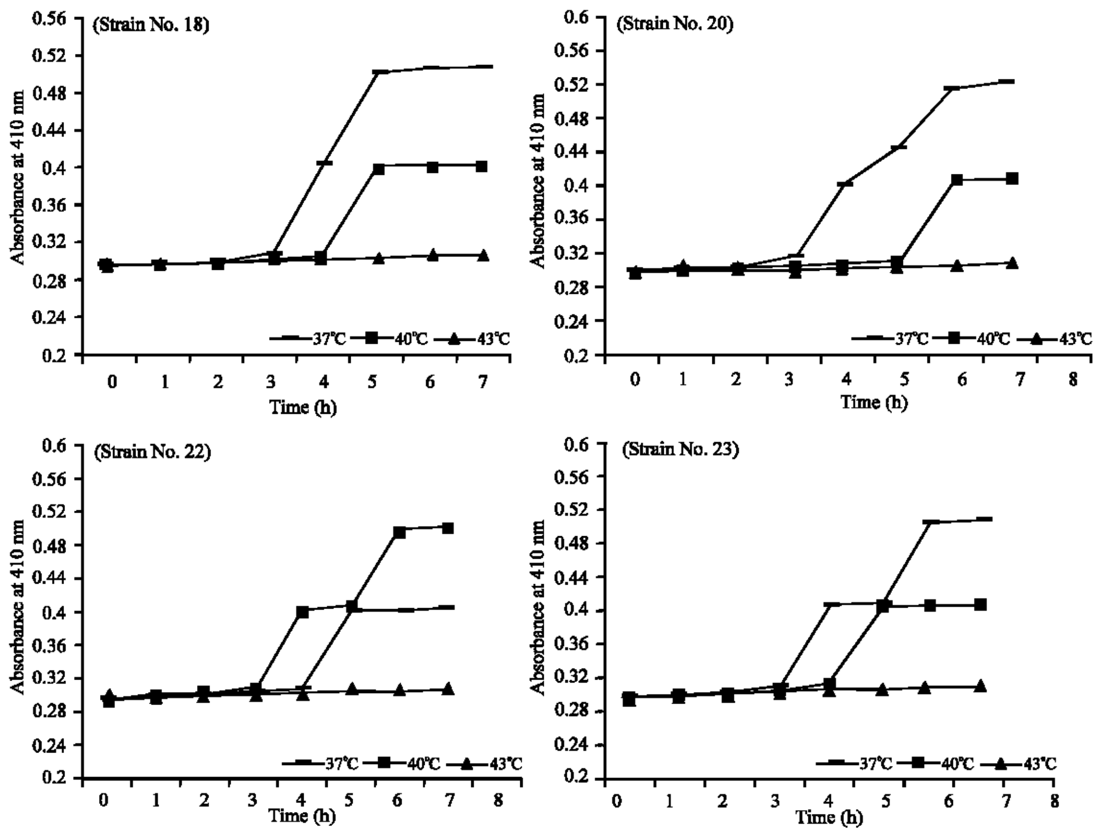

Fig. 7: Growth of *L. acidophilus* at different temperatures

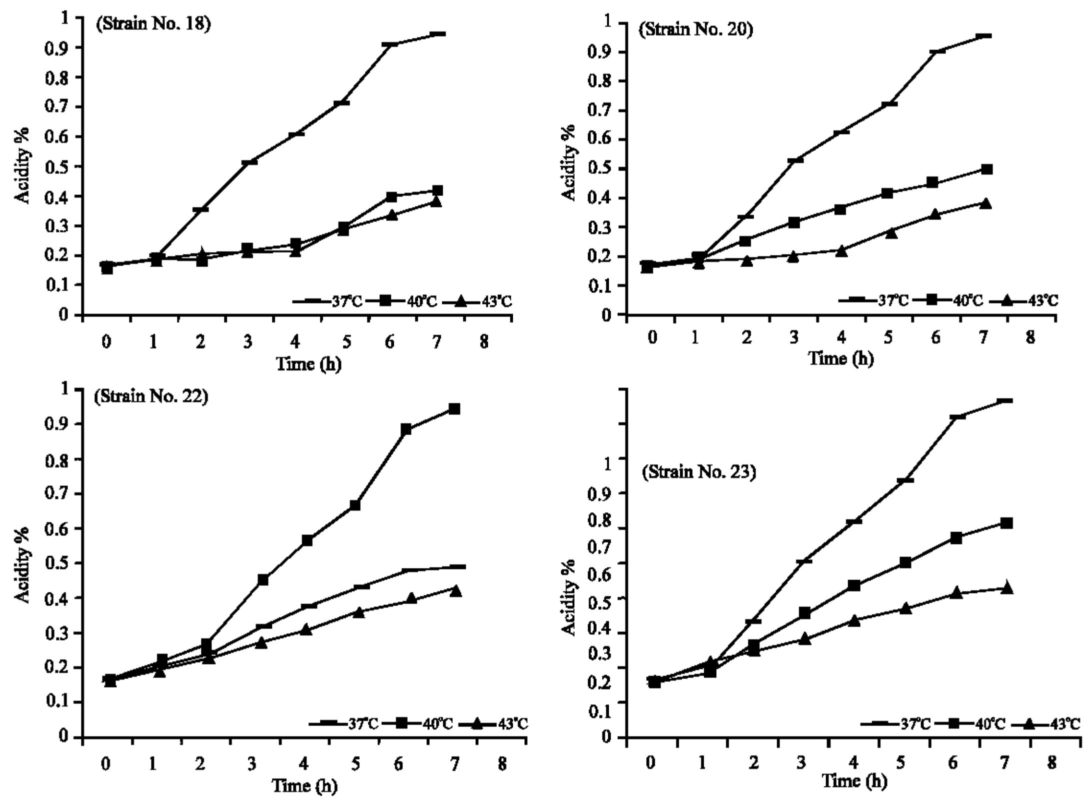

Fig. 8: Change in acidity of *L. acidophilus* at different temperatures

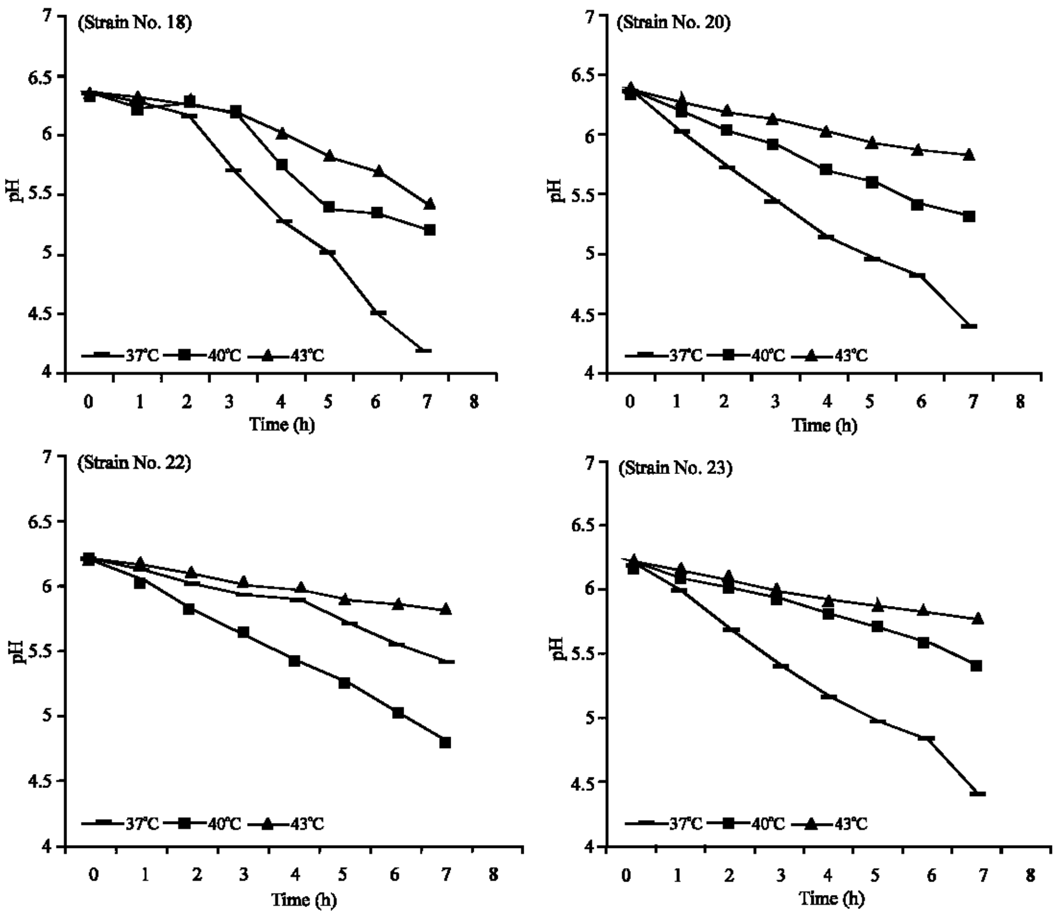

Fig. 9: Change in pH of *L. acidophilus* at different temperatures

pH and acidity. At optimum temperature, the strains showed maximum acidity. The acidification of three strains of *L. lactis* varied from 0.15 to 0.58% while one strain showed maximum acidity and lowest pH values at 40°C. In case of *S. cremoris* the acidification varies from 0.15 to 0.55% and lowest pH values at 37°C. The acidification of three strains of *L. acidophilus* varies from 0.15 to 0.96 % at 37°C while one strain showed maximum acidity and lowest pH values at 40°C.

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