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# BIOCHEMICAL CHARACTERISTICS OF LACTIC ACID PRODUCING BACTERIA AND PREPARATION OF CAMEL MILK CHEESE BY USING STARTER CULTURE

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## ABSTRACT

Lactic acid bacteria (LAB) were isolated from camel milk by culturing the milk on specific media and pure culture was obtained by sub-culturing. Purification of culture was confirmed by Gram's staining and identified by different biochemical tests. Camel milk contained lactic acid producing bacteria like *Streptococci* such as *S. cremoris* and *S. lactis* and *Lactobacilli* such as *L. acidophilus. L. acidophilus* grew more rapidly in camel milk than others as its growth was supported by camel milk. Ability of each strain was tested to convert lactose of milk into lactic acid. It was observed that 66% lactose was converted by *S. lactis* 20, whereas *S. cremoris* 22 and *L. acidophilus* 23 converted 56 and 74% lactose into lactic acid, respectively. Effect of freeze-drying was also recorded and the results showed that in all cases there was a slight decrease in the cell count before and after the freeze-drying. The decrease was approximately 0.47, 0.078 and 0.86% for *S. lactis* 20, *S. cremoris* 22 and *L. acidophilus* 23, respectively. Starter culture was prepared from strains isolated from camel milk. Camel and buffalo milk cheese was prepared by using starter culture. The strains isolated from camel milk were best for acid production and coagulated the milk in less time. It is concluded that cheese can be prepared successfully from camel milk and better results can be obtained by coagulating milk with starter culture.

**Key Words:** Lactic acid bacteria, camel milk, lactose utilization, lyophilization.

## INTRODUCTION

Micro-organisms are important in dairy products. One of the most important groups of acid producing bacteria in the food industry is the Lactic Acid Bacteria (LAB) which are used in making starter culture for dairy products. The proper selection and balance for starter culture is critical for the manufacture of fermented products of desirable texture and flavour. The microbiological quality of milk and milk products is influenced by the initial flora of raw milk (Ritcher and Vadamuthu, 2001). When camel milk is left to stand, its acidity rapidly increases due to presence of LAB (Ohris and Joshi, 1961). It has also been recognized that LAB are capable of producing inhibitory substances other than organic acids (lactate and acetate) that are antagonistic toward other micro-organisms (Daeschel, 1989).

In Pakistan, the production of milk for human use is approximately 71% from buffaloes, 24% from cows and 5% from camel and other species (Anonymous, 1994). Although this share from camels is very low but milk produced by camels in drought areas can be a valuable source of food for human population. The camel is used for several purposes including transporting goods and people as well as for milk and meat. In severe drought, sheep, goats and cattle die while camel remains relatively unaffected and serves as the only provider of food (Sweet, 1965). In many arid areas, camels play a central role as producer of milk. The comparative advantage of the camel as a dairy animal over the other species in the same environment is difficult to quantify; however, it is widely recognised that in absolute terms, the camel produces more milk, and for a longer period of time, than any other milch animal kept under the same conditions (Farah, 1996).

Pakistan is a country where vast arid regions exist. In these regions, long spells of dry period without any rain are common. Under such conditions, the only livestock, which can successfully survive and can produce substantial quantities of good quality milk, is the camel. But the camel milk has no access to the market and due to its poor keeping quality it cannot be used as fresh and goes wasted. During peak production season, it can be saved and effectively utilized through converting it into cheese by using starter culture of LAB. The present study was aimed to characterize the LAB and to develop cheese making technology from camel milk by using starter culture of LAB and to transfer this technology to camel keepers in arid regions of Pakistan.

## MATERIALS AND METHODS

**Sample collection**

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 and brought to the Dairy Technology Research Laboratory (DTRL) of Animal Sciences Institute, National Agriculture Research Centre (NARC), Islamabad in ice box for microbiological study and evaluation for different biochemical properties and cheese production.

**Bacteriological analysis**

Each sample was immediately cultured on MRS broth (code CM 359) M17 agar (code CM 785) and nutrient agar plates. Mosaics of colonies were obtained on all three media. Then desired glistening colonies were picked up from the MRS, M17 and nutrient agar plates by sterile platinum loop and subculturing was continued until the pure culture was obtained.

Purification of the culture was confirmed by Gram staining. Pure colonies were again cultured on specific media for *Lactobacilli* and *Streptococci* i.e. MRS and M17 agar plates and in the MRS and M17 broth and stored at 4°C in refrigerator until used.

After obtaining pure culture, following bio-chemical tests were performed for identification purposes:

1. Catalase test.
2. Gram's staining.
3. Motility.
4. Acidification of sugars (sugar tests).
5. Growth in 4.0 and 6.5% NaCl.
6. Growth on 0.3% methylene blue.
7. Temperature tolerance test.

**Lactose estimation**

After isolation and identification of LAB, ability of each strain for conversion of lactose into lactic acid was tested by the method of Ahmed *et al*. (1988).

**Freeze-drying**

Selected strains were freeze-dried by using Freeze-Drier FD-1 (EYELA Company, Japan). Three freeze-drying sterilized flasks, each containing 100 ml of 10% non-fat skim milk, were innoculated with 2g pure cultures of selected strains such as *L. acidophilus, S. lactis and S. cremoris* separately. These flasks were kept at 37°C till coagulation. The coagulated cultures were homogenized by manual shaking. From each flask, 1 ml of homogenized culture was taken for bacterial counts before freeze drying. Frozen layers of homogenized culture in the flask were prepared by placing the flasks in refrigerator at -50°C for three days. Then these flasks were subjected to vaccum-drier for 12 hours for lyophilization of pure cultures. These lyophilized cultures were transferred to sterilized containers, 1 g of each dried culture was diluted with 9 ml of 1.25% (w/v) sodium citrate solution and bacterial counts were made.

**Camel and buffalo milk cheese preparation**

After isolation and identification of LAB, starter culture (*S. lactis* and *S. cremoris*) was prepared in the ratio of 95:5. Buffalo milk cheese was prepared by using already prepared starter culture by the method of Athar *et al*. (1989) and camel milk cheese was prepared with some modifications, which are as follows:

Quality of camel milk was recorded through different tests like acidity, pH and other qualitative tests like fat, proteins etc. Milk was pasteurized at 62°C for 15 minutes and cooled to 30°C and then starter culture of LAB (*S. cremoris* and *S. lactis* in a ratio of 95:5) was added at the rate of 5% of milk. After about one and a half hour, rennet was added at the rate of 0.03 g/L of milk. The milk was allowed to coagulate and was transferred to a muslin cloth. The whey was allowed to drain by hanging the muslin cloth for about 24 hour and the remaining curd was pressed to form cheese. Total titratable acidity, expressed as % lactic acid, was measured by the method of Atherton and Newlander (1977) and pH was recorded by electric pH meter (model Beckman # 44 ).

## RESULTS AND DISCUSSION

The presence of nonpathogenic bacteria in milk is not a serious matter but when camel milk is left to stand, the acidity rapidly increases due to the presence of lactic acid producing bacteria. Camel milk also supported the growth of *Lactobacillus acidophilus* and other *Streptococci*, which were isolated and identified for making starter culture of fermented milk products like cheese. Mostly, lactic acid producing bacteria that grow in camel milk are *Lactobacilli (Lactobacillus acidophilus*) and *Streptococci* (*S. cremoris* and *S. lactis*), *which* are used as starters in dairy products. More growth of *L. acidophilus* was observed in camel milk as compared to others, as in every camel milk sample *L. acidophilus* was found. These findings are in accordance with Abu-Tarboush (1994), who reported that camel milk provided support to the growth of *L. acidophilus.*

Different strains isolated from camel milk are shown in Figures 1, 2 and 3, while the results of different biochemical tests are given in Table 1. These results showed that all three strains isolated were Gram positive and non-motile. Moreover, none of the strain

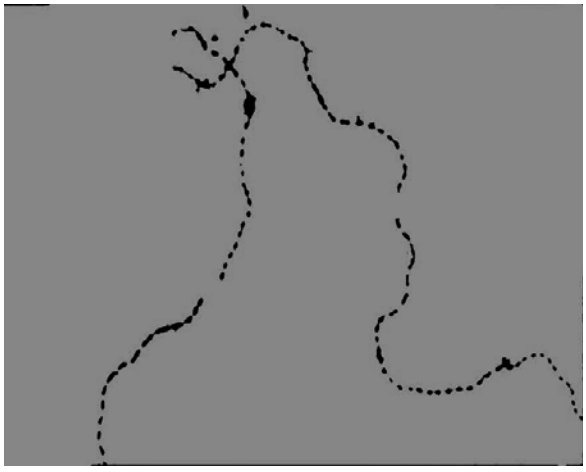

**Fig.1: Medium chains of *S. cremoris***

**Fig. 2: Chain of *L. acidophilus***

**Fig 3: Short chains of *S. lactis***

showed any catalase activity. It was further observed that in 0.3% methylene blue, one of the tests that distinguish *S. lactis* from *S. cremoris*, the former showed no growth while proper growth was observed in case of latter. All the selected strains showed proper growth in 4% NaCl, while no growth was observed in 6.5% NaCl except *L. acidophilus.*

All the isolated strains were further confirmed by sugar tests and the results are presented in Table 2. These results indicate that *S. lactis* showed positive reactions for lactose, sucrose, glucose, maltose, galactose and fructose, except mannitol. The results for *S. cremoris* indicate that all of these strains gave positive reactions with lactose, glucose, galactose and fructose while gave negative reactions with sucrose, maltose, and mannitol. *L. acidophilus* gave positive reactions with lactose, glucose, maltose, galactose sucrose and fructose and negative reactions with mannitol. These results are similar to the Bergey's manual of determinative bacteriology (Anonymous, 1974). Similar results of sugar test for *L. acidophilus* were reported by Abu-Tarboush (1994).

**Lactose estimation**

The strains which produced maximum acidity were used in lactose estimation. Since *S. lactis* 20 *S. cremoris* 22 and *L. acidophilus* 23 strains produced maximum acidity during first five hours, they were selected for assessment of maximum ability of microbes to use lactose and convert it into lactic acid. The concentration of lactose was assessed from standard curve (Fig. 4).

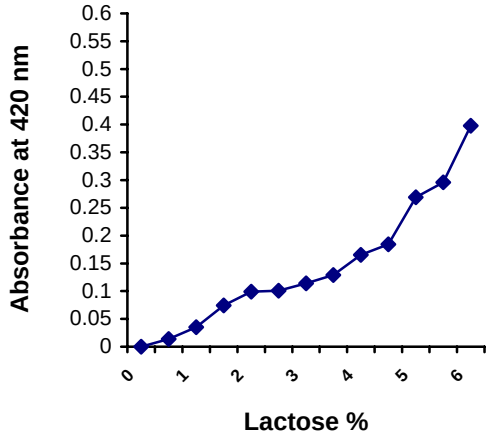


**Fig. 4. Lactose standard curve**

**Table 1: Biochemical and physiological properties of lactic acid producing bacteria**

| Properties | *S. lactis* | *S. cremoris* | *L. acidophilus* |
|---|---|---|---|
| Morphology | Spherical, short chains | Spherical medium chains | Rods pairs, chains |
| Growth at 10°C | + | + | - |
| Growth at 45°C | - | - | + |
| Gram's staining | + | + | + |
| Catalase test | - | - | - |
| Motility | - | - | - |
| Growth in 4% NaCl | + | + | + |
| Growth on 0.3% methylene blue | - | + | + |
| Growth in 6.5% NaCl | - | - | + |

+ = Positive result - = Negative result

**Table 2: Results of sugar test for species identification**

| Species | Sucrose | Maltose | Lactose | Mannitol | Glucose | Glactose | Fructose |
|---|---|---|---|---|---|---|---|
| *S. lactis* | + | + | + | - | + | + | + |
| *S. cremoris* | - | - | + | - | + | + | + |
| *L. acidophilus* | + | + | + | - | + | + | + |

+ = Acid production – = No acid production

**Table 3: Ability of some selected strains to convert lactose into lactic acid at optimum temperature**

| *Strains* | Lactose initially (%) (0 hr) | Lactose after coagulation (%) (5 hr) | Conversion of lactose into lactic acid (%) |
|---|---|---|---|
| *S. lactis* 20 | 5.1 ± 0.01 | 1.7 ± 0.01 | 66 |
| *S. cremoris* 22 | 5.1 ± 0.01 | 2.2 ± 0.01 | 56 |
| *L. acidophilus* 23 | 5.1 ± 0.01 | 1.3 ± 0.01 | 74 |

S = Streptococcus L = Lactobacillus

The results are summarized in Table 3. It is clear from the table that 66% lactose was converted by *S. lactis* 20, whereas *S. cremoris* 22 and *L. acidophilus* 23 converted 56 and 74% lactose into lactic acid, respectively.

**Freeze-drying**

In order to preserve the selected strains (starter culture i.e *S. lactis, S. cremoris* and *L. acidophilus*) for a longer time, the effect of freeze-drying on the viability of each selected strain was studied. The results of this experiment are summarized in Table 4. It is clear from the results that in all cases, there was a slight decrease in the bacterial count before and after freeze-drying. The decrease was approximately 0.47 and 0.078% for *S. lactis* 20, *S. cremoris* 22 and 0.45% for *L. acidophilus* 23. The isolates are best and vigorous for fermentation process and can coagulate the milk in less time by producing maximum acidity. Comparative trials were also conducted with relation to acid production in the buffalo and camel milk and the results. showed that acid production of lactic acid bacteria (*S. cremoris and S. lactis*) in buffaloe milk took less time (3 hrs) than camel milk (4 hrs) at which milk completely curdled, as given in Table 5.

**Conclusion**

Based on the finding of the present study, it is concluded that cheese can be prepared successfully from camel milk and better results can be obtained by coagulating milk with starter culture. This cheese making technology can also help the camel keepers in dry areas to improve their economic conditions by finding a suitable market for camel milk cheese.

**Acknowledgement**

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**Table** 4: **Effect of freeze-drying on the viability of selected strains**

| Strains | Bacterial count before freeze-drying | Bacterial count after freeze-drying | Reduction after freeze-drying |
|---|---|---|---|
| *S. lactis* 20 | $2.98 \times 10^{7}$ | $1.36 \times 10^{5}$ | 0.470 |
| *S. cremoris* 22 | $2.40 \times 10^{7}$ | $1.89 \times 10^{4}$ | 0.078 |
| *L. acidophilus* 23 | $2.95 \times 10^{7}$ | $1.30 \times 10^{5}$ | 0.450 |

**Table 5: Comparison between camel and buffalo milk with relation to acid production at 37°C**

| Time (hrs) | Camel milk *acidity (%)* | pH | Buffalo milk acidity (%) | pH |
|---|---|---|---|---|
| 0 | 0.12 | 6.4 | 0.11 | 6.7 |
| 1 | 0.19 | 6.0 | 0.15 | 6.2 |
| 2 | 0.24 | 5.6 | 0.24 | 5.6 |
| 3 | 0.31 | 5.3 | 0.29 | 5.2 |
| 4 | 0.34 | 4.8 | - | - |

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