ISOLATION AND IDENTIFICATION OF LACTIC ACID PRODUCING BACTERIA FROM CAMEL MILK

Toqeer Ahmed, Rashida Kanwal, Izhar Hussain Athar[1] and Najma Ayub
Department of Biological Sciences, Quaid-I-Azam University, Islamabad. [1]Dairy Technology Research Laboratory, National Agricultural Research Centre, Islamabad.

ABSTRACT

Lactic acid bacteria (LAB) were isolated from camel milk by culturing the camel 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 bio-chemical tests. Camel milk contains lactic acid producing bacteria including *Streptococci* such as *S. cremoris* and *S. lactis* and *Lactobacilli* such as *L. acidophilus*. *L. acidophilus* grows more rapidly in camel milk than others as its growth is supported by camel milk. A variety of food can be preserved by lactic acid fermentation, so starter culture was prepared from strains which were isolated from camel milk. Camel and buffalo's milk cheese was prepared by using starter culture. The strains isolated from camel milk were best for acid production and can coagulate the milk in less time. Camel milk cheese was prepared and compared with buffalo's milk cheese. 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, starter culture, camel milk.

INTRODUCTION

Milk is highly nutritious and is considered a nearly balanced diet and complete food. It contains adequate amount of all the essential components, including proteins and minerals, necessary for growth and maintaining health. Milk proteins can well supplement the vegetable proteins to meet the human dietary requirements (McGillivary and Porter 1958). Milk is the main food obtained from a herd of camels (Dahl and Hjort, 1979).

In Pakistan, the production of milk for human use is 71% from buffaloes, 24% from cows and 5% from camels 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 such as transporting goods and people as well as for milk and meat. In severe drought conditions animals like sheep, goat 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 milk suppliers. 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 held under the same conditions (Farah, 1996).

Camel milk is nutritionally and medicinally superior to cow milk and milk from other species. Camel milk is used therapeutically against jaundice, problems of spleen, tuberculosis, anaemia and piles (Rao *et al.*, 1970). Patients with chronic hepatitis had improved liver function after being treated with camel milk (Sharmanov *et al.*, 1978). Camel milk also has slimming properties (Yasin and Wahid, 1957).

When camel milk is left to stand, its acidity rapidly increases (Ohris and Joshi, 1961) due to presence of lactic acid bacteria. Camel milk also has germicidal property, which is of great importance in the preservation of milk. This property is due to the presence of lactic acid producing bacteria i.e. *Lactobacilli* and *Streptococci*. *Lactobacillus acidophilus* strains showed inhibitory activity towards *Salmonella typhi, Staphylococcus aureus, Escherichia coli, Proteus vulgaris* and *Yersinia enterocolitica*.

A wide variety of food is preserved by lactic acid fermentation, including milk, meat, fruits and vegetables. Reduction of pH and removal of large amount of carbohydrates by fermentation are primary preserving actions that lactic acid bacteria (LAB) provide to a fermented food. 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 microorganisms (Daeschel, 1989).

The antibacterial properties of LAB are well documented (Daeschel, 1989). Several LAB, typically

of the genera *Streptococcus* and *Lactobacillus*, are well known to produce antibacterial substances. These substances include hydrogen peroxide, diacetyl, bacteriocins, and other undefined inhibitory substances. Antifungal properties of LAB have received little attention; however, several metabolites of LAB have been reported to have antifungal activity (Daeschel, 1989; Batish *et al.*, 1989).

Pakistan is a country where vast arid regions exist. In these regions, long spells of dry period without any rains 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 by converting it into cheese by using starter culture of lactic acid bacteria. This study was carried out to isolate and identify the lactic acid producing bacteria from camel milk for fermented milk products.

MATERIALS AND METHODS

Twelve camel milk samples were collected in sterile tubes from Barani Livestock Production Research Institute (BLPRI), Kheri Murat, Fateh Jang, and District Attock. Camel milk samples were brought to Dairy Technology Research Laboratories (DTRL), National Agricultural Research Centre (NARC) Islamabad, for microbiological and biochemical tests, under refrigerator conditions.

Each sample was immediately cultured on M17 MRS, and nutrient agar plates and incubated on all three media. Mosaic of colonies were obtained on all three media, glistening colonies were picked up from the MRS and M17 agar plates by sterile platinum loop and subculture was continued until the pure culture was obtained. Purification of the culture was confirmed by Gram staining. Pure colonies were again cultured on 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 biochemical tests were performed:

Catalase test

Acidification of sugars (Sugar tests).

Growth in 4% and 6.5% sodium chloride.

Growth on 0.3% methylene blue.

After isolation and identification of lactic acid bacteria, starter culture (*S. lactis* and *S. cremoris*) was prepared in the ratio of 95:5. Camel and buffalo's milk cheese was prepared by using already prepared starter culture according to the method of Athar *et al.*, (1989). Total titratable acidity expressed as % lactic acid was measured by the method of Atherton and Newlander (1977) and pH was determined by electric pH meter (model Beckman # 44).

RESULTS AND DISCUSSION

The presence of non – pathogenic 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 the earlier reports. Abu-Tarboush (1994) 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. The results of different biochemical tests are given in Table 1.

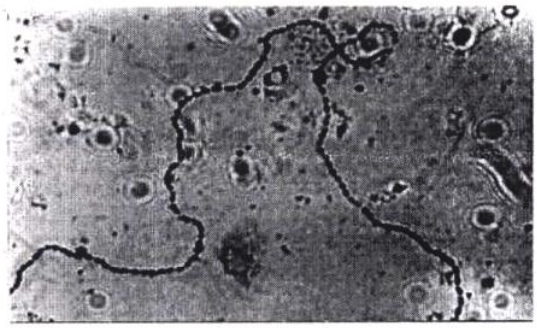

Fig 1: Medium chains of S. cremoris

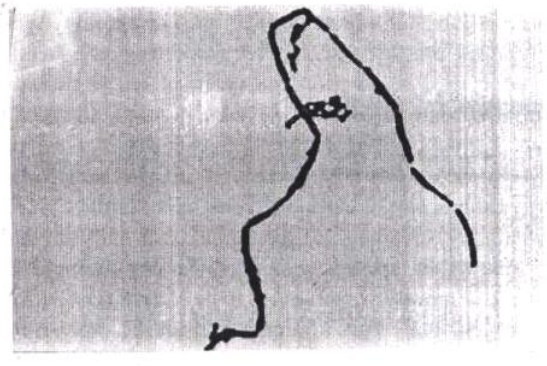

Fig 2: Chain of L. acidophilus

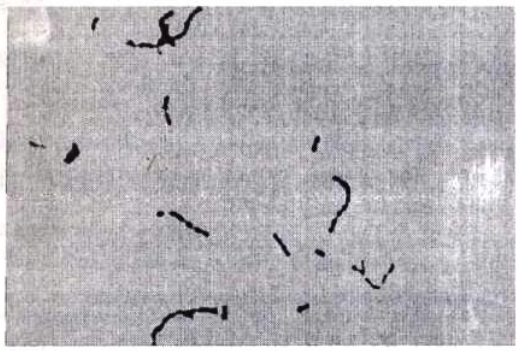

Fig 3: Small chains of S. lactis

while no growth was observed in 6.5% NaCl except *L. acidophilus* (Table 1).

All the isolated strains were further confirmed by sugars tests and the results are presented in Table 2. The 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 maltose, lactose, glucose, galactose, and fructose while gave negative reactions with sucrose and mannitol. The results of *L. acidophilus* show that these strains gave positive reactions with sucrose, lactose, glucose, maltose, galactose, and fructose and gave negative reactions with mannitol. Similar results of sugar test for *L. acidophilus* was given by Abu- Tarboush (1994).

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 tests	-	-	-
Growth in 4 % NaCl	+	+	+
Growth on 0.3% methylene blue	-	+	+
Growth in 6.5 % NaCl	-	-	+

+ = Positive result - = Negative result S = *Streptococcus* L = *Lactobacillus*

Table 2: Results of sugar test for species identification

Species	Sucrose	Maltose	Lactose	Manitol	Glucose	Galactose	Fructose
S. lactis	+	+	+	-	+	+	+
S. cremoris	-	+	+	-	+	+	+
L. acidophilus	+	+	+	-	+	+	+

+ = Acid production – = No acid production S = *Streptococcus* L = *Lactobacillus*

Table 3: Comparison between camel and buffalo's milk with relation to acid production

	Camel milk		Buffalo's milk	
TIME (hours)	*Acidity (%)*	pH	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	----	----

The results show that all three strains isolated were Gram positive. Moreover none of the strain showed any catalase activity. It was further observed that in 0.3% methylene blue, one of the tests 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

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 for acid production in buffaloes and camel milk and results showed that acid production of lactic acid bacteria (*S. cremoris and S. lactis*) in buffaloes milk took three hours and camel milk took

four hours at which milk completely curdled as given in Table 3.

The strains isolated from camel milk are the best for fermentation process and can be used as starter for fermented milk products like cheese in dairy and also in the desert areas. Therefore, 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 proper market of camel milk cheese.

ACKNOWLEDGEMENT

This research work was supported by Dairy Technology and Research Laboratories (DTRL), NARC, Islamabad and Camel Applied Research Development Network by providing research facilities, media and other chemicals.

REFERENCES

Abu-Tarboush, H.M., 1994. Growth behaviour of *Lactobacillus acidophilus* and biochemical characteristics and acceptability of acidophilus milk made from camel milk. Department of Food Science, College of Agriculture, King Saud University, Riyadh, Saudi Arabia.

Anonymous, 1994. Economic Survey of Pakistan. Economic Advisory Wing, Ministry of Finance, Govt. of Pakistan, Islamabad.

Athar, I.H., T. Masud and A. Ali., 1989. Manufacturing of indigenous cheese, using starter culture and direct acidification process. Pakistan J. Sci. Ind. Res., 32(5): 355-357.

Atherton, H.V. and Newlander, 1977. Chemistry and Testing of Dairy Products. 4th ed., AVI Publishing Co., Westport, Connecticut, USA.

Batish, V.K., S. Grover and R. Lal, 1989. Screening lactic starter culture for antifungal activity, Cultured Dairy Prod. J., 24(2): 21-25.

Daeschel, M.A., 1989. Antimicrobial substances from lactic acid bacteria for use as food preservatives. Food Technol., 43: 164-166.

Dahl, G. and A. Hjort, 1979. Dromedary pastoralism in Africa and Arabia. In: IFS Symposium on Camels. Saudan 447-458.

Farah, Z., 1996. Camel milk, properties and products. Ed. 1st Laboratory of Dairy Sci. Swiss Federal Institute of Technology.

McGillivary, W.A. and J.W.G. Porter, 1958. Review of the progress of dairy science sector. D. Nutritive value of milk and milk products. J. Dairy Res., 25: 344-363.

Ohris, S.P. and B.K. Joshi, 1961. Composition of camel milk. Indian Vet. J., 38(a): 514-516.

Rao, M.B., R.C. Gupta and N.N. Dastur, 1970. Camels milk and milk products. Indian J. Dairy Sci., 23: 71-78.

Sharmanov, T. Sh., R. Kh. Kadyrova, O.E. Shlygina and R.D. Zhaksylykava, 1978. Changes in the indicators of radioactive isotope studies of the liver of patients with chronic hepatitis during treatment with whole camel's and mare's milk. Voprosy Pitaniya 1: 9-13.

Sweet, L.E., 1965. Camel pastoralism in North Arabia. In: Man, Culture and Animals. Edited by A. Leeds and A.O. Vayda. AAAS Washington, D.C. 129-152.

Yasin, S.A. and A. Wahid, 1957. Pakistan camels. A preliminary survey. Agric. Pakistan, 8: 289-297.