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Rate of Acid Production by Some Lactic Streptococci Individually and in Combination in Buffalo Whole and Skim Milk at Different Temperatures

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ABSTRACT

The present study was planned to examine the rate of acid production by *Streptococcus lactis*, *Str. cremoris* and *Str. diacetylactis* (DRC_1) singly and in combination in buffalo skim and whole milk at 25 and 30°C. The cultures were inoculated at 5% rate (V/V) in both the types of sterilized milk and incubated at 25 and 30°C for 10 hours. For combinations cultures were grown separately and mixed before use. The mixed cultures inoculated at 5% rate in 1 : 1 ratio as above. Hourly samples were drawn to measure titratable acidity expressed as per cent lactic acid (% L.A.) upto 10 hrs. of incubation.

The cultures when grown singly or in combination performed better at 30°C than at 25°C. The acid production was higher in skim milk than in whole milk at both the temperatures. Individual cultures produce more lactic acid than mixed cultures in both the type of milk and at both the temperatures. Thus it is proved that these streptococci are non-symbiotic. Maximum acid production (1.2% L.A.) at the end of 10th hour was achieved by *Str. lactis* in skim milk at 30°C.

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INTRODUCTION

Rate of acid production by starter bacteria is an important characteristic from the point of preparation of fermented milk products. It is affected by several factors including the temperature of incubation, composition of milk and associative growth of more than one species/strains of bacteria.

The present study was planned to examine three different lactic streptococci for their behaviour in buffalo whole and skimmed milk, when inoculated singly and in combinations and incubated at 25° and 30° C.

MATERIALS AND METHODS

Three cultures namely *Str. lactis* (isolated and maintained at our Dairy Microbiology Department), *Str. cremoris* (Hansen's Laboratory Denmark); and *Str. diacetylactis* (DRC_1) procured from National Dairy Research Institute, Karnal were used in the study. The cultures were propagated in sterilized (121° C/20 min) skim milk (less than 0.5% fat) of buffalo and stored in refrigerator till used. The cultures were transferred weekly in fresh skim milk as above. Initially cultures were activated by three successive transfers be-

fore use. Sixteen hours old cultures of *Str. lactis*, *Str. cremoris* and *Str. diacetylactis* incubated at 30° C were inoculated into 100 ml sterilized skim and whole milk of buffalo having less than 0.5% fat and with 6.0% fat, respectively at the rate of 5% (V/V) and 10 ml quantities were distributed in 18 x 150 mm sterile test tubes. The milk inoculated with the above cultures and their combinations were incubated at 25° and 30° C.

Hourly samples were drawn by taking out a set of test tubes from the incubator up to ten hours to measure the titratable acidity by titrating 10g of sample against 0.1 N NaOH using phenolphthelin indicator and expressed as per cent lactic acid (% L.A.).

In preparing combinations cultures were grown separately and mixed before inoculation. The mixed cultures were then inoculated at 5% rate in 1 : 1 ratio and incubated at 25° and 30° C.

RESULTS AND DISCUSSION

The rate of acid prodouction by three lactic streptococci singly and in combinations in buffalo whole and skim milk at 25° and 30° C have been represented in Tables 1 to 5 and figures 1 to 5.

TABLE 1

Effect of incubation temperature and types of milk on rate of acid production by *Str. lactis*.

Time of incubation hours	*Streptococcus lactis*			
	Skim milk		*Whole milk*	
	25°C	*30°C*	*25°C*	*30°C*
0	0.207	0.207	0.189	0.189
1	0.312	0.315	0.318	0.318
2	0.345	0.357	0.354	0.363
3	0.384	0.420	0.381	0.402
4	0.513	0.579	0.510	0.558
5	0.648	0.774	0.615	0.744
6	0.774	0.891	0.669	0.858
7	0.912	1.017	0.825	0.918
8	1.020	1.112	0.948	0.990
9	1.077	1.200	0.975	1.011
10	1.113	1.213	1.005	1.086

The figures represent average of three trials.

Table-1 and figure-1 shows the acid production by *Str. lactis* grown in buffalo skim and whole milk at 25° and 30° C for a period of ten hours. It can be observed that the acid production by *Str. lactis* showed an increase from 0.2% L.A. to 0.4% L.A. in a period of 3 hours at both temperatures in buffalo skim and whole milk at 30° C. However, the rate of acid production increased from 0.4% L.A. to 1.2% in skim milk incubated at 30° C

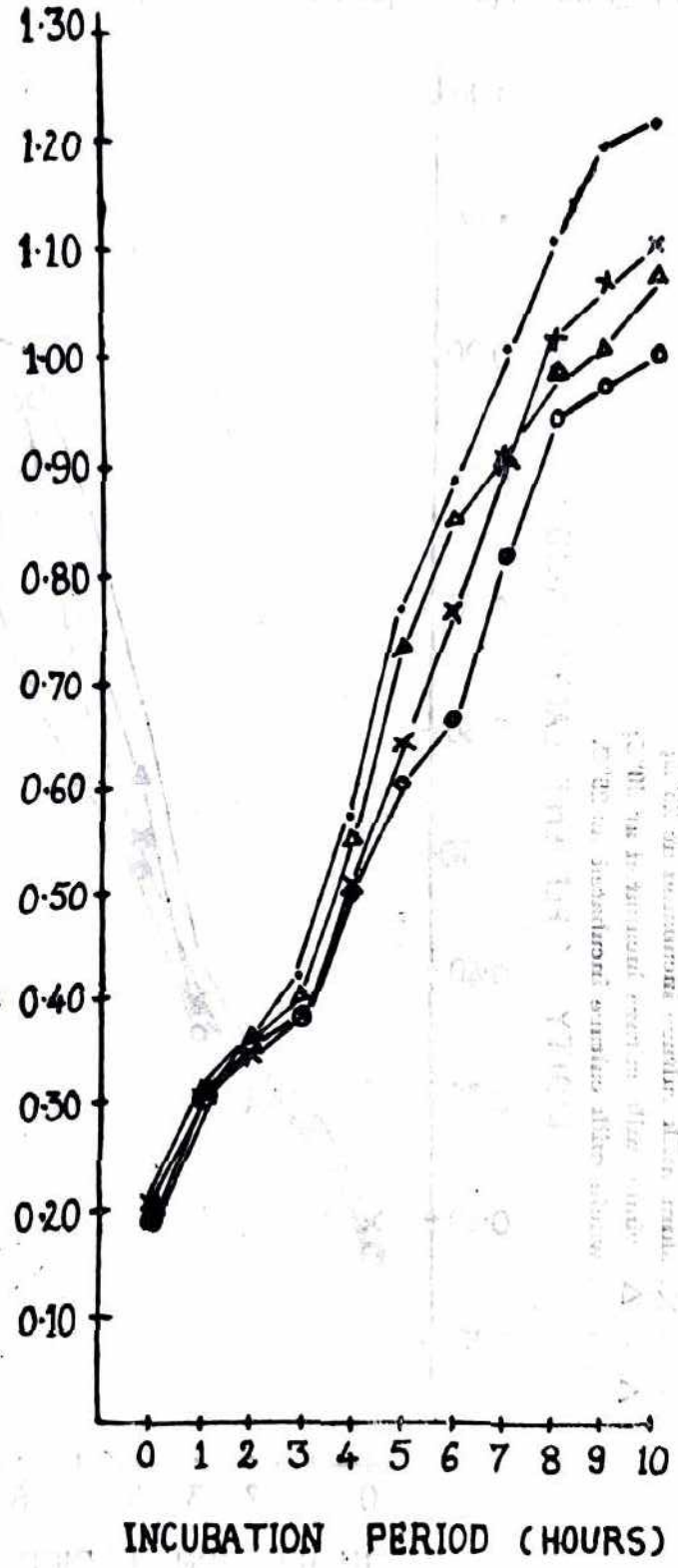

Figure 1: Effect of incubation temperature and type of milk on rate of acid production by *Str. lactis*.

●—●, skim milk culture incubated at 30°C;
×—×, skim milk culture incubated at 25°C;
△—△, whole milk culture incubated at 30°C;
○—○, whole milk culture incubated at 25°C.

from 3 hours to 9 hrs. It was interesting to note that both whole milk and skim milk showed almost similar increase in acidity upto a period of 5 hrs. Subsequent incubation showed higher acid production in skim milk than whole milk.

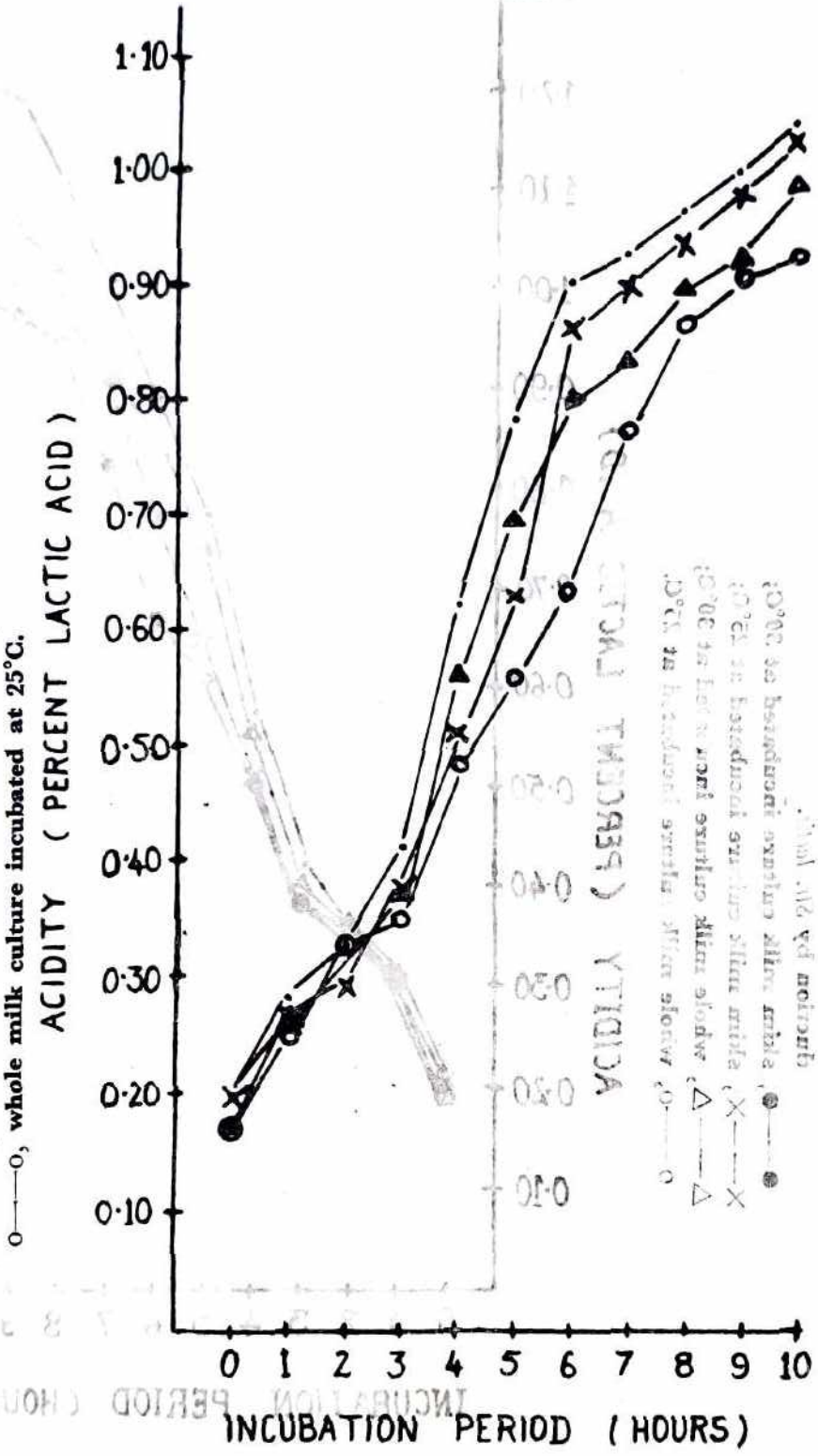

Figure 2: Effect of incubation temperature and type of milk on rate of acid production by *Str. diacetylactis* (DRC_1).

●—●, **skim milk culture incubated at 30°C;**
×—×, **skim milk culture incubated at 25°C;**
△—△, **whole milk culture incubated at 30°C;**
○—○, **whole milk culture incubated at 25°C.**

TABLE 2

Effect of incubation temperature and types of milk on the rate of acid production by *Str. diacetylactis* (DRC_1).

Time of incubation hours	*Streptococcus diacetylactis* DRC_1)			
	Skim milk		*Whole milk*	
	25°C	*30°C*	*25°C*	*30°C*
0	0.198	0.198	0.171	0.171
1	0.267	0.282	0.249	0.267
2	0.294	0.327	0.330	0.339
3	0.375	0.401	0.357	0.372
4	0.519	0.621	0.480	0.564
5	0.636	0.783	0.558	0.699
6	0.864	0.900	0.633	0.801
7	0.897	0.921	0.774	0.831
8	0.939	0.957	0.864	0.891
9	0.975	0.990	0.900	0.918
10	1.020	1.029	0.915	0.987

The figures represent average of three trials.

Table-2 and figure-2 indicates the rate of acid production by *Str. diacetylactis* (DRC_1). It was observed that the acid production by this culture was 0.35% L.A. in 3 hrs. and it showed a rapid increase in acid content between 3 and 6 hrs. As in *Str. lactis* it showed better acid production in skim milk after 3 hours than whole milk. However, the acid production by this culture at the end of 10 hours was only 1.02% which was lower than that of *S. lactis* for the same period. An interesting observation in this culture is that it shows reduced rate of acid production after 6 hrs. as against 8 to 9 hrs. observed with *Str. lactis*.

In *Str. cremoris* the rate of acid production was almost similar in both skim and whole milk at the two tem-

TABLE 3

Effect of incubation temperature and types of milk on rate of acid production by *Str. cremoris*.

Time of incubation hours	*Streptococcus cremoris*			
	Skim milk		*Whole milk*	
	25°C	*30°C*	*25°C*	*30°C*
0	0.198	0.198	0.180	0.180
1	0.276	0.282	0.273	0.279
2	0.303	0.327	0.297	0.327
3	0.339	0.361	0.336	0.363
4	0.363	0.441	0.387	0.438
5	0.426	0.579	0.420	0.519
6	0.468	0.672	0.465	0.642
7	0.543	0.828	0.615	0.756
8	0.762	0.873	0.753	0.842
9	0.888	0.921	0.888	0.924
10	0.924	0.984	0.933	0.969

The figures represent average of three trials.

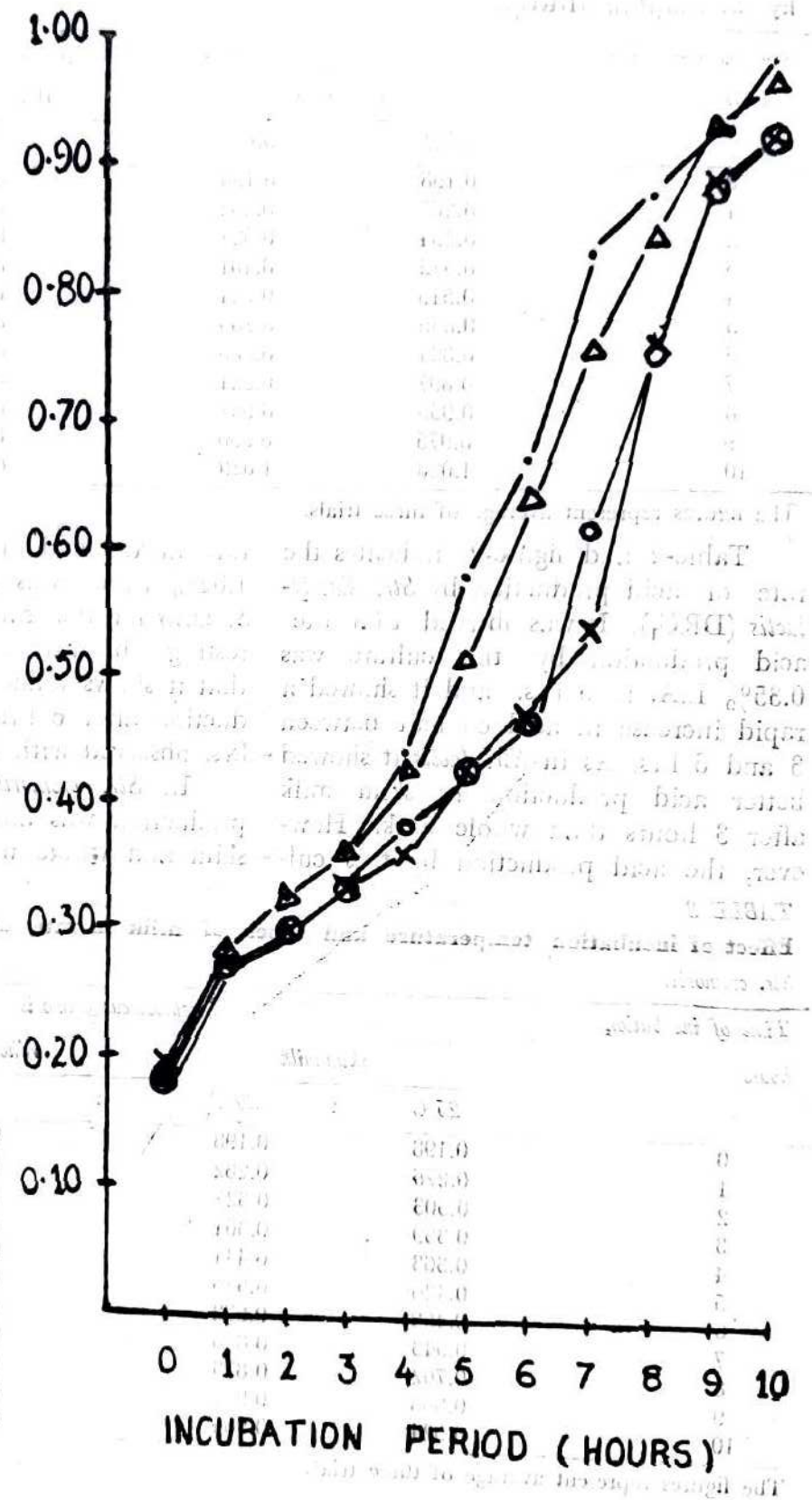

Figure 3: Effect of incubation temperature and types of milk on rate of acid production by *Str. cremoris*.

●—●, **skim milk culture incubated at 30°C;**
×—×, **skim milk culture incubated at 25°C;**
△—△, **whole milk culture incubated at 30°C;**
o—o, **whole milk culture incubated at 25°C.**

peratures unlike the above two cultures (Table-3 and figure 3). However, the acid production was better at 30° C than at 25° C. The rate of acid development in the two milks at the two temperatures were similar upto one hour of incubation only, subsequently cultures incubated at 30° C showed a higher acid than at 25° C. It was also observed that like *Str. lactis* and *Str. daicetylactis* this culture also showed fast rate of acid production after 3 h. of incubation. The total acidity at the end of 10 hrs. was however, less than 1.0% L.A. in this culture at both incubation temperatures in skim as well as whole milk.

TABLE 4

Effect of incubation temperature and types of milk on rate of acid production by *Str. lactis*+*Str. diacetylactis.*

Time of incubation hours	*Str. lactis+Str. diacetylactis*			
	Skim milk		*Whole milk*	
	25°C	*30°C*	*25°C*	*30°C*
0	0.198	0.198	0.180	0.180
1	0.207	0.225	0.198	0.225
2	0.270	0.282	0.261	0.270
3	0.342	0.351	0.315	0.333
4	0.387	0.432	0.369	0.420
5	0.477	0.540	0.405	0.522
6	0.522	0.648	0.504	0.630
7	0.576	0.702	0.519	0.675
8	0.630	0.747	0.603	0.723
9	0.711	0.801	0.702	0.774
10	0.774	0.837	0.747	0.801

The figures represent average of three trials.

Among the combinations tested *Str. lactis* + *Str. diacetylactis* (Table-4 and figure-4) and *Str. cremoris* + *Str. diacetylactis* (Table-5 and figure-5) showed a reduction in acid production than the individual cultures. However, the rate of acid production was almost constantly increasing throughout the incubation period in both cultures. It can also be seen from these figures that *Str. lactis* + *Str. diacetylactis* combination in both whole and skim milk, showed similar rate of acid production. *Str. cremoris* + *Str. diacetilactis* showed higher acid production in skim milk than in whole milk, at the two temperatures. The acidity obtained by *Str. lactis* + *Str. diacetylactis* after 10 hrs. was 0.77% L.A. and 0.84% L.A. in skim milk while in whole milk it is 0.75% L.A. and 0.80% L.A. at 25° and 30° C. respectively.

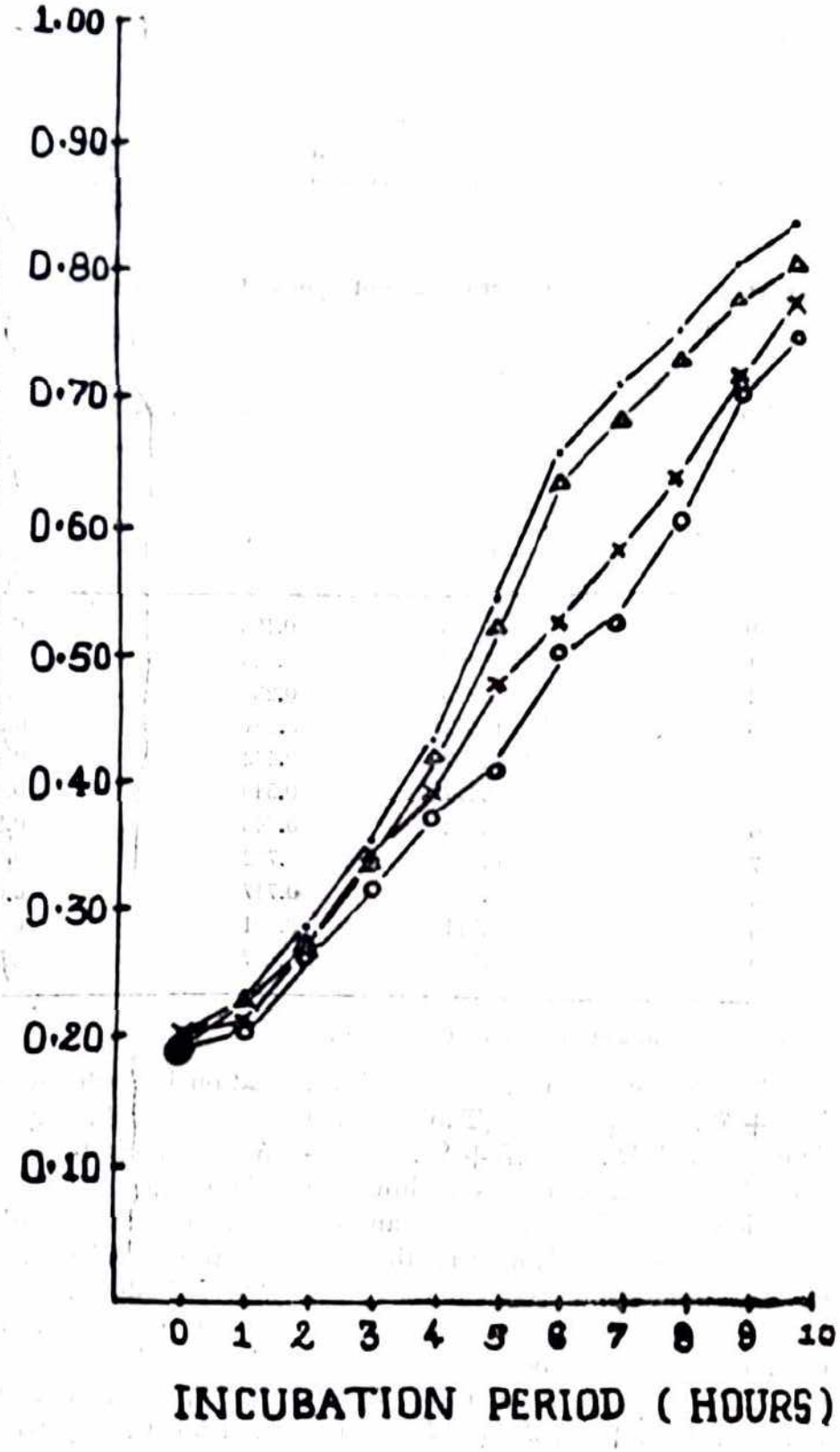

Figure 4: Effect of incubation temperature and type of milk on rate of acid production by *Str. lactis* + *Str. diacetylactis*.
●—●, skim milk cultures incubated at 30°C;
×—×, skim milk cultures incubated at 25°C;
Δ—Δ, whole milk cultures incubated at 30°C;
○—○, whole milk cultures incubated at 25°C.

TABLE 5

Effect of incubation temperature and types of milk on rate of acid production by *Str. cremaris*+*Str. diacetylactis.*

Time of incubation hours	*Str. cremaris*+*Str. diacetylactis*			
	Skim milk		*Whole milk*	
	25°C	*30°C*	*25°C*	*30°C*
0	0.198	0.198	0.180	0.180
1	0.216	0.270	0.216	0.270
2	0.234	0.324	0.234	0.306
3	0.342	0.351	0.324	0.342
4	0.360	0.432	0.351	0.420
5	0.504	0.603	0.432	0.549
6	0.549	0.675	0.474	0.576
7	0.621	0.747	0.579	0.672
8	0.693	0.810	0.636	0.747
9	0.720	0.864	0.693	0.792
10	0.792	0.900	0.774	0.828

The figures represent average of three trials.

From the foregoing study it is evident that the lactic streptococci strains tested in present study were slightly inhibitory to each other in regard to acid production. The other important observation made which indicates higher acid production in skim milk than whole milk in most of the combinations and individual cultures, are contradicting the findings of Habaj *et al.* (1974) who observed higher rate of acid production in whole milk than in skim milk with mixed strain lactic streptococci. This inhibitory effect observed may be because of apparent difference in the total solids content or probably due to the lower chain volatile fatty acids liberated by these cultures during their growth in whole milk. Lawrence *et al.* (1976) have in their review in cheese starters have indicated that C_4–C_{12} fatty acids and oleic acid showed inhibitory effect on starter bacteria. Present observation on slightly reduced rate of acid production after few hours of incubation observed with cultures grown in whole milk suggest that possibly it may be due to the release of some of the lower chain fatty acids after few hours of incubation. However, this needs further confirmation as Foster *et al.* (1958) and Hammer and Babel (1957) have indicated that milk fat does not play much role in acid production. Since buffalo whole milk contains higher quantity of fat it will be interesting to study the cause for this inhibition observed in whole milk.

The observations in regard to temperature of incubation used on acid production it was found that 30° C is better for acid production than 25° C in the three lactic streptococci or their

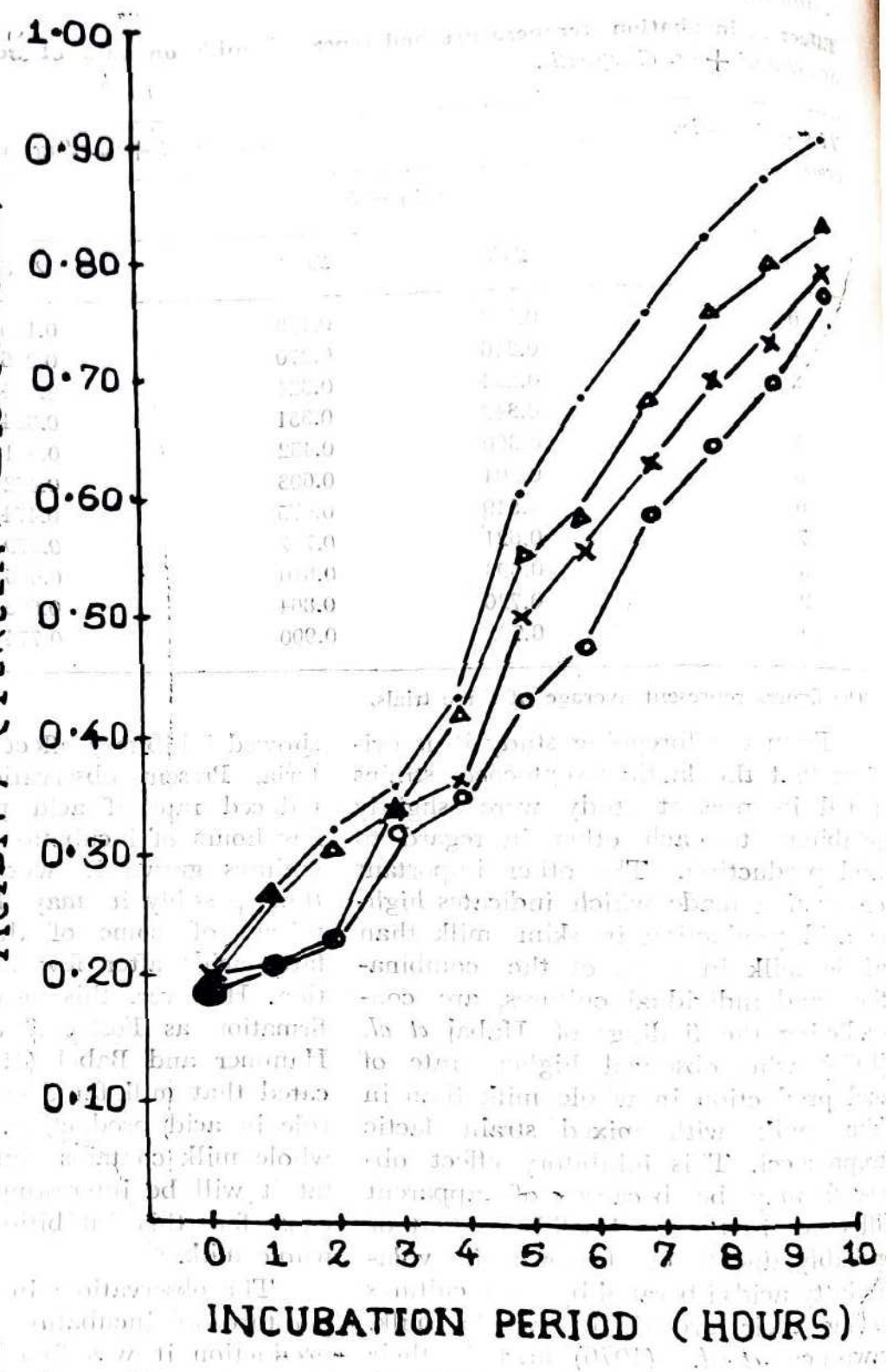

Figure 5: Effect of incubation temperature and type of milk on rate of acid production by *Str. cremoris* + *Str. diacetylactis*.

●—●, skim milk cultures incubated at 30°C;
×—×, skim milk cultures incubated at 25°C;
Δ—Δ, whole milk cultures incubated at 30°C;
o—o, whole milk cultures incubated at 25°C.

combinations studied. This confirms the observations of Golding *et al* (1951) Habaj *et al* (1979) with the lactic streptococci used by them. Dutta *et al.* (1972) found 30° C to give higher acid production than 22° C in *Str. lactis* and *Str. diacetylactis* but in *Str. cremoris* they showed slightly lower final acidity at 30° C than at 22° C. The higher acidity at 30° C observed in the present study is because 30 C° is optimum temperature for the growth of lactic streptococci. However, in present study observations were made only upto 10 hrs. of incubation while Dutta *et al.* (1972 and 1973) reported the values after 18 hours of incubation. Variations in incubation temperature for acid production has been reported by several workers. (Babel, 1946; Dahlberg and Ferris, 1945; Golding *et al.*, 1951; Harrison and Dearden, 1941 and Sharma & Jain, 1974 and 1975).

These observations suggest the need for selecting the incubation periods and temperatures and also combinations of starter cultures while manufacturing a fermented milk with higher per cent inoculum (5% v/v) used in present study. The results in the present study suggest the need for confirming the observations regarding the role played by milk fat for growth and acid production by starter cultures.

REFERENCES

Babl, F. J. 1946. Factors influencing acid production by cheese cultures I. Effect of cooking temperature on acid production in the manufacture of Cheddar cheese. *J. Dairy Sci.* **29:** 589-596.

Dahlberg, A. C., and F. Ferris. 1945. Influence of the frequency of transfer of lactic starters upon rate of acid development and quality of Cheddar cheese. *J. Dairy Sci.* **28:** 771-778.

Dutta, S. M., Kuila, R. K., Arora, B. C. and B. Ranganathan. 1972. A research note. Effect of incubation temperature on acid and flavour production in milk by lactic acid bacteria. *J. Milk and Food Technol.* **35** (4) : 242-244.

Dutta, S. M., Kuila, R. K., B. Rangnathan, 1973. Effect of different heat treatments of milk on acid and flavour production by five single strain cultures. *Milchwissenschaft*, **28** (4) : 231-233.

Foster, E. M., Nelson, F. E., Speck, M. L., Doetsch, R. N. and J. C. Olson Jr. 1958. "Dairy Microbiology" MacMillan and Co. Ltd., London.

Golding, N. S., H. Amundson and R. O. Wagenuar. 1943. Factors affecting the development of acidity in pasteurized skim milk inoculated with commercial lactic starters. *J. Dairy Sci.* **26:** 909-919.

Habaj, B., Czkierda, A. and L. Stepaniak. 1974. Acid and gas producing activity of mesophilic lactic streptococci when grown in whole milk or skim milk. XIX *Int. Dairy Congr.* **IE**, 425-426.

Hammer, B. W. and F. J. Babel. 1957. "Dairy Bacteriology", 4th edn., John Wiley & Sons Inc., New York.

Harrison, J., and D. V. Dearden. 1941. Slowness in cheese making. *J. Dairy Res.* **12:** 35-43.

Lawrence, R. C., Thomas, T. D. and B. E. Terzaghi. 1976. Reviews of progress of dairy science: Cheese starters. *J. Dairy Res.* **43:** 141-193.

Sharma, C. K. and S. C. Jain. 1974. Effect of starter cultures and incubation period and temperature on the acidity of dahi (curd), Part-I. *J. Food Sci. and Technol.* **11:** 277-280.

Sharma, C. K. and S. C. Jain, 1975. Effect of starter cultures and incubation (period and temp.) on the acidity of dahi (curd), Part-II. *J. Food Sci. and Technol.* **12:** 81-83.