# Selection of Effeicient Strains of Indigenous Lactic Acid Bacteria for Cheese Making

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## Introduction

Cheese is a highly nutritious and delicious fermented food. Cheese making is concerned with the formation of lactic acid in milk for curdling and other products for flavour by lactic acid bacteria. The most important lactic acid bacteria in commercial use are isolated from traditional products and are not known to exist outside the dairy environment[4]. These lactic acid bacteria include lactic group of *Streptococcus* and *Lactobacillus*[9]. The species and strains of these two genera vary widely in their acid producing capacity and also in generàl characters[5,9].

The characteristics of the strains influence the quality of the products. Hence, the selection of efficient strains of lactic acid bacteria is very important. There are many examples of strain selection, done usually on empirical basis and rarely on rational basis. For the selection on empirical basis, a large number of strains are studied to get a few efficient ones.

The screening of a large number of isolates in order to detect promising strains, has often been on random "trial and error" basis. Artificial mutation and gene manipulation can bring about improvement in the traditional strains, but such manipulation processes are expensive and require modern laboratory. Other selection techniques can, however, remove the difficulties and can reduce the labour, time and troubles[7].

The present work was undertaken to isolate a number of the strains of lactic scid bacteria from local cottage cheese, and also to select efficient ones using some inexpensive techniques.

## Materials and Methods

**1)** ***Isolation of the strains :*** Yeast glucose lemco agar and broth media[10] were used for the isolation of the strains of *Streptococcus*. For the isolation of the strains of *Lactobacillus* solid and liquid media of Rogosa[11] and MRS[8] were used. Spreading and streaking plate techniques were followed for the purpose of isolation. The plates were incubated at 30°C for 24-48 hours.

**2)** ***Identification of the strains :*** The isolated strains were studied thoroughly for their cultural, morphological, physiological and biochemical characteristics. The characterized isolates were identified according to Bergey's manual of determinative bacteriology[1], Foster et al.[3], Garvie[4], and Harrigan and McCance[6].

**3)** ***Selection of efficient strains :*** For the selection of efficient strains, 10 ml aliquots of 10% sterile reconstituted skim

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milk of pH 6.3 in test tubes were inoculated with 1 ml suspension of each strain of *Lactobacillus* and *Streptococcus* in normal saline. The inoculated tubes were incubated at 37°C for 24-48 hours. After inoculation the time and nature of curdling were observed and recorded. The amount of whey produced was measured. The curd was filtered; the residue i.e., solid curd was dried in an oven at 45°C till it showed a constant weight. The pH of the curd was determined by a pH meter.

For testing the ability of the strains of *Streptococcus* to produce acid in the milk culture, the activity test was performed. For this test. 100 ml sterile skim milk in conical flask was inoculated with 1 ml culture suspension of each strain of *Streptococcus*, and was incubated at 30°C in a water bath for 6 hours. After the incubation, acidity of fermented milk was determined by titration with N/9 NaOH(6). Uninoculated blank was also titrated for necessary correction. The acidity was expressed in terms of lactic acid.

***4) Production trial :*** From the efficiency test, 5 strains out of 14 were selected for a trial on the production of cheese. For the trial both skim and cream milks were used in order to see the quality i.e., texture, colour, flavour etc. of the cheese. The trial was designed with the following combinations of the selected strains.:

*Starter No. 1 : Lactobacillus plantarum*-1+ *Streptococcus cremoris*-2 in skim milk.

*Starter No. 2 : Lactobacillus plantarum*-2+*Streptococcus* lactis sub sp. *diacetylactis*-1+*Lactobacilus acidophilus* in cream milk.

*Starter No. 3 : Streptococcus cremoris*-2+*Streptococcus lactis* sub sp. *diacetylactis*-2+*Lactobacilus* acidophilus in cream milk.

*Starter No. 4 : Streptococcus lactis* sub sp. *diacetylactis*-1+*Lactobacilus plantarum*-1 in cream milk.

Five hundred millilitre aliquots of 10% milk were inoculated with the starters of the above combinations and incubated at 40°C for 24-48 hours in semi-anaerobic condition. After incubation the formed curd was pressed to remove the whey. The pressed and formed curd was then ready for ripening. The ripening process was maintained for 3 days at 10°C.

**Results :**

Forteen strains of lactic acid bacteria were isolated (Table-1). Of the 14 strains, 6 belonged to *Lactobacillus* and 8 to *Streptococcus*, Out of 6 strains, the strains RB, RK, RF1, RF2 and TRF were identified as homofermentative *Lactobacillus plantarum*-1, *Lactobacillus plantarum*-2, *Lactobacillus plantarum*-3 and *Lactobacillus plantarum*-4 and *Lactobacillus plantarum*-5 respectively and the strain RM was identified as *Lactobacillus acidophilus*.

Six strains (YK, YKM, YM1, YM2 Ym3 & YF) of *Streptococcus* were placed under N-lactic group & two strains (YB & YB2) under viridans group. Out of 6 strains of

N-lactic group, the strains YF & YM2 were identified as *Streptococcus cremoris-1* and *Streptococcus cremoris-2* respectively and the other four strains (YK, YKM, YM1 and YM3) were identified as the strains of *Streptococcus lactis*. Of the strains of *Streptococcus lactis*, strains YM1 & YM3 were further differentiated i to *Streptococcus lactis-1* and *Streptococcus lactis-2* and the strains YK and YKM into *Streptococcus lactis* sub sp. *diacetylactis-1* and *Streptococcus lactis* sub sp. *diacetylactis-2*.

Table-1 and Plate-1 show the efficiency of the strains of *Lactobacillus* and *Streptococcus* in curd formation. Among the 14 strains, *Streptococcus plantarum-1* produced the highest amount (0.58 g) of curd and the lowest amount (0.26 g) of curd was produced by *Streptococcus lactis-2*. These two strains caused the highest and lowerst drop of pH i.e., from 6.3 to 3.2 and from 6.3 to 5.8 respectively. Accordingly the highest amount (5.3 ml) of whey was produced by *Streptococcus plantarum-1*, whereas *Streptococcus lactis-2* could not produce any whey.

Of the strains of N-lactic group of *Streptococcus, Streptococcus lactis* sub sp. *diacetylactis-1* and *Streptococcus cremoris-2*, produced the highest amount of acid in milk culture and the value was 0.41 g acid estimated as lactic acid per 100 ml culture (Fig. 1). Lowest amount of acid (0.25 g/100 ml) was produced by *Streptococcus lactis-1*. *Streptococcus lactis* sub sp. *diacetylactis-2*, *Streptococcus lactis-2*, *Streptococcus cremoris-1* and viridans streptococci-1 produced at least 0.30 g acid/100 ml culture.

Combination of the strains of *Lactobacillus* and *Streptococcus* was found better than the strains of the individual genus in curd formation. In production trial curd formation was better with skim milk than with cream milk. Cheeses formed with the combination of starter No.2 were firmer and harder and were with low moisture content, whereas cheeses produced from cream milk were spongy in nature with high moisture content.

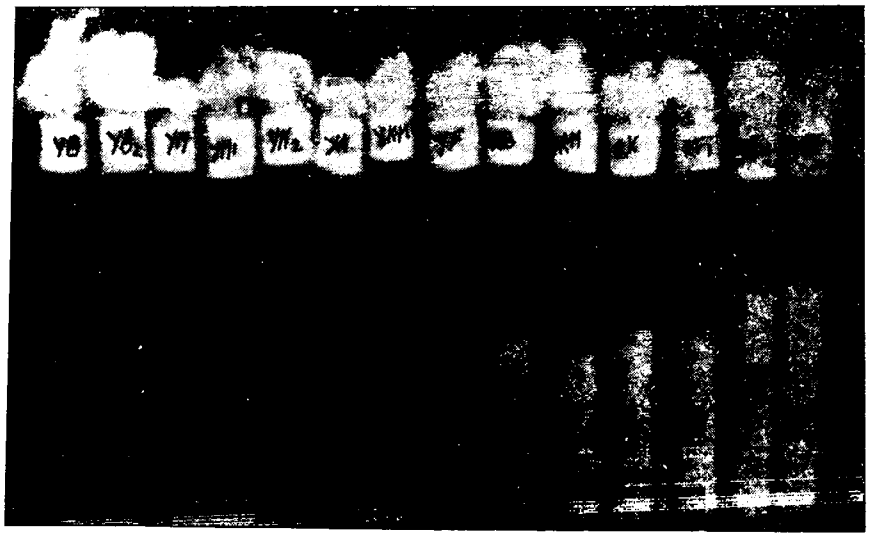

**Plate-1 :** *Efficiency of the 14 strains of lactic bacteria in curd formation.*

**Table 1 :** Efficiency of the 14 strains in reducing pH of the culture and in curd formation.

| Strain | Dry weight of the curd (g) | Whey (ml) | pH | |
|---|---|---|---|---|
| | | | Milk | Curd |
| *Lactobacillus plantarum*-1 | 0.85 | 5.3 | 6.3 | 3.2 |
| *Lactobacillus plantarum*-2 | 0.48 | 5.0 | 6.3 | 4.0 |
| *Streptococcus lactis* sub sp. *diacetylactis*-1 | 0.48 | 5.2 | 6.3 | 3.9 |
| *Lactobacillus acidophilus* | 0.43 | 5.2 | 6.3 | 3.8 |
| *Streptococcus lactis sub sp. diacetylactis*-2 | 0.40 | 5.2 | 6.3 | 4.0 |
| *Streptococcus cremoris*-1 | 0.40 | 5.0 | 6.3 | 3.9 |
| *Viridans streptococcci*-2 | 0.39 | 4.9 | 6.3 | 3.9 |
| *Lactobacillus plantrarum*-3 | 0.36 | 5.2 | 6.3 | 3.6 |
| *Viridans streptococcci*-1 | 0.36 | 4.2 | 6.3 | 4.0 |
| *Streptococcus lactis*-1 | 0.36 | 5.0 | 6.3 | 5.0 |
| *Streptococcus cremoris*-2 | 0.30 | 3.9 | 6.3 | 5.0 |
| *Lactobacillus plantarum*-4 | 0.30 | 4.0 | 6.3 | 4.2 |
| *Lactobacillus plantarmu*-5 | 0.29 | Trace | 6.3 | 5.2 |
| *Streptococcus lactis*-2 | 0.26 | Trace | 6.3 | 5.8 |

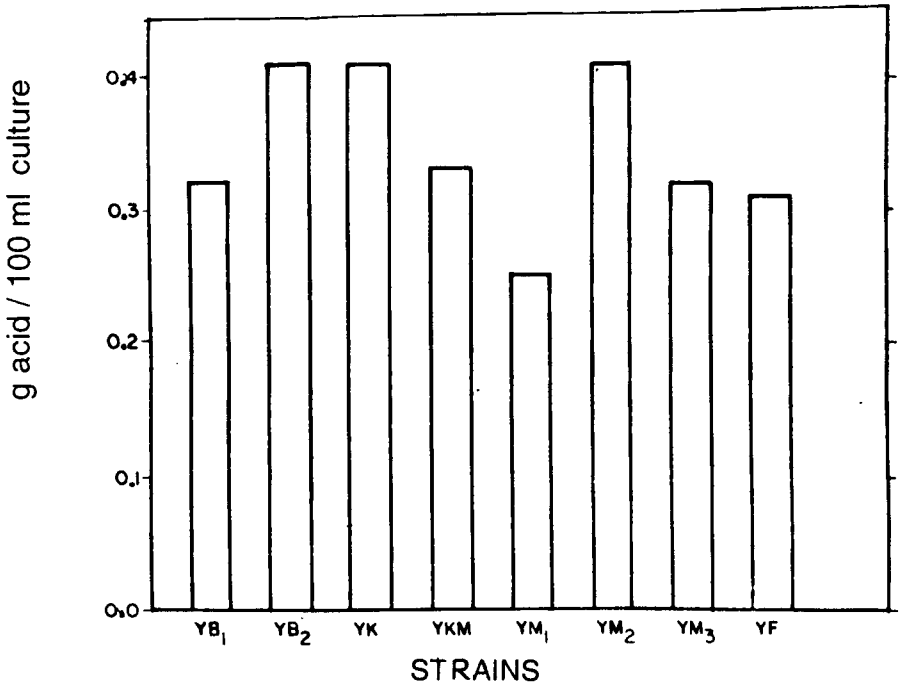


**Fig. 1** : *Production of lactic acid by 8 strains of Streptococcus in milk culture.*

**Discussion**

Among the 14 strains tested, *Lactobacillus plantarum*-1 gave rise to highest curd formation releasing the highest amount of whey (Table-1). The drop of pH from 6.3 to 3.2 in the culture due to acid production by this strain was the highest. The lowest amount of curd was produced by *Streptococcus cremoris*-2 forming no clear whey.

From the efficiency test, it was found that among the strains of *Lactobacillus* and *Streptococcus*, the strains of *Lactobacillus* showed more efficiency in the formation of curd which is the prime prerequisite condition of cheese making.

It was seen that among the strains of *Streptococcus, Streptococcus lactis* sub sp. *diacetylactis-1*, *Streptococcus cremoris*-2, and viridans streptococci-2, produced the highest amount of acid. These results also support the results of efficiency test. Sheikh *et al* (12) found that *Streptococcus* produced least quantity of acid while the mixed culture of *Lactobacillus* and *Streptococcus* produced higher quantity of acid in the curd. In the present study, performance of the combination of *Lactobacillus* and *Streptococcus* strains in curd formation was better than that of the individual strain. This also agrees well with the finding of Foster *et al.*(3) and Garvie(4). Although viridans streptococci-1 (Strain YB1) and viridans streptococci-2 (Strain YB2) showed better efficiency in acid production (Fig. 1), they were not considered as the efficient strains in the production trial because of their non-lactic nature.

**Summary**

Fourteen strains of lactic acid bacteria were isolated from local cottage cheese. The strains were charactrized thoroughly and were identified. Of the 14 strains, 6 beloged to *Lactobacillus* and 8 to *Streptococcus*. Combination of the strains of *Lactobacillus* and *Streptococcus* showed better activity than the strains of individual genus in producing curd. Three strains of *Lactobacillus* and two strains of *Streptococcus* were finally selected for using in making cheese, on the basis of their performance in producing acid in milk culture, and in forming curd and quality cheese.

**References**

1. Bergery's Manual of Determinative Bacteriology, (R.E. Buchanan, and N. E. Gibons co-editors). The williams and Wilkin Co., Baltimore 1974.
2. Feresu, S.B. and Muzondo, M.I. Identification of some lactic acid bacteria from two Zimbawean fermented milk products, World J. Microbiology and Biotechnology, 1990, 6: 178.
3. Foster, E.M., Nelson, F.E., Speck, M.L., Doetsch, R.N. and Olson J.C. Dairy Microbiology. Macmillan & Co. Ltd. London, 1958.
4. Garvie, E.I. Taxonomy and Identification of Bacteria Important in Cheese and fermented Dairy Products. In : Advances in the Microbiology and Biochemistry of cheese and fermented milk (F.L. Davies and B.A. Law editors). Elsevier Applied Science Publishers, London : 1984, 35-66.
5. Hammer B.W. Dairy Bacteriology. Chapman & Hall Ltd., London, 1948.
6. Harrigan, W.F. and McCance M.E. Laboratory Methods in Food and Dairy Microbiology. Revised edition prepared by Harrigan, W. F. Academic press, London 1976.
7. Johnston, J.R. Strain selection and Improvement. In : Microbiology of fermented foods. Vol-2 (Brain, Z.B. Wood editor). Elsevier applied science publishers, New York 271, 1985.
8. Man, J.C. de, Rogosa, M. and Sharpe, M.E.A. Medium for the cultivation of lactobacilli. J. Appl. Bact. 1960, 23: 130.
9. Marshall, V.M.E. and Law, B.A. The physiology and growth of dairy lactic acid bacteria. In : Advances in the Microbiology and Biochemistry of Cheese and Fermented Milk, (F.L. Davies, and B.A. Law. editors), Elsevier applied science publishers. New York, 67, 1984.
10. Naylar, J. and Sharpe, M.E. Lactobacilli in cheddar cheese. I. The use of selective media for isolation and of serological typing for indentification. J. Dairy Res. 1958, 25: 92.
11. Rogosa, M., Mitchell, J.A. and Wiseman, R.F. A selective medium for the isolation and enumeration of oral and faecal streptococci. J. Bact. 1951, 62: 132.
12. Sheikh, N.M., Joarder G.K. and Khatoon M. Bacteriology of Dadhi. Part I. The studies on the production of curd and acid by typical dadhi producing bacteria and their quantitative changes during fermentation. Sci. Res. 1970, 7: 97.