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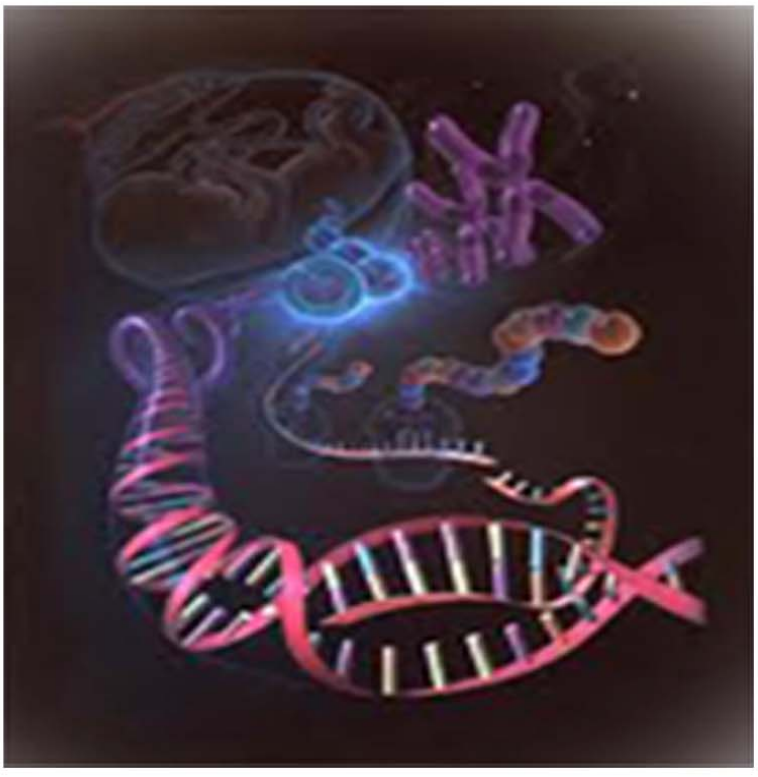

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Research Paper

GROWTH AND SURVIVAL OF PATHOGENIC *E. COLI* DURING CURDLING OF MILK

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The presence of pathogenic serotypes of *E. coli* in milk and milk products causes food borne illness. Curd is prepared by fermenting milk with Lactic Acid Bacteria (LAB). Among LAB, *Lactobacillus acidophilus L. delbrueckii, L. bulgaricus, Streptococcus thermophilus* and *Lactococcus lactis*, are the main organisms used for the preparation of curd in India. Apart from producing non-specific antimicrobial substances such as lactic acid and H_2O_2, LAB also capable of synthesizing more specific bacteriostatic and bactericidal agents. The inhibitory effect of LAB during curdling of milk on growth of pathogenic and non-pathogenic *E. coli* was studied. The two *E. coli* strains had been found to behave differently during fermentation of milk with LAB at 37°C. Growth of *E. coli* 026 was reduced greatly during curdling of milk whereas inhibition of growth was not significant for *E. coli* 10536. Death rate of *E. coli* 026 in curd was found to be much higher than *E. coli* 10536.

Keywords: Fermentation, Food born illness, Pathogenic serotypes, Lactic Acid Bacteria (LAB)

INTRODUCTION

Curd, a popular Indian fermented milk product, is well known for its palatability and nutritive value. *Lactobacillus acidophilus L., delbrueckii L. bulgaricus*, *Streptococcus thermophilus* and *Lactococcus lactis* are the main organism used for the preparation of curd in India. During the fermentation of milk to curd, lactose, present in milk is converted to lactic acid by thermophilic lactic acid bacteria. As the pH of milk is lowered due to acid production by the bacteria, the micellar calcium phosphate, which holds casein micelles together, is solubilized, resulting in precipitation of the case in molecules at their isoelectric point, around pH 4.6 to 4.7.

The bacteria in general, remain viable for up to one month with the curd kept at 40°C. The heat treatment of milk before curd preparation, kills undesirable microorganisms and reduces redox potential, which favor the growth of curdling bacteria. It has been reported earlier by Gandhi and Namburdipad (1975), and Reddy *et.al.* (1983) that 62% of market and home made curd sample possess antibacterial properties, which are

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associated with lactic acid bacteria and have been found to control various intestinal disorders such as diarrhoea, constipation and vomiting.

Production of these antibacterial agents is extremely dependent on the strain and its growth environment. Their action spectra are also variable. As can be seen, very little is known about bacteriostatic or bactericidal agents produced by curdling bacteria compared to data accumulated on other lactic acid bacteria. Despite only a few reports are available, many researchers still believe that curd possesses antibacterial properties that are not solely dependent on low pH values.

The presence of coliform bacteria in milk product generally provides an index of the hygienic standard and its keeping quality. In addition, coliform present in the milk product may include certain strains of *E. coli*, which cause foodborne illness. Out breaks of foodborne illness caused by *E. coli* have been associated with consumption of milk and other diary products. The *E. coli* seems to survive despite inhibition and or inactivation by the lactic acid bacteria present in this product. Survival of *E. coli* in fermented dairy product is variable and depends on the species of the lactic acid bacteria used to prepare the product, strains of sub species level of inoculums used, incubation temperature at which fermentation is carried out, amount and speed of acid production, resulting pH, temperature at which the product is stored after fermentation and composition of the product.

In India, curd is a popular fermented food product. Sucrose at a concentration of around 10% is added to the milk and then fermented with lactic acid bacteria. In practice, milk is kept in earthen pot loosely covered by a plate and inoculated with a small amount of curd prepared earlier. The fermentation is carried out at ambient temperature. There is every chance that undesirable microorganisms including pathogenic one may be introduced into the milk through earthen pot, food handlers, starter culture, etc. Present study will determine and compare the behavior of two different strains of *E. coli* pathogenic and nonpathogenic during preparation and storage of curd at room temperature (30^{o}C).

METHODOLOGY

E. coil 026 and *E. coli* 10536 were obtained from Central Research Institute, Kasuali and Central Drug laboratory, Calcutta, India, respectively. Lactic acid bacteria were isolated and identified from curd sample purchased from local sweetmeat shop.

A small amount of curd with a standard 3 mm loop was streaked on the surface of MRS agar plate, which was previously dried. The inoculated plates were incubated at 37^{o}C for 48 h. Suspected colonies were isolated and further purified by repeating the process. Finally, purified colonies were sub-cultured on MRS agar slants for maintenance and identification. Identification of the isolated LAB was done biochemically.

E. coli was also monitored by 5 tube most probable number technique used MacConkey broth. Diluted curd samples were inoculated at 44^{o}C for 24 h. Production of acid and gas in the tubes were considered as *E. coli* positive. This was further confirmed by noting these IMViC patterns of the isolated colonies on EMS plates.

Milk, collected from local sweet meat shop manufacturer, was dispensed into Erlenmeyer flask and sterilized at 121^{o}C for 15 min. The milk was cooled to ambient temperature to 100 ml of

sterile milk in 250 ml Erlenmeyer flask, 1 ml of 24 h Nutrient broth culture of *E. coli* was added. This was incubated at 37°C. Viable count of *E. coli* was determined at regular intervals till the organism entered death phase. In another set of experiment along with *E. coli*, 1 ml of lactic acid bacterial culture also added to 100 ml of sterile milk. This was incubated at 37°C and *E. coli* count was enumerated as above.

To 100 g of curd sample, viable *E. coli* cell suspension was added and was homogenized by blending under aseptic condition. This was kept at 37°C and viable count of *E. coli* was taken every hour up to 4 h.

RESULTS AND DISCUSSION

All the isolated strains were belonging to the genus *Lactobacillus* and *Lactococcus*. These bacteria were grown in sterile milk at 37°C for 18 h and were used as inoculums for curdling experiments. The growth curves of pathogenic *E. coli* 026 and nonpathogenic *E. coli* 10536 in sterile milk incubated at 37°C for the duration of the experiment (25 h) are presented in Figure 1 respectively. Both the strains attained maximum growth after incubation of 16 h. Maximum colony forming unit was obtained (Log 8.5 cfu/ml) with the strain *E. coli* 026 than *E. coli* 10536 (log 7.7 cfu/ml). However, count of *E. coli* 10536 declined sharply than *E. coli* 026 after entering death phase.

In order to study the growth pattern of *E. coli* strains during curdling, 100 ml of sterile milk was inoculated with *E. coli* (log 3.5 cfu/ml) as well as lactic acid bacteria (log 4 cfu/ml). Inoculated milk was incubated at 37°C under stationary condition. Growth of *E. coli* at different interval of time was determined by MPN technique as described in materials and methods section.

In every case, *E. coli* was confirmed by isolation of the organism through MacConkey agar plates and studying the IMViC patterns of the isolates. It appears that growth pattern of *E. coli* strains during curdling of milk with lactic acid bacteria is different. *E. coli* 026 attained maximum growth in 10 h and count fell rapidly thereafter. Maximum colony count attained was also much lower than obtained with pure culture in sterile milk. This indicates that LAB inhibits the growth of *E. coli* 026. In contrast, time required for attaining maximum growth of *E. coli* 10536 (Figure 4) during curdling with lactic acid bacteria is almost same in comparison to monoculture growth in sterile milk. However, maximum cell number attained was one log cycle less in comparison to when allowed to grow alone (Figures 1 and 2) and death rate was also found

Figure 1: Showing Growth of *E. coli* 026 (Series-1 and *E. coli* 10536 (Series-2) in Sterile Milk

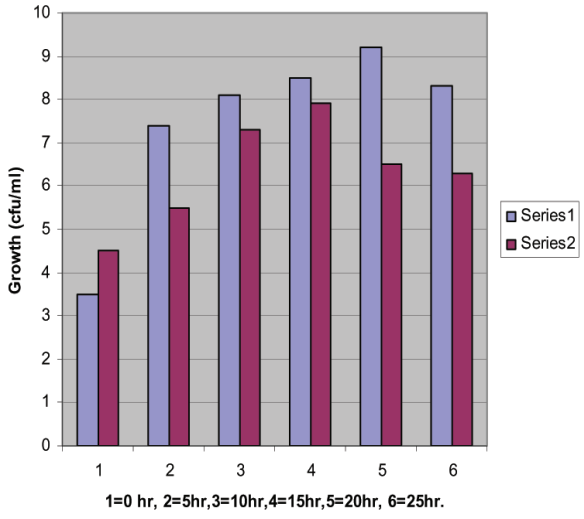
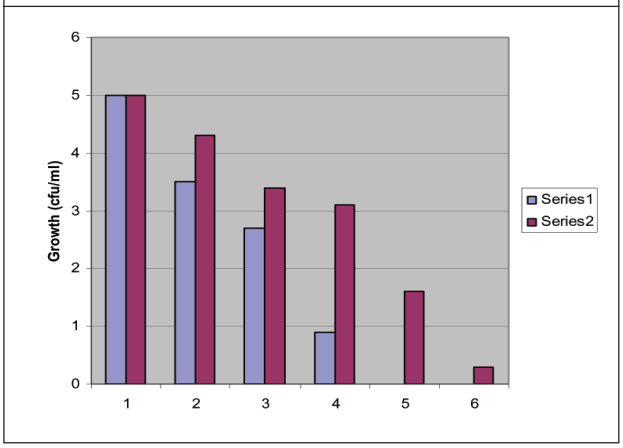


Figure 2: Showing Growth of *E. coli* 026 (Series-1 and *E. coli* 10536 (Series-2) After Curdling of Milk (x axis Shows Hours of Incubation)



to be higher. The two *E. coli* strains behave differently during curdling of milk with lactic acid bacteria at 37°C for 24 h.

The inhibitory effect of lactic acid bacteria on growth of *E. coli* 026 particularly was not due to lowering of pH, nor the action of acid normally produced by *Lactobacilli*, addition hydrochloric, lactic and acetic acid to the media (milk) in amounts to bring the pH to 5-6 did not impede the normal growth of *E. coli*. Another possible reason for that is competition for nutrients may be discarded, as they were present in excess amount. It is likely that inhibitory substances are normal products of metabolic activities of *Lactobacilli*. There are plenty of reports available that lactic acid bacteria produces different anti microbial compounds such as hydrogen peroxide, lactic acid and volatile fatty acids. Difference in response by two *E. coli* strains used in this experiment was probably due to resistance offered by *E. coli* 10536 against their inhibitory substances.

In order to study whether *E. coli* can survive in curd, i.e., if it is contaminated after curdling of milk, curd sample (100 g) was mixed with *E.coli* at a concentration of log 5 cfu/g. Curd incubated at 37°C up to 4 h. Count of *E. coli* was taken every hour. Results are presented in Figure 2. Count of *E. coli* fell rapidly. *E. coli* 026 could not be detected in curd sample during 3 h. *E. coli* 10536 concentration in curd also fell rapidly by 3 log cycle within 4 h. This further proves that *E. coli* 10536 is not sensitive to anti-microbial compounds produced by lactic acid bacteria than *E. coli* 026. The almost similar type of result was found by Rathi *et. al.* (1990) and Balasubramanyam and Bharadaraj (1994).

CONCLUSION

It may be concluded from this study that lactic acid bacteria during curdling of milk produces some anti microbial substances, which is effective against *E. coli*. Response of *E. coli* varies from strain to strain. If any accidental contamination of curd with *E. coli* occurs, there is every chance that the pathogen will be eliminated from the curd within a short period.

REFERENCES

1. Balasubramanyam B V and Bharadaraj M C (1994), *J.food Sci. Technol*., Vol. 31, pp. 241-244.
2. Gandhi D N and Namburdipad V K N (1975), *Indian J. Dairy Sci.,* Vol. 28, pp. 72-77.
3. Rathi S D, Deshmukh D K, Ingle U N and Syed H M (1990), *Ind. J.Dairy Sci*., Vol. 43, p. 249.
4. Reddy G V, Shahani K M, Friend B E and Chandan R C (1983), *Cult. Dairy Prod. J.,* Vol. 18, pp. 15-21.
5. Shah N and Jelen P (1990), *J. Food Sci.,* Vol. 55, pp. 506-509.

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