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The plasmid profiling of different strains of *Lactococcus lactis* Subsp. *Lactis*

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Abstract
Plasmid biology has become an important area of investigation in dairy starters. Genes located on plasmid DNA code some properties vital for successful milk fermentations. Some of the metabolic properties observed in lactic streptococci have been clearly established as being plasmid mediated, which makes them an unstable character during subsequent subculturing. Strains of *L.lactis* subsp. *lactis* ML3, ML8 and C2 were taken for the present study. The plasmid profiling of these cultures were evaluated after every subculturing. It was observed from plasmid profiling that both characters *i.e.* lactose utilization and protein degradation characters were plasmid encoded.

Keywords: Psychiatric disorders, suicide, suicide attempt; first admission; recurrent admission; schizophrenia; bipolar disorder; depression; substance abuse disorder

Introduction
In Lactic streptococci several metabolic properties vital for successful dairy fermentation are unstable. With the advent of techniques for studying genetic composition of dairy streptococci, it has become possible to provide explanation for this unstable phenomenon. These organisms characteristically harbor many plasmid species. The number observed ranges from two to eleven, but most strains appear to contain four to seven distinct plasmid species. Most of the plasmids observed in these organisms are cryptic, but some carry identifiable traits.

When a bacterial cell divides, each daughter cell receives a copy of the chromosomal DNA along with a copy or copies of the plasmids from the parent. Because plasmid DNA replicates independently of the chromosome, however, any mutation resulting in failure of plasmid replication results in a daughter cell that doesn't receive a copy of that plasmid and this is unable to perform the function dictated by the plasmid. For this reason, plasmid – associated traits may be more unstable than functions controlled by chromosomal genes. The high spontaneous loss of a metabolic property therefore suggests plasmid DNA involvement. This spontaneous loss is only presumptive evidence, however, and confirmation of the role of plasmids will depend on physical and genetic studies.

Materials and methods
The cultures used in this study were obtained from National Collection of Dairy Cultures (NCDC), Dairy Microbiology Division, National Dairy Research Institute, Karnal. The following cultures were taken into consideration for this study:

1. Lactococcus lactis subsp. lactis ML 3
2. Lactococcus lactis subsp. lactis ML 8
3. Lactococcus lactis subsp. lactis C2

All the standard cultures were transferred in skim milk and incubated at 30°C. The cultures were propagated up to 21 transfers. In all the cases, one per cent culture showing 0.3 O.D. was inoculated in reconstituted skim milk. The metabolic characteristics i.e. pH, titratable acidity, and proteolytic activity of the standard cultures were evaluated after 0,4,8,12,16 and 24 h of incubation.

Isolation, Purification and Characterization of Plasmid DNA
Growth of Cells
The cultures meant for plasmid isolation were propagated overnight at 32°C in an appropriate M 17 broth containing lactose or glucose.

The resulting culture provided a 2 per cent inoculum for lysis broth, strains were propagated at 32 °C for 4 h.
The alkaline lysis procedure recommended by Anderson and McKay (1983) [6] was followed with minor modifications.

Agarose Gel Electrophoresis
Agarose at a concentration of 0.6 per cent in TAE buffer (1X) and 0.3 per cent ethidium bromide was added and poured on the gel plate fitted with comb and height of the gel was adjusted to 6 mm. After 45 min, the comb was carefully removed from set gel and the gel plate was carefully placed in submarine horizontal electrophoresis. The gel was covered by pouring enough TAE buffer (1X).
10 µl of DNA sample was mixed with 5 µl of tracking dye. This mixture was added to sample wells in 10 – 12 µl quantities. Electrophoresis was performed at 50V for 5h. The gel was examined under UV transilluminator for visualization of bands and compared with Hind III digested 1 kb ladder as standard.

Result and Discussion
Plasmid profiling
A single high molecular weight band was obtained in each of the standard culture, which was compared with 1kb marker. After 21 transfers also it resulted the presence of only one high molecular weight plasmid similar to the one present in the parent cultures
Most lactococci possess a wide range of plasmid molecules, which can be resolved by gel electrophoresis to give a characteristic plasmid profiles. The plasmid profiles of the cultures during propagation were same as of standard cultures. Hence these plasmids of the standard strains might be correlated with the lactose utilizing and proteolytic activities.
The plasmid profiles of the strain *L. lactis* subsp. *lactis* ML3 corroborated the earlier findings of Klaenhammer *et al.* (1978) [4] and Kuhl *et al.* (1979) [5]. The plasmid profile of strain 712 was in slight deviation with the report of Gasson (1983) [3]. However, it was consistent with an earlier report of Davies and Gasson (1981) [2], who suggested that the plasmid complements might vary slightly in response to different environments. McKay (1983) [6] reported that high molecular weight plasmid encodes essential metabolic properties in lactococci.
Klaenhammer *et al.* (1978) [4] identified five plasmid species 1, 2, 5, 10 and 30 Mdal, with the later carrying genetic determinants for lactose metabolism in *S. lactis* C2. Similarly, Thompson and Collins (1989) [11] compared the plasmid profiles of lactic streptococci isolated from fermented milk and dried starter and reported the presence of low molecular weight (2 to 3.5 Mdal) plasmids with unknown function in dried starter, while it was absent in lactic streptococci isolated from fermented milk. The presence of plasmids ranged from 0 to 6 per cell of sizes 2.1 to 76.5 kb was reported in some strains of mesophilic lactic acid bacteria (TsongRong *et al.*, 1996) [12]. In a related report lactose negative mutants were missing a 34 Mdal plasmid, while citrate negative and protease negative were missing a 6 Mdal and 23 Mdal plasmids, respectively (Nakamura *et al.*, 1992) [7]. Parashar *et al.* (1998) [9] reported the presence of 7 plasmids ranging in size from 3.3 to 25 Mdal in *L.lactis* subsp. *lactis* PM 23 and stated that a 25 Mdal plasmid encodes for EPS production. AkCelik (1999) [1] observed that lactose and proteinase utilization were plasmid encoded which are of 41.0 and 28.2 kb sizes, respectively in *L. lactis* subsp. *lactis* LL140. The presence of heat stress protein gene (HAP) in *S.thermophilus* on plasmid ranging from 2.8 to 11 kb in sizes and on 7.5 kb plasmid in *L.lactis* subsp. *cremoris* was observed by Somkuti and Steinberg (1999) [10].

Conclusions
In all the lactococci it was established that the genes involved in the lactose and proteinase activities are plasmid mediated. These biochemical and molecular characters of the standard cultures are quite stable during subsequent propagation upto 21 transfers and could be used in dairy and food industries for fermentation.

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