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Research note :

Effect of subculturing on metabolic activities of different strains of *Lactococcus lactis*

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In Lactic streptococci several metabolic properties which are 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 (AkCelik, 1999). 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 (Klaenhammer et al., 1978). 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 (Gasson 1983). 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 (McKay, 1983).

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

An experiment was undertaken at Department of Biotechnology, IGAU, Raipur, Chhattisgarh, India. 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. pH Development of acidity in the culture was monitored by periodic measurement of the pH with a Systronics pH meter. The samples were homogenized and pH measurement was taken after equilibration for 20 seconds. The titratable acidity in milk cultures was determined by titrating 10 ml sample with 0.1N NaOH using phenolphthalein as an indicator after every 4h of incubation. The result has been expressed as per cent lactic acid. The proteolytic activity was determined according to the method of Hull (1947) with slight modifications.

Three different strains of lactococci were grown in reconstituted skim milk at 30° C for 21 subsequent transfers. The titratable acidity and proteolytic activity of these cultures were estimated (Tables 1 – 3). Phenotypically all the three cultures showed almost similar acid production and proteolytic activity during subsequent propagation. *Lactococcus lactis* subsp. *lactis* C2 was low acid producer among all the cultures. The acid production was consistent in all the cultures during propagations except in 7^{th} to 11^{th} transfers. The maximum proteolysis of 8600 ìg/5ml was exhibited by *L.lactis* subsp. *lactis* ML3 in 7^{th} transfer after 24h of incubation whereas the lowest of 2300 ìg/5ml was exhibited by *L.lactis* subsp. *lactis* ML8 during 14^{th} transfer (Figs 1 to 3). The proteolytic activity also increases with the incubation period. No major differences were observed in acid producing ability and degree of proteolysis of isolates and standard strains of lactococci (Padmanabha *et al.*, 1994). With the

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Table 1 : Effect of subculturing on pH and Titratable Acidity of *Lactococcus lactis* subsp.*lactis* ML 3.

No.of Transfer	pH						Titratable Acidity (% Lactic Acid)					
	0h	4h	8h	12h	16h	24h	0h	4h	8h	12h	16h	24h
1.	6.3	5.9	5.05	4.7	4.6	4.5	0.24	0.6	1.0	1.0	1.12	1.13
2.	6.3	6.05	5.0	4.7	4.65	4.45	0.24	0.42	0.95	1.05	1.08	1.09
3.	6.3	5.8	4.9	4.7	4.6	4.5	0.24	0.6	0.97	1.08	1.1	1.2
4.	6.4	6.05	5.0	4.7	4.6	4.6	0.23	0.45	0.85	0.95	1.08	1.08
5.	6.4	6.1	5.1	5.0	5.0	4.8	0.23	0.5	1.01	1.05	1.1	1.1
6.	6.3	6.1	5.0	4.7	4.5	4.45	0.24	0.42	0.9	0.95	0.99	1.1
7.	6.4	6.0	4.9	4.7	4.6	4.4	0.25	0.45	0.92	0.92	1.1	1.15
8.	6.3	5.5	5.0	5.0	4.6	4.5	0.24	0.46	0.8	1.12	1.2	1.25
9.	6.3	5.95	5.05	4.95	4.55	4.5	0.24	0.52	0.82	1.0	1.05	1.1
10.	6.4	5.5	4.9	4.7	4.5	4.4	0.24	0.5	0.72	1.01	1.0	1.11
11.	6.4	6.05	5.0	4.7	4.5	4.5	0.23	0.48	1.08	1.09	1.09	1.12
12.	6.4	6.05	5.1	4.75	4.6	4.55	0.25	0.5	1.07	1.08	1.15	1.3
13.	6.3	6.0	5.0	4.65	4.6	4.55	0.25	0.5	0.75	1.02	1.12	1.28
14.	6.4	6.0	4.9	4.6	4.4	4.4	0.23	0.55	0.85	1.0	1.08	1.15
15.	6.35	6.0	4.9	4.75	4.55	4.5	0.23	0.4	0.86	0.9	0.92	1.0
16.	6.4	6.1	4.8	4.7	4.6	4.5	0.23	0.42	0.85	0.9	0.91	1.01
17.	6.3	6.1	4.9	4.85	4.55	4.4	0.25	0.44	0.86	0.9	0.93	0.95
18.	6.5	5.85	5.1	4.7	4.5	4.5	0.23	0.42	0.87	0.91	0.95	0.96
19.	6.3	6.1	4.9	4.7	4.6	4.5	0.25	0.38	0.85	0.9	0.93	0.95
20.	6.4	5.95	5.0	4.7	4.5	4.5	0.23	0.4	0.76	0.91	0.99	1.02
21.	6.4	6.05	4.8	4.7	4.5	4.5	0.24	0.6	0.9	0.91	1.0	1.03

Table 2 : Effect of subculturing on pH and Titratable Acidity of *Lactococcus lactis* subsp.*lactis* ML 8.

No. of Transfer	pH						Titratable Acidity (% Lactic Acid)					
	0h	4h	8h	12h	16h	24h	0h	4h	8h	12h	16h	24h
1.	6.3	6.2	5.5	4.6	4.55	4.4	0.24	0.42	1.0	1.13	1.15	1.15
2.	6.3	6.0	4.9	4.55	4.5	4.4	0.23	0.40	1.10	1.10	1.12	1.15
3.	6.3	6.05	4.8	4.6	4.45	4.3	0.24	0.62	0.89	1.0	1.0	1.02
4.	6.4	6.05	4.85	4.5	4.4	4.4	0.25	0.40	0.8	0.83	1.05	1.1
5.	6.4	6.25	5.2	4.8	4.7	4.5	0.25	0.60	0.91	0.98	1.18	1.2
6.	6.4	6.0	5.0	4.6	4.5	4.4	0.22	0.48	0.88	1.0	1.02	1.11
7.	6.4	5.95	4.7	4.55	4.5	4.4	0.23	0.46	0.96	1.0	1.11	1.2
8.	6.3	5.9	5.1	4.8	4.6	4.5	0.24	0.51	0.7	1.02	1.2	1.25
9.	6.4	5.9	4.85	4.7	4.5	4.5	0.22	0.48	0.98	1.2	1.22	1.25
10.	6.4	5.9	5.25	4.7	4.6	4.5	0.25	0.50	0.85	1.0	1.02	1.1

Contd....

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No. of Transfer	pH						Titratable Acidity (% Lactic Acid)					
	0h	4h	8h	12h	16h	24h	0h	4h	8h	12h	16h	24h
11	6.4	6.15	5.0	4.6	4.5	4.5	0.25	0.40	0.82	1.01	1.05	1.05
12	6.3	6.1	5.0	4.6	4.5	4.5	0.25	0.40	0.8	1.0	1.18	1.22
13	6.4	6.1	5.1	4.7	4.6	4.5	0.23	0.41	0.8	1.0	1.01	1.2
14	6.3	6.1	5.0	4.65	4.5	4.5	0.25	0.50	0.84	0.98	1.02	1.08
15	6.3	6.1	4.85	4.7	4.65	4.55	0.23	0.42	0.91	0.98	0.99	1.0
16	6.4	6.05	4.9	4.8	4.5	4.5	0.23	0.40	0.9	0.97	1.0	1.01
17	6.3	6.0	4.8	4.75	4.7	4.55	0.25	0.41	0.89	0.9	0.9	0.98
18	6.5	6.05	5.2	4.8	4.55	4.45	0.25	0.40	0.82	0.9	1.01	1.05
19	6.3	6.1	4.9	4.8	4.65	4.5	0.24	0.43	0.79	0.91	0.95	1.01
20	6.4	6.15	4.8	4.7	4.65	4.5	0.23	0.37	0.9	0.93	0.98	1.02
21	6.4	6.15	5.1	5.1	4.7	4.5	0.24	0.50	0.82	0.85	0.99	1.01

Table 3 : Effect of subculturing on pH and Titratable Acidity of *Lactococcus lactis* subsp.*lactis* C 2.

No. of Transfer	pH						Titratable Acidity (% Lactic Acid)					
	0 h	4h	8h	12h	16h	24h	0h	4h	8h	12h	16h	24h
1.	6.3	6.1	5.8	5.45	5.3	5.1	0.19	0.50	0.70	0.79	1.0	1.0
2.	6.3	6.15	5.8	5.45	5.3	5.2	0.23	0.39	0.75	0.76	0.80	0.91
3.	6.3	6.25	5.7	5.6	5.4	5.3	0.24	0.43	0.72	0.75	0.80	0.82
4.	6.4	6.2	5.7	5.6	5.5	5.5	0.23	0.39	0.55	0.68	0.81	0.83
5.	6.4	6.3	5.75	5.5	5.5	5.35	0.25	0.50	0.62	0.70	0.90	1.0
6.	6.3	6.1	5.7	5.5	5.4	5.2	0.24	0.41	0.72	0.8	0.86	0.89
7.	6.4	6.3	5.8	5.7	5.65	5.5	0.22	0.25	0.50	0.50	0.55	0.60
8.	6.3	6.2	5.7	5.6	5.55	5.5	0.21	0.31	0.60	0.65	0.70	0.75
9.	6.3	6.2	5.6	5.5	5.5	5.45	0.21	0.31	0.59	0.62	0.71	0.72
10.	6.4	6.1	5.65	5.4	5.4	5.3	0.23	0.48	0.60	0.65	0.74	0.79
11.	6.4	6.1	5.7	5.4	5.3	5.2	0.25	0.50	0.52	0.65	0.71	0.80
12.	6.4	6.15	5.7	5.4	5.3	5.3	0.23	0.50	0.52	0.63	1.01	1.1
13.	6.3	6.1	5.6	5.5	5.4	5.05	0.23	0.51	0.55	0.59	0.65	1.12
14.	6.45	6.2	5.5	5.5	5.2	5.2	0.21	0.50	0.51	0.67	0.80	0.81
15.	6.35	6.2	5.75	5.5	5.1	5.1	0.23	0.35	0.60	0.71	0.79	0.80
16.	6.4	6.2	5.7	5.6	5.1	5.1	0.23	0.31	0.61	0.70	0.79	0.82
17.	6.3	6.2	5.6	5.4	5.2	5.0	0.23	0.32	0.60	0.71	0.79	0.91
18.	6.5	6.15	5.8	5.6	5.4	5.2	0.23	0.38	0.60	0.71	0.75	0.82
19.	6.3	6.3	5.65	5.55	5.05	5.0	0.25	0.35	0.60	0.72	0.75	0.81
20.	6.45	6.15	5.65	5.3	5.2	5.05	0.24	0.40	0.52	0.75	0.79	0.85
21.	6.4	6.1	5.4	5.4	5.2	4.9	0.24	0.50	0.79	0.82	0.91	0.95

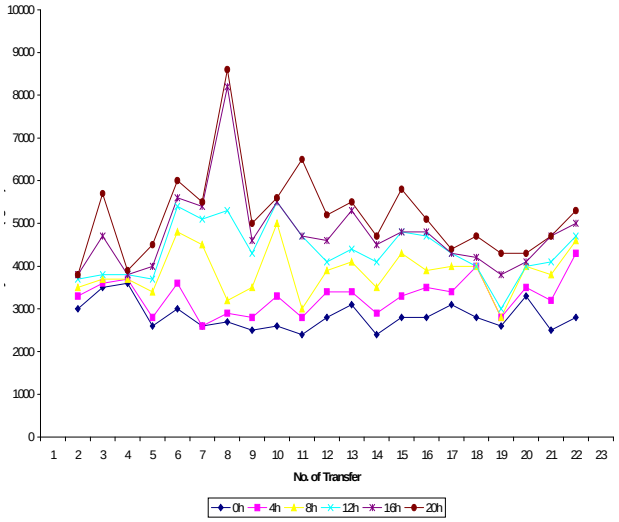

Fig. 1 : Effect of Subsculturing on Proteolytic Activity of lactococcus lactis subsp. lactis ML3.

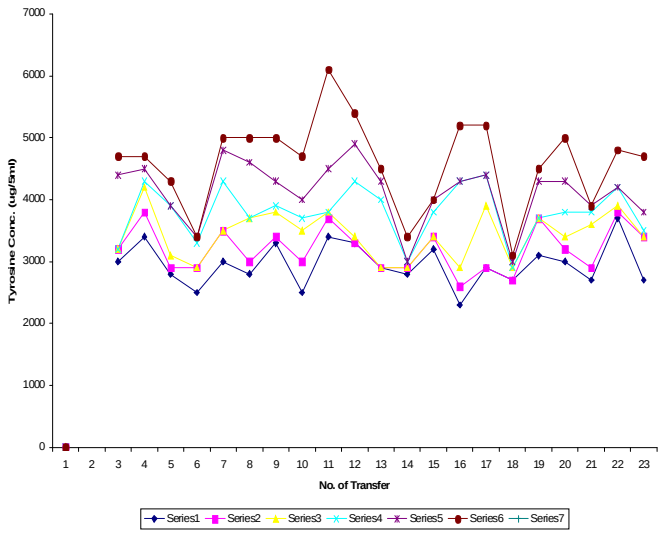

Fig. 2 : Effect of Subsculturing on Proteolytic Activity of lactococcus lactis subsp. lactis ML8.

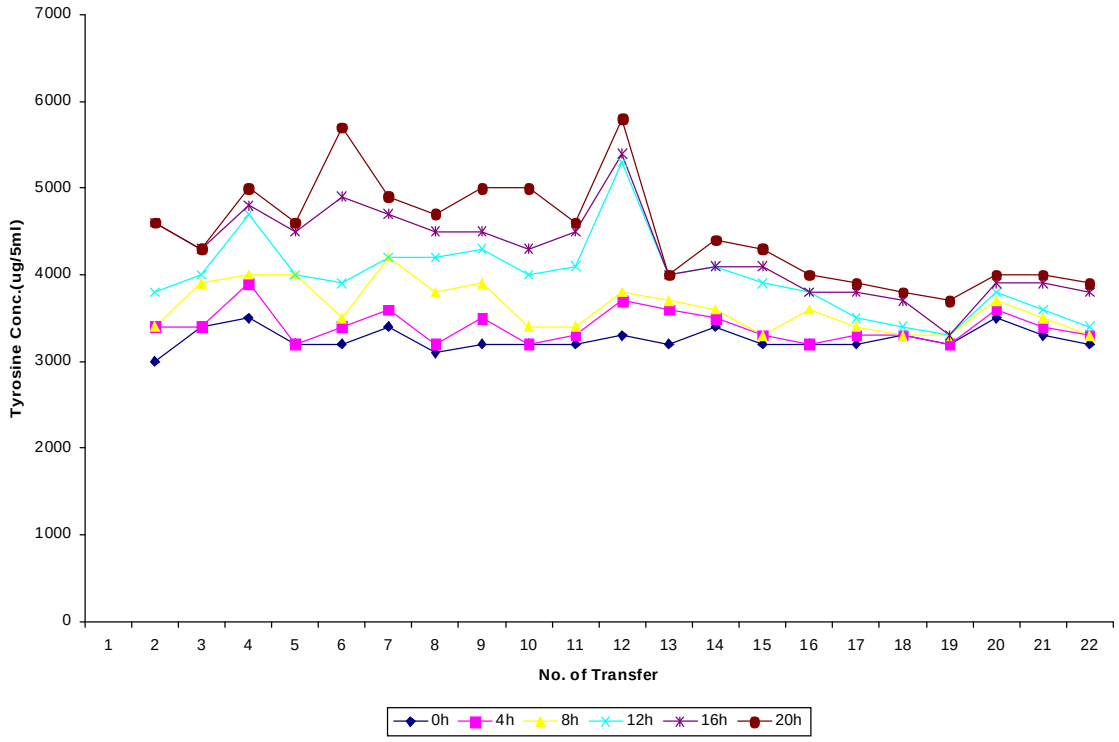

Fig. 3 : Effect of Subsculturing on Proteolytic Activity of lactococcus lactis subsp. lactis C2.

increase in incubation period the isolates exhibits more or less similar degree of lactose metabolizing and protein hydrolyzing abilities comparable to that of standard cultures.

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