Screening and selection of cultures based on techno-functional properties for ripening of cream

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Abstract: The aim of the present study was to screen and select diacetyl producing *Lactococcus lactis* culture(s) based on techno-functional properties for ripening of cream to formulate defined strain starter for preparation of ripened cream. Diacetyl production (determined by Creatine test) of 13 *Lactococcus* cultures from National Collection of Dairy Cultures, ICAR-NDRI, Karnal was studied in skim milk at 30°C for 0, 6, 8 and 10 h. Four potent diacetyl producing cultures, namely *L. lactis* NCDC 97, 127, 128, 193 were selected on this basis for further study along-with diacetyl negative *L. lactis* NCDC 314. Growth, acidification and diacetyl production profiles of selected cultures were evaluated in skim milk and cream (40% fat) at 30°C for 0, 6, 8 and 10 h. *L. lactis* NCDC 128 and 193 started diacetyl production at 6 h of fermentation in skim milk as well as in cream, which was further increased upto 8 h of fermentation. Diacetyl production in cow cream (40% fat) at 8 and 10 h of fermentation were comparable. The pH of cream and skim milk dropped from 6.2 to 4.5. Viable counts were observed to be increased by 2 log from initial count of 7 log cfu/ml. Growth, acidification and diacetyl production were higher in skim milk as compared to cream.

Keywords: Cream, Diacetyl, Fermentation, *Lactococcus lactis*

Introduction

Fermentation of cream by lactic acid bacteria majorly includes catabolism of sugars present in the food matrix along with citrate. Citrate has its abundance in nature, and it is major potential source of energy for microorganisms to act and multiply in food system. This citrate fermentation leads to C₄ carbon products, mainly acetoin, diacetyl and butanediol provided the acidic conditions are maintained. These compounds are flavour and aromatic compounds adds consumer appeal to dairy product. Among these, diacetyl is main responsible for buttery aroma in dairy matrix. Flavour production by lactic acid bacteria through citric acid fermentation in dairy matrix is a complex phenomenon which includes cobweb of multiple aroma and flavour producing compounds. These compounds contribute to flavour of product which enhance their market value in dairy and food industry. The flavour production by desired micro-organisms in dairy products, makes them a value added product in terms of nutritional functionality as well as consumer acceptability is increased. Fermented cream is used primarily as a condiment in cooking and baking: an ingredient in sauces, toppings, dips, and dressings and low-fat spread. It is obtained through the fermentation of standardized, homogenized, heat-treated sweet cream using certain strains of lactic acid bacteria, which deliberately sour and thicken the cream. As a result of lactic acid fermentation, the nutritional quality of the cream improves and the microbial safety increases, in association with the reduction of probable toxic compounds and the production of antimicrobial substances such as lactic acid, hydrogen peroxide, and bacteriocins. Cream fermented with suitable lactic cultures may have the potential for use as a probiotic carrier because it provides an excellent environment for probiotic bacteria, with the added advantage of having nutritional value as well as yield a good flavor product (butter or ghee). Jiao ke is a homemade naturally fermented cream in Inner Mongolia of China. The main lactic acid bacteria (LAB) strains isolated from Jiao ke are *Lactobacillus* spp., *Lactococcus* spp., and *Enterococcus* spp. Jiao ke has a lower cholesterol content than other cream products (Gong et al. 2010). Some lactic acid bacteria have a potential to lower cholesterol content (Anderson, 1999; Sanders, 2000; Jones et al. 2004). In Ethiopia, ghee is used in different traditional diets and culinary

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purposes in different communities (Gemechu, 2017). Strain *Lactobacillus plantarum* KLDS 1.0344 isolated from home made fermented cream in Inner Mongolia of China has the most potent use as a dietary supplement for lowering human serum cholesterol. According to Joseph and Srinivasan (1980) among the four commonly used dairy starters namely *Streptococcus lactis* ssp. *diaceytillactis*, *S. lactis*, *S. thermophilus* and *Lactobacillus bulgaricus*. Ghee produced by *Streptococcus lactis* ssp. *Diaceytillactis* had relatively better flavour. In cream, cultures of streptococci developed total titratable acidity ranging from 0.44 to 0.67% LA, 0.52 to 0.70 % LA and 0.67 to 0.80%LA at the end of 24, 48 and 72 h of fermentation, respectively. *S. diaceytillactis* DRC-1 culture produced maximum titratable acidity 0.80% LA, among streptococci group. *L. bulgaricus* LBW produced the maximum amount of titratable acidity 1.25% LA, among all the four organisms studied. Joseph and Srinivasan (1980) made a comparison between four starter cultures namely *Streptococcus lactis* C-10, *S. lactis* ssp. *diacetilactis* DRC-1, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* for preparation of ripened cream ghee.

Materials and methods

Cultures

Thirteen *Lactococcus* spp. cultures such as *L. lactis* ssp. *diaceytillactis* NCDC 60, 61, 62, 64, 289 and *L. lactis* ssp. *lactis* NCDC 94, 97, 125, 127, 128, 193, 313 and 314 were collected from National Collection of Dairy Cultures (NCDC), ICAR-NDRI, Karnal. Freeze dried cultures were activated in chalk litmus milk (30°C/ 15-18 h) and stored at 7°C. Sub-culturing was done in M17 broth at 30°C for 16-18 h and maintained at refrigeration conditions. Chalk litmus milk activated culture was propagated in M17 broth twice (30°C/ 15 h) to get active culture. The morphological and biochemical identification of cultures for purity was carried out by tests such as negative staining, gram staining, catalase test, and growth in selective medium.

Evaluation of *Lactococcus* cultures for diacetyl production in skim milk

Chalk litmus milk activated culture was propagated in M17 broth twice (30°C/ 15h) to get active culture. Sterile skim milk was prepared by autoclaving 10 ml skim milk in test tube. Activated M17 broth culture was inoculated @ 2% in 10 ml skim milk. The test tube was incubated @ 30°C for 15 h and the curd was allowed to set. After incubation, 2 ml of curd and 2 ml of NaOH solution (40% w/v) were mixed in a clean test tube. Pinch of creatine monohydrate powder was mixed to it. The tube was kept in dark for 10 min. Appearance of pink colour band showed the indication for diacetyl flavour production (Ivanova et al. 2012).

Growth, acidification and diacetyl production profile of *Lactococcus* cultures in skim milk and cream.

After initial screening of diacetyl production, five selected *Lactococcus* cultures (4 strong diacetyl producers (NCDC 193, 97, 127, 128) and 1 diacetyl negative (NCDC 314), were evaluated for growth, acidification and diacetyl production in skim milk and cream at 30°C for 10 h. Four flasks of sterile skim milk (100 ml each) were inoculated with active skim milk culture (@ 2%) and incubated at 30°C for 10 h. At 0, 6, 8 and 10 h samples were analyzed for pH, titratable acidity, lactococci count, coliforms, yeasts and mold counts. Similarly, *Lactococcus* cultures were also evaluated for growth, acidification and diacetyl production profile in cow cream (40% fat). Throughout the study, cow cream of 40% fat is used.

Analysis of fermented skim milk/cream for pH and titratable acidity

pH of sample was determined with the help of calibrated pH meter (Thermo scientific). Titratable acidity was determined by transferring well homogenized sample (10 ml) was in 100 ml conical flask. Phenolphthalein indicator solution (3 to 5 drops) was added to it and titrated against N/10 NaOH solution with continuous stirring till light pink colour persists. Volume of NaOH solution required was measured and titratable acidity (% lactic acid) was calculated (Oladipo et al. 2014).

Enumeration of total lactic count, coliform and yeast and mold count

Total lactic count (log cfu/ml) was enumerated by plating on M17 agar (30°C/ 24-48 h) (AOAC, 1980) after preparation of suitable decimal dilutions. Coliform count was enumerated by plating 1 ml sample on violet red bile agar plate (37°C/ 24 h). Yeasts and molds count was obtained by plating 1 ml sample on potato dextrose agar plate (25°C/ 3-5 days) (AOAC, 1980).

Results and discussion

Acidification and diacetyl production of thirteen *Lactococcus* cultures were evaluated in skim milk (@ 2%) at 30°C for 15 h (Table 1). Maximum pH was dropped by NCDC 60 (4.25) and minimum pH drop (4.74) was observed by NCDC 64. Whereas all the other cultures, depicted a pH reduction in the range of 4.2-4.7. Titratable acidity of fermented skim milk by NCDC 64 was highest (0.96 % LA) among all the cultures. Least acid development (0.70 % LA) was observed with NCDC 125 (Figure 1). Nine cultures were found as diacetyl producing, among which five were weak diacetyl producers (NCDC 125, 313, 60, 61 and 62) and four cultures were identified as strong diacetyl producers (NCDC 97, 127, 128 and 193). Four *Lactococcus* cultures were found as non-diacetyl producers namely NCDC 94, 289, 314 and 64 (Figure 4.1). Marino et al. 2003 reported the acidification ability of *L. lactis* strains in ranges of 0.76 to 0.79 % LA, and 1.13 to 1.24% LA after 6 and 24 h of fermentation in skim milk.

Fig. 1 Creatine test for diacetyl production by *Lactococcus* cultures in skim milk
Pink colour indicate positive for diacetyl production

Table 1 Acidification and diacetyl production of *Lactococcus* cultures at 30°C for 15 h

Cultures	pH	Titratable acidity (% LA)	Diacetyl
<i>L. lactis</i> ssp. <i>diacetylactis</i> NCDC 60	4.25±0.04	0.81±0.03	+
<i>L. lactis</i> ssp. <i>diacetylactis</i> NCDC 61	4.35±0.03	0.80±0.01	+
<i>L. lactis</i> ssp. <i>diacetylactis</i> NCDC 62	4.26±0.02	0.78±0.02	+
<i>L. lactis</i> ssp. <i>diacetylactis</i> NCDC 64	4.74±0.02	0.96±0.06	-
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 94	4.35±0.04	0.85±0.04	-
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 97	4.28±0.04	0.72±0.02	+++
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 125	4.34±0.06	0.70±0.05	+
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 127	4.27±0.05	0.86±0.02	+++
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 128	4.37±0.04	0.82±0.02	+++
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 193	4.27±0.05	0.86±0.01	+++
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 313	4.31±0.07	0.88±0.03	+
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 314	4.54±0.06	0.85±0.04	-
<i>L. lactis</i> ssp. <i>lactis</i> NCDC 289	4.45±0.09	0.85±0.05	-

Data are Mean ± SEM, n=3

(-) Negative, (+) Weakly Positive, (+++) Strongly positive
(Qualitative : Visual observation)

Growth, acidification and diacetyl production profile of selected *Lactococcus* cultures in skim milk

Growth, acidification and diacetyl profile of four selected strong diacetyl producing *Lactococcus* cultures and one diacetyl negative *Lactococcus* culture in skim milk was studied at 30°C for 10 h (Table 2). As the incubation started, there was decrease in pH and increase in titratable acidity vis-à-vis increase in viable counts with time intervals (Table 2). Two cultures started diacetyl production at 6 h of fermentation (*L. lactis* NCDC 193 and *L. lactis* NCDC 128) whereas other two diacetyl positive *L. lactis* NCDC97 and *L. lactis* NCDC127 cultures started diacetyl production at 8 h of fermentation (Figure 2). Count was increased by 2 log from initial log count of 7 log cfu/ml. *L. lactis* NCDC193, started diacetyl production at 6 h of incubation as citric acid fermentation by citrate permease starts at pH range 4.8-5.0. *L. lactis* ssp. *lactis* NCDC 193 reduced milk pH to 4.8 at 6 h, which was further dropped down to 4.60 at 10 h (Table 2). Diacetyl content was increased as titratable acidity increased, from 0.16 to 0.68% LA. Diacetyl content rises along with the viable count.

L. lactis NCDC128, showed similar response as that of culture NCDC 193. Diacetyl increased consistently starting from 6 h to 8 h of fermentation (Figure2). In the similar pattern, it showed a steep decline in pH and titratable acidity from 6.20 to 4.6 and 0.23 to 0.45% LA respectively from 0 to 10 h of fermentation at 30°C (Table 2). Viable count at 0, 6, 8 and 10 h by *L. lactis* NCDC 128 were 7.14, 8.31, 8.62 and 9.77 log cfu/ml, respectively. *L. lactis* NCDC 97 and *L. lactis* NCDC 127, did not start diacetyl production even at 6 h of incubation (Figure 2). *L. lactis* NCDC 97 and *L. lactis* NCDC 127, both did not produce diacetyl content at par with NCDC 193 and NCDC 128. *L. lactis* NCDC 314 did not produced diacetyl after 10 h of fermentation (Figure2).

Growth, acidification and diacetyl production profile of *Lactococcus* cultures in cow cream (40% fat)

Growth, acidification and diacetyl profile of five selected *Lactococcus* cultures was also evaluated in cow cream (40% fat) at 30°C for 10 h. Diacetyl production by *L. lactis* NCDC 128 and *L. lactis* NCDC 193 started at 6 h of fermentation (Figure3). The

Fig. 2 Diacetyl production profile (Creatine test) of *Lactococcus* cultures in skim milk at 30C for 10 h

Table 2. pH, titratable acidity, viable count and diacetyl production of selected *Lactococcus* cultures in skim milk at 30°C for 10 h

Cultures	Incubation Time (h)	pH	Titratable acidity (% LA)	Viable count (log cfu/ml)	Diacetyl
<i>L. lactis</i> NCDC 97	0	6.20±0.07	0.18±0.23	6.06±0.32	-
	6	5.17±0.18	0.72±0.43	7.03±0.92	-
	8	4.62±0.41	0.76±0.76	8.01±0.28	++
	10	4.59±0.81	0.82±0.32	8.70±0.72	++
<i>L. lactis</i> NCDC 127	0	6.18±0.09	0.18±0.43	7.79±0.07	-
	6	5.17±0.32	0.62±0.61	8.56±0.92	-
	8	4.62±0.54	0.55±0.18	8.63±0.23	++
	10	4.59±0.19	0.65±0.07	8.71±0.15	++
<i>L. lactis</i> NCDC 128	0	6.20±0.76	0.23±0.03	7.14±0.32	-
	6	4.80±0.31	0.36±0.43	8.31±0.08	+
	8	4.50±0.94	0.42±0.40	8.62±0.64	+++
	10	4.60±0.32	0.45±0.54	9.77±0.32	+++
<i>L. lactis</i> NCDC 193	0	6.20±0.64	0.16±0.42	7.60±0.83	-
	6	4.80±0.05	0.60±0.32	8.47±0.32	+
	8	4.50±0.43	0.63±0.03	8.57±0.76	+++
	10	4.60±0.05	0.68±0.15	8.91±0.45	+++
<i>L. lactis</i> NCDC 314	0	6.40±0.34	0.17±0.43	7.73±0.21	-
	6	5.28±0.65	0.35±0.38	8.51±0.54	-
	8	4.50±0.13	0.64±0.72	8.90±0.06	-
	10	4.43±0.17	0.87±0.65	8.91±0.07	-

Table 3 pH, titratable acidity, viable count and diacetyl production of *Lactococcus* cultures in cow cream (40% fat) at 30°C for 10 h

Cultures	Incubation Time (h)	pH	Titratable acidity (% LA)	Viable count (log cfu/ml)	Diacetyl
<i>L. lactis</i> NCDC 97	0	6.20±0.07	0.18±0.23	6.06±0.32	-
	6	5.17±0.18	0.72±0.43	7.03±0.92	-
	8	4.62±0.41	0.76±0.76	8.01±0.28	++
	10	4.59±0.81	0.82±0.32	8.70±0.72	++
<i>L. lactis</i> NCDC 127	0	6.18±0.09	0.18±0.43	7.79±0.07	-
	6	5.17±0.32	0.62±0.61	8.56±0.92	-
	8	4.62±0.54	0.55±0.18	8.63±0.23	++
	10	4.59±0.19	0.65±0.07	8.71±0.15	++
<i>L. lactis</i> NCDC 128	0	6.20±0.76	0.23±0.03	7.14±0.32	-
	6	4.80±0.31	0.36±0.43	8.31±0.08	+
	8	4.50±0.94	0.42±0.40	8.62±0.64	+++
	10	4.60±0.32	0.45±0.54	9.77±0.32	+++
<i>L. lactis</i> NCDC 193	0	6.20±0.64	0.16±0.42	7.60±0.83	-
	6	4.80±0.05	0.60±0.32	8.47±0.32	+
	8	4.50±0.43	0.63±0.03	8.57±0.76	+++
	10	4.60±0.05	0.68±0.15	8.91±0.45	+++
<i>L. lactis</i> NCDC 314	0	6.40±0.34	0.17±0.43	7.73±0.21	-
	6	5.28±0.65	0.35±0.38	8.51±0.54	-
	8	4.50±0.13	0.64±0.72	8.90±0.06	-
	10	4.43±0.17	0.87±0.65	8.91±0.07	-

Data are Mean ± SEM, n=3

(-) Negative, (+/++) Weakly Positive, (+++) Strongly positive

(Qualitative : Visual observation)

similar pattern was observed in case of skim milk by both the two cultures. Diacetyl production increased along with time interval,

vis-à-vis supported by growth profile in terms of viable count. Fermentation of cow cream by *L. lactis* NCDC 193, increased the

Fig. 3 Diacetyl production profile (Creatine test) of *Lactococcus* cultures in cow cream (40% fat) at 30°C for 10 h

viable count from 7.38, 8.08, 8.13 and 9.57 log cfu/ml at 0, 6, 8 and 10 h, respectively (Table 3). Acidification profile of NCDC 193 also showed promising results, pH was dropped from 6.18 to 4.52 after 10 h of fermentation. *L. lactis* NCDC 128, behaved in a similar pattern in cream as skim milk. Titratable acidity achieved at 10 h of fermentation was 0.75 % LA. Viable count obtained after 10 h of fermentation was highest among all the cultures in cream (9.22 log cfu/ml) (Table 3). *L. lactis* NCDC 97 and *L. lactis* NCDC 127, started diacetyl production at 8 h of fermentation. The pH obtained by both these cultures at 6 h of fermentation was 5.01 and 5.32, respectively. At 8 h, both these cultures (NCDC 97 and NCDC 127) showed less diacetyl production as compared to *L. lactis* NCDC 193 and *L. lactis* NCDC 128, at same time interval (Figure 3). *L. lactis* NCDC 97 and *L. lactis* NCDC 127, had less viable count after 10 h of fermentation i.e. 8.3 and 8.06 log cfu/ml, whereas other two strong diacetyl producers showed a much better growth (Figure 3). Diacetyl production by *L. lactis* NCDC 193 and *L. lactis* NCDC 128 at 8 and 10 h of interval were comparable (Figure 3).

Joseph and Srinivasan (1980) studied the fermentation profile of cream (40% fat) with *S. lactis* C-10 or *S. diacetylactis* DRC-1 at 30°C for 72 h and with *S. thermophilus* HST, *L. bulgaricus* LBW at 37°C for 72 h. Titrable acidities were 0.4, 0.46, 0.42 and 0.68 %LA after 12 h; 0.52, 0.70, 0.65 and 1.10 % LA after 48 h; 0.67, 0.80, 0.74 and 1.25% LA after 72 h. Ripened buffalo cream (15% fat) with different lactic cultures single and in combination and reported that titratable acidity (% LA) were 0.91, 1, 1.06, 1.08 and 1.06 %LA with *S. lactis*, *S. cremoris*, *S. diacetylactis*, *S. lactis* + *S. diacetylactis*, and *S. cremoris* + *S. diacetylactis* respectively.

Conclusions

Nine *Lactococcus* cultures were positive for diacetyl production, among them four were potent diacetyl producing cultures, namely *L. lactis* NCDC 97, 127, 128 and 193 were selected on this basis along-with diacetyl negative *L. lactis* NCDC 314. pH of skim milk was reduced in the range of 4.43-4.59 and titratable acidity was increased in the range of 0.65-0.87% lactic acid. Approximately 2 log increase of viable counts were observed. *L. lactis* NCDC 193 and *L. lactis* NCDC 128 started diacetyl production at 6 h, whereas *L. lactis* NCDC 97 and *L. lactis* NCDC 127 cultures started diacetyl production after 8 h. At 8 and 10 h, diacetyl production by *L. lactis* NCDC 193 and *L. lactis* NCDC 128 were higher than *L. lactis* NCDC 97 and *L. lactis* NCDC 127. Growth, acidification and diacetyl profile of the five *Lactococcus* cultures in cream (40% fat) was also evaluated at 30°C for 10 h. Diacetyl production

by *L. lactis* NCDC 128 and *Lactococcus lactis* NCDC 193 was also observed at 6 h. Further, diacetyl production was increased upon increasing fermentation upto 8 h. Viable counts of *L. lactis* NCDC 193 were increased from 7.38 to 9.57 log cfu/ml and pH was dropped from 6.18 to 4.52 and acidity was increased from 0.16 to 0.6 %LA at the end of 10 h. Diacetyl production by *L. lactis* NCDC 193 and *L. lactis* NCDC 128 at 8 and 10 h were comparable. Ripened cream of optimum quality was produced from two different cultures *L. lactis* NCDC 193 and *L. lactis* NCDC 128.

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