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Activity profile of starter cultures maintained in whey based medium

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Abstract
Acid production, proteolytic and lipolytic activities are the major characteristics of lactic acid bacteria (LAB) that affects the flavor development in fermented dairy products. Based on these activities, the LAB used for each dairy product is specific. In this study we analyzed, whether the activity levels of starter LAB depends on the medium used for their growth or maintenance. Whey supplemented with one percent yeast extract (1WYE) was the treatment medium and control media were commercial broth and skim milk for this analysis. The starters used were *Lactococcus lactis* 676, *Lactococcus lactis ssp. diacetylactis* 621 and *Lactobacillus rhamnosus* 296. The study revealed that, the proteolytic and lipolytic activity of starters are independent of the medium used for their growth. Therefore, whey based medium can be utilized as the cost-effective growth and maintenance medium of starters for fermented dairy product sector.

Keywords: Starter cultures, whey, yeast extract, proteolytic activity, lipolytic activity

1. Introduction
Lactic acid production, proteolytic and lipolytic abilities are the major activity measures of starter cultures; which were considered as the heart of fermented dairy products. Proteolytic activity by starter cultures improves the nutritional quality and enhances milk digestibility in fermented products (Zourari *et al*., 1992) [1]. Whereas, degradation of lipids (lipolytic activity) by lactic acid bacteria is responsible for the development of flavour in fermented foods like cheese (Gaddi and Marth, 1970) [2]. By monitoring the lactic acid production, we can analyse the efficiency of bacterial strains for fermentation (Accolas *et al*., 1977) [3].
The objective of our study was to analyse whether these activities of starter cultures are dependent on the medium used for its growth and maintenance. For that, we utilized whey as the treatment growth medium. Whey is a byproduct obtained from cheese and paneer industry, which was usually discarded. But majority of the water soluble nutrients of milk are available in whey which makes it a potent pollutant to the environment (Macwan *et al*., 2016) [4]. The proper utilization of these nutrients for starter culture production can make the starters more affordable for small scale entrepreneurs in fermented dairy food sector and also reduces the environmental pollution. In this study, we analysed the growth of lactic acid bacteria in whey based media and compared its activity level to that grown in control media like commercial broth and skim milk.

2. Materials and Methods
2.1 Procurement of starter cultures
Three pure LAB cultures like *Lactobacillus rhamnosus* 296, *Lactococcus lactis* 676 and *Lactococcus lactis* ssp. *diacetylactis* 621 were procured from National Collection of Dairy Cultures (NCDC), Karnal. These freeze dried cultures were further propagated in commercial broths like MRS (for lactobacilli) or M17 (for Lactococcus) broth and stored at 4°C.

2.2 Preparation of whey based media
From University dairy plant, KVASU, Mannuthy, fresh skim milk was collected, coagulated with rennet (0.003%) and incubated at 31 °C for 40 minutes. The coagulum was cut and cooked, followed by the collection of whey.
The collected fresh whey was supplemented with commonly available exogenous nitrogen sources like yeast extract or peptone at varying concentrations *viz*., Whey + 0.3% yeast extract (0.3 WYE), Whey + 0.5% yeast extract (0.5 WYE), Whey + 1% yeast extract (1 WYE), Whey + 0.3% peptone (0.3 WP), Whey + 0.5% peptone (0.5 WP) and Whey + 1% peptone (1 WP).

2.3 Estimation of growth rate of starters
Starter cultures were inoculated into the autoclaved commercial broth, skim milk (control media) and whey based media at 2 per cent level. These were further incubated for 18 hours at optimum temperatures for each culture and the growth rate of cultures was estimated as cell count by microbial plate count method (Wehr and Frank, 2004) [5] at suitable dilutions in MRS or M17 agar and expressed in log cfu/ml. The whey based media showing the highest growth rate of organisms was selected as the optimized whey based treatment media for the activity studies.

2.4 Activity tests for starters
The activity level of starters maintained in broth, skim milk and optimised whey medium was analysed based on the parameters like titratable acidity, proteolytic activity and lipolytic activity.

2.4.1 Titratable acidity
As per method described in IS 1973 [6], lactic acid production was analysed by titrimeteric method. The sample was titrated against N/10 NaOH, using phenolphthalein as indicator.

2.4.2 Calculation
Titratable acidity as % Lactic acid
= 9 AN/W

Where,
A = Volume of standard NaOH required for titration
N = Normality of Standard NaOH solution
W = weight of the sample taken for test

2.4.2 Proteolytic activity
Proteolytic activity was estimated by measuring zone of hydrolysis in skim milk agar according to the method described by Benaissa *et al*. (2017) [7], bacterial cultures to be tested were inoculated on skim milk agar plates and incubated at the optimum temperatures for 48 hours, proteolytic activity was revealed by the appearance of a clear halo around each inoculated points. The diameter of this halo was later measured in mm.

2.4.3 Lipolytic activity
The bacterial cultures were streaked on tributyrin agar plate and incubated at optimum temperatures for 48 hours. Activity was observed as clear zone of hydrolysis around the inoculated points, which was later measured in cm (Katz *et al.*, 2002) [8].

3. Result and Discussion
3.1 Growth rate of starters
The growth rate of starter cultures as log cfu/ml is depicted in chart 1. By statistical analysis of the data, we selected whey supplemented with one percent yeast extract (1WYE) as the optimized whey based medium for further activity studies, due to a significantly higher growth rate of starters in 1 WYE when compared to other whey based media. Burns *et al.* (2008) [9] also identified yeast extract as the best growth promoter when compared to meat extract, soy peptone, tryptone and casein acid hydrolysate.

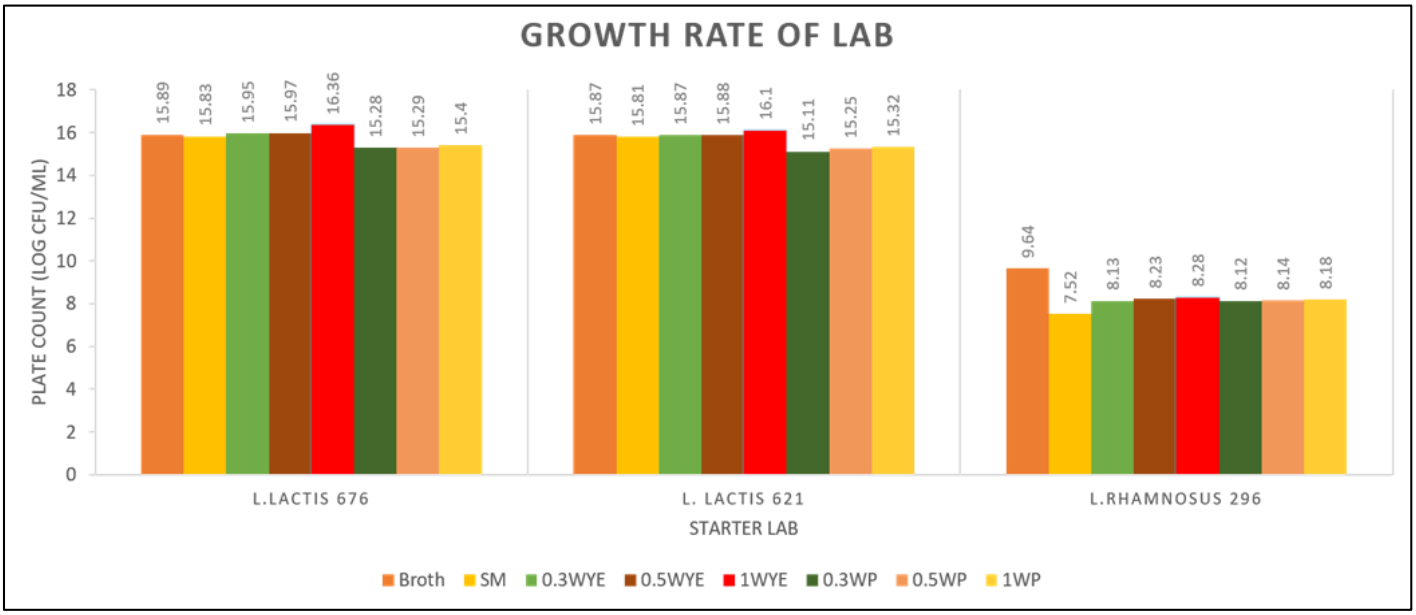

Chart 1: Growth rate of LAB (log cfu/ml)

3.2 Activity tests for starters
3.2.1 Titratable acidity
The titratable acidity expressed as the percentage of lactic acid is given in table 1. It is evident from the table that the titratable acidity of the medium increased correspondingly with the increased growth rate of the starter cultures in the medium. A significantly higher titratable acidity was observed in the whey based medium when compared to the skim milk medium. A similar finding was observed by Boudjou *et al.* (2014) [10], that the bacterial count shows a positive correlation with lactic acid production in the medium.

Table 1: Titratable acidity after 18 hours of incubation in percentage of lactic acid

Treatment	*L. lactis* 676	*L. lactis* 621	*L. rhamnosus* 296
Broth	1.01 ± 0.018^{ab}	0.88 ± 0.012^{ab}	0.80 ± 0.007^{a}
Skim milk	0.96 ± 0.020^{b}	0.86 ± 0.014^{b}	0.71 ± 0.010^{c}
Whey $_{optimised}$	1.06 ± 0.014^{a}	0.91 ± 0.009^{a}	0.77 ± 0.007^{b}

Each value is a mean of six observations with SE
Means with different superscript in same column differ significantly ($p \leq 0.001$)

3.2.2 Proteolytic activity

The proteolytic activity of the cultures estimated in mm is given in table 2. The proteolytic activity of the cultures were found to be same irrespective of the medium used for their growth. In this method, the concentration of cultures (10^7 cfu/ml) inoculated in the wells of skim milk agar were almost similar. A similar finding was indicated by Benaissa *et al*. (2017), that the proteolytic ability of *Lactobacillus* spp. grown in whey based media and control MRS media were the same.

On comparison of proteolytic activity between cultures, *Lactobacillus rhamnosus* 296 shows a significantly higher proteolytic activity than lactococci spp. In a similar study Pailin *et al*. (2001) [11], also observed that *Lactobacillus* sp. shows higher proteolytic activity than cocci lactic acid bacteria.

Table 2: Proteolytic activity observed as zone of hydrolysis, mm

Treatment	*L. lactis* 676	*L. lactis* 621	*L. rhamnosus* 296
Broth	1.87 ± 0.105 ^{nsB}	1.53 ± 0.105 ^{nsC}	3.52 ± 0.060 ^{nsA}
Skim milk	1.73 ± 0.071 ^{nsB}	1.65 ± 0.076 ^{nsB}	3.53 ± 0.061 ^{nsA}
Whey $_{optimised}$	1.77 ± 0.088 ^{nsB}	1.62 ± 0.048 ^{nsB}	3.58 ± 0.065 ^{nsA}

Each value is a mean of six observations with SE

Means having different capital letters as superscript differs significantly within a row ($p \leq 0.01$)

ns – no significant difference within a column

3.2.3 Lipolytic activity

The lipolytic activity of the cultures is indicated in table 3, which was the measure of zone of hydrolysis expressed in cm. From the table it was evident that the lipolytic potential of the organisms will not vary based on the medium of growth. On comparison between cultures, *L. lactis* 676 ranked first in lipolytic activity which was followed by *L. lactis ssp. diacetylactis* 621 and *L. rhamnosus* 296 in descending order. In a study by Searle *et al*. (1970) [12] observed that *Lactococcus* spp. showed higher lipolytic activity than *Lactobacillus* spp. Our results were in agreement with the above finding.

Even though cultures *L. lactis* 676 and *L. lactis ssp. diacetylactis* 621 belong to same species, they showed significant difference in the lipolytic activity. It may be due to the difference in strains. Katz *et al*. (2002) had a similar finding that, the lipolytic activities of LAB were species and strain specific.

Table 3: Lipolytic activity observed as zone of hydrolysis, cm

Treatment	*L. lactis* 676	*L. lactis* 621	*L. rhamnosus* 296
Broth	2.433 ± 0.026 ^{nsA}	2.323 ± 0.012 ^{nsB}	1.578 ± 0.019 ^{nsC}
Skim milk	2.435 ± 0.025 ^{nsA}	2.325 ± 0.018 ^{nsB}	1.568 ± 0.013 ^{nsC}
Whey$_{optimised}$	2.437 ± 0.028 ^{nsA}	2.320 ± 0.017 ^{nsB}	1.575 ± 0.006 ^{nsC}

Each value is a mean of six observations with SE

Means having different capital letters as superscript differs significantly within a row ($p \leq 0.01$)

ns – no significant difference within a column

4. Conclusion

Based on the growth performance of starter cultures, whey incorporated with one per cent yeast extract (1 WYE) was selected as the optimized whey medium. It was observed that the increase in titratable acidity of the medium depends on the growth of starter cultures in the medium. The proteolytic and lipolytic activity of starters showed no significant difference in any of the media used for their growth. Thus, whey based medium can be utilized effectively for the growth and maintenance of starter cultures without any reduction in their activity levels. Accordingly, the utilization of whey can lower the cost of production of starter cultures, which will be a benefit for small scale fermented dairy product entrepreneurs.

5. References

1. Zourari A, Accolas JP, Desmazeaud MJ. Metabolism and biochemical characteristics of yogurt bacteria. A review. Le lait. 1992; **72(1):**1-34.2
2. Gaddi AL, Marth EH. Growth and activity of lactic acid bacteria in soymilk. Department of Food Science and the Food Research Institute, University of Wisconsin, Madison, Wisconsin 53706, 1970.
3. Accolas JP, Bloquel R, Didienne R, Regnier J. Propriétés acidifiantes des bactéries lactiques thermophiles en relation avec la fabrication du yoghourt. Le lait. 1977; 57:1-23.
4. Macwan SR, Dabhi BK, Parmar SC, Aparnathi KD. Whey and its utilization. Int. J. of Curr. Microbiol. Appl. Sci. 2016; 5(8):134-155.
5. Wehr HM, Frank JF. Standard Methods for the Examination of Dairy Products. Edn 17. American public health association, Washington, 2004, 261.
6. IS: 1166, Determination of titratable acidity. Milk products. Bureau of Indian Standards, New Delhi, 1973.
7. Benaissa M, Halima ZK, Karam NE. Development of a sweet whey- based medium for culture of *Lactobacillus*. Afr. J. Biotech. 2017; **16**:1630-1637.
8. Katz M, Medina R, Gonzalez S, Oliver G. Esterolytic and lipolytic activities of lactic acid bacteria isolated from ewe's milk and cheese. J Food Prot. 2002; 65(12):1997-2001.
9. Burns P, Vinderola G, Fernando M, Reinheimer J. Suitability of whey and buttermilk for the growth and frozen storage of probiotic lactobacilli. Int. J. Dairy Tech. 2008; 61:156-164.
10. Boudjou S, Zaidi F, Hosseinian F, Oomah BD. Effects of faba bean (*Vicia faba*) flour on viability of probiotic bacteria during kefir storage. J. Food Res. 2014; 3(6):13.
11. Pailin T, Kang DH, Schmidt K, Fung DYC. Detection of extracellular bound proteinase in EPS- producing lactic acid bacteria cultures on skim milk agar. Letters in Appl. Microbiol. 2001; 33(1):45-49.
12. Searle MA, Argyle PJ, Chandan RC, Gordon JF. Lipolytic and proteolytic activities of lactic cultures. In: XVIII Int. Dairy Congr. IE. 1970, 111.