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Assessment of post acidification behaviour of different strains of *Lactiplantibacillus plantarum* and *Lacticaseibacillus rhamnosus* isolated from indigenous sources

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Abstract
In this study, four strains of *Lactiplantibacillus plantarum*, four strains of *Lacticaseibacillus rhamnosus* and one strain of *Lacticaseibacillus paracasei* isolated from various indigenous sources were assessed for their post acidification nature. Significant inter-species and inter-strain diversity (p<0.01) were observed in the time taken by the cultures to reduce the pH of cow milk to 4.5. The difference between the initial pH (4.5) and the pH after 48 h of extended fermentation (ΔpH_{48}), of all the tested cultures were more than 0.3 units and thereby all them were graded as high post acidifying. Findings of the reported study underscore the previous reports of low level occurrence of natural low post acidifying LAB cultures defined as those limiting drops to ≤ 0.30 pH units (p<0.01).

Keywords: Post-acidification, Indigenous starter culture

Introduction
Post acidification, a process by which the quality of the fermented products deteriorates during storage due to metabolic activities of the starter culture is identified as a major shelf-life limiting phenomenon in fermented dairy products. This process causes many detrimental effects like wheying-off, textural flaws, excessive sourness, and suppression of the perception of aroma in fermented milk products [1, 2]. Among the different factors contributing to post- acidification in fermented dairy products [3, 4], the starter cultures used for fermentation are considered to be of prime relevance. So it is very important that screening for post acidification behaviour is essentially conducted while assessing the technological attributes of putative starter cultures. Among the various dairy starter cultures, members of the family *Lactobacillaceae are of special significance and are o*rganized into different categories based on relevance to the industry [5]. Within this family, *Lactobacillus, Lacticaseibacillus, Lactiplantibacillus, Limosilactobacillus and Levilactobacillus are identified as t*he five most extensively studied genera. Among the various species of *Lactiplantibacillus, Lactiplantibacillus plantarum* is the most comprehensively studied species, particularly in dairy products, fruits, and vegetables [6].It is a versatile microorganism found in a wide range of ecological niches from the human gastrointestinal (GI) tract to different fermented foods [7]. *Lacticaseibacillus paracasei*, known for its probiotic properties and flavor-producing potential, is generally considered as an adjunct starter culture. In contrast to traditional starter strains, *Lcb. paracasei* exhibits a comparatively lower fermentation capacity [8]. *Lacticaseibacillus rhamnosus*, another member of *this genus is* widely recognized for health promoting properties and hence broadly applied in the production of food and dietary supplements [9].Though all these cultures are commonly used for dairy fermentations, prior research has fallen short in delineating the strain and species wise variations in the post acidification behaviour of these industrially relevant dairy starter cultures. This study aimed to bridge this knowledge gap by assessing the post-acidification behaviour of four strains of *Lpb. plantarum, four strains of Lcb. rhamnosus and a strain of Lcb. paracasei* isolated from various indigenous sources.

Materials and methods

Cultures used

Nine cultures from the culture stock of Department of Dairy Microbiology, Verghese Kurien Institute of Dairy and Food Technology (VKIDFT), Mannuthy were used in this study. Details of the cultures used are given in Table 1. For the experiments, these cultures were activated in reconstituted skimmed milk (10% w/v, Sagar, Amul) by inoculation at one per cent level of the substrate and subsequently incubated at 37 ℃ till a count of 7 $\log_{10}$ CFU/ml was attained.

Table 1: Details of the cultures used

Sl No.	Isolate name	NCBI Accession No:	Source
1	*Lpb. plantarum* DM02-V2-2025	PV249427	Vechur cow milk
2	*Lpb. plantarum* DMAG02	OR105028	Malabari goat milk
3	*Lpb. plantarum* DM122024	PQ725712	Vechur cow milk
4	*Lpb. plantarum* IDK 120	MT211513	Curd
5	*Lcb. paracasei* DMB11	OR905857	Murrah Buffalo milk
6	*Lcb. rhamnosus* PTA 1502	MK729020	Curd
7	*Lcb. rhamnosus* AMB 120	MT180552	Curd
8	*Lcb. rhamnosus* DMBS07	OR905853	Beans
9	*Lcb. rhamnosus* DMBR05	OR905852	Appam Batter

Assessment of Post Acidification behaviour of the cultures

Activated starter cultures (7 $\log_{10}$ CFU/ml) were inoculated at one per cent level into 50 ml homogenized, standardised (3.2% fat and 8.5% SNF) heat treated (85 ℃/15 min) and cooled cow milk collected in polypropylene (PP) bottles. The inoculated milk samples were aerobically incubated at 37℃. After attaining a pH of 4.5 (a pH confirmative of coagulation as the isoelectric point of milk is 4.6), the samples were incubated for another 24 h/48 h at the same temperature. Extent of acidification was monitored over extended periods, with pH_{24} and pH_{48} representing the values obtained after 24 and 48 h of extended fermentation. Differences between the initial (4.5) and final pH of fermentation (pH_{24} and pH_{48}) were taken as ΔpH_{24} and ΔpH_{48} respectively. Cultures with $\Delta pH_{48} \leq 0.3$ units, were graded as low post acidifying cultures and those with $\Delta pH_{48} > 0.3$ units as high post acidifying cultures [10].

Statistical analysis

The post acidification evaluation was conducted in three independent batches. The data obtained were analysed using Statistical Package for Social Sciences (SPSS, Version 22) and are presented as the mean ± standard error. Paired t-test and one way ANOVA were used for comparing between periods within each sample treatment and the changes between the samples in each period. In ANOVA, Tukey's Honest Significant Difference (HSD) was used to find the difference between groups.

Results and Discussion

Sl No:	Culture	Time taken for reaching pH 4.5 (h)	24 h of extended incubation		48 h of extended incubation		t value
			Titratable acidity (% LA)	ΔpH_{24} (4.5-pH24)	Titratable acidity	ΔpH_{48} (4.5-pH48)	
1.	DM02-V2-2025	25.83 ± 0.17^{a}	0.939 ± 0.03^{ab}	0.4667 ± 0.033^{abx}	$1.05 \pm .015^{a}$	0.7667 ± 0.033^{by}	-5.196*
2.	DMAG02	46.17 ± 0.17^{b}	0.977 ± 0.006^{ab}	0.4000 ± 0.058^{ax}	1.028 ± 0.034^{a}	0.6333 ± 0.033^{ax}	-2.646^{ns}
3.	DM122024	27.00 ± 0.0^{c}	0.996 ± 0.006^{ab}	0.5333 ± 0.033^{abx}	1.119 ± 0.006^{b}	0.8667 ± 0.033^{bcy}	-10.000**
4.	IDK 120	17.83 ± 0.16^{d}	2.593 ± 0.0396^{d}	1.2667 ± 0.033^{ex}	2.745 ± 0.007^{d}	1.4333 ± 0.033^{dy}	-5.000*
5.	DMB11	24.67 ± 0.33^{e}	0.873 ± 0.0138^{a}	0.6333 ± 0.033^{bc}	1.089 ± 0.009^{ab}	0.8333 ± 0.033^{bc}	-[#]
6.	PTA 1502	36.17 ± 0.17^{f}	1.375 ± 0.018^{c}	0.7333 ± 0.033^{cdx}	1.528 ± 0.0098^{c}	0.9667 ± 0.033^{cy}	-7.000*
7.	AMB 120	38.17 ± 0.17^{g}	1.074 ± 0.038^{b}	0.8333 ± 0.067^{d}	1.089 ± 0.009^{ab}	0.9667 ± 0.033^{c}	-4.000 ns
8.	DMBS07	40.17 ± 0.17^{h}	1.339 ± 0.014^{c}	0.7667 ± 0.033^{cdx}	1.530 ± 0.007^{bc}	0.9333 ± 0.033^{cy}	-5.000*
9.	DMBR05	29.50 ± 0.29^{i}	1.327 ± 0.062^{c}	0.6333 ± 0.033^{bcx}	1.535 ± 0.0028^{c}	0.9667 ± 0.033^{cy}	-10.000**
	F value	2012.019**	289.660**	38.00**	1502.56**	43.361**	

Figures are mean ± standard error of 3 replications, **- Significant at one per cent level (p<0.01), *- Significant at five per cent level (p<0.05), ns- non-Significant (p>0.05), [a-i]: means with different superscripts vary significantly within column, [xy]- means with different superscripts vary significantly within row, [#]-the correlation and t cannot be computed because the standard error of the difference is 0. Among the nine cultures used, four were distinct strains of *Lpb. plantarum*, *Lcb. rhamnosus* and one was a strain of *Lcb. paracasei* isolated from various indigenous sources. Significant (p<0.01) inter strain as well as species variations were observed in the time taken by the cultures to reduce the pH to 4.5(Table 1). Among the *Lpb. plantarum* cultures, the time taken to reduce the pH to 4.5 was lowest for strain IDK 120 while among the *Lcb. rhamnosus*, it was the lowest for the strain DMBR05 (29.50 ± 0.29 h). It is interesting to note

that IDK 120, the culture which attained pH 4.5 within the shortest period was the highest (2.593 ± 0.0396) acid producer among the tested cultures. These observations strongly agree with the previous reports of strain specific variations in the rate of acid production of lactic acid bacteria [11]. None of them exhibited $\Delta pH_{48} \leq 0.3$ units and thereby all the tested cultures were graded as high post-acidifying (Table 1). This observation of low occurrence of natural low post-acidifiers is supported by the previous report of absence of low post-acidifiers among the different strains of *Lpb. plantarum*, *Levilactobacillus brevis*, *Lactiplantibacillus pentosus* isolated from traditional fermented vegetables [12]. Shori et al. (2022) reported higher degree of post fermentative activity of *Lpb. plantarum* than *Lcb. rhamnosus* and *L. casei* during refrigerated storage of cashew milk for 21 days [13]. Similarly, Shori (2024) reported that *Lcb. rhamnosus L. casei* demonstrated more significant ($p < 0.05$) post acidification compared with *Lpb. plantarum* in fermented camel milk during storage [14]. However, no such clear demarcation was observed in the present study. The strong acidification nature of members of the species *Lpb. plantarum* and *Lcb. rhamnosus* also could be a reason for the absence of low post acidifiers among the cultures tested in the study. The species *Lacticaseibacillus paracasei* is generally considered as a slow acid producer than *Lcb. rhamnosus*, another species of the same genus. Differing from this general presumption, *Lacticaseibacillus paracasei* DMB11 took significantly ($p<0.01$) shorter time than all the strains of *Lcb. rhamnosus* used in this study to reduce the pH level to 4.5. The fastest and highest acid producer among the tested strains, IDK 120 was found to be highest post acidifier as it exhibited the highest ΔpH_{24} (1.2667±0.033) and ΔpH_{48} (1.4333± 0.033). Ability of this strain to produce high level of lactic acid and tolerate high level of acid could be effectively made use of in the preparation of high acid fermented milk products. ΔpH_{48} was found to be significantly higher than ΔpH_{24} in the case of all the cultures other than DMAG02 and AMB120. In the case of these cultures, though ΔpH_{48} was found to be higher than ΔpH_{24}, the increase was not statistically significant ($p>0.05$). As reported in a previous study [15], The present study also observed major decrease in pH from 4.5 during the first 24 hours compared to the subsequent 24 hours.

Conclusion

The findings of the reported study highlights marked strain and species-specific differences in the extent of post acidification by Lactic acid bacteria (LAB). This observation highlights the relevance of including post acidification profile screening as an inevitable criterion while evaluating the techno- functional attributes of putative dairy starter cultures. Among all the cultures evaluated, *Lpb. plantarum* IDK 120 exhibited the fastest acidification and the highest extent of post acidification. Considering the comparatively shorter time taken to attain pH 4.5 and the lower extent of post acidification DMB11, DM02-V2-2025 and DM122024 could be more suitable for development of mild acidic fermented products. Additionally, the study also underscored the previous reports of low level of occurrence of natural low post acidifying LAB cultures, defined as those limiting pH drops to ≤ 0.30 units.

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