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Temperature-Dependent Variation in API 50 CH Fermentation Profiles of *Lactobacillus* Species

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Abstract. API 50 CH fermentation profiles of 45 *Lactobacillus*, one *Atopobium*, and three *Weissella* strains incubated at 30°C and 37°C were evaluated. *Atopobium uli* and ten species of *Lactobacillus* showed stable patterns despite the change in temperature. The rest of the type strains showed discrepancy between the two incubation temperatures: 18 strains lost, 12 additionally fermented another sugar, and 7 others fermented a different one in lieu. The variation was maximum in *L. delbrueckii* subsp. *delbrueckii*. *L. malefermentans* failed to ferment any of the substrates at 37°C. Majority of the food and plant-associated strains (mainly heterofermenters) retained distinctive traits at 30°C, while most of the animal-associated strains (mostly homofermenters) did so at 37°C. No general trend was observed; 30°C appeared to promote heterofermentation, while 37°C favored homofermenters. Use of API 50 CH profiles for taxonomic purpose in most lactobacilli appears reproducible if a specific temperature for a species is strictly followed.

The genus *Lactobacillus* currently consists of over 60 species and subspecies based on phylogenetic data [3, 7, 12]. Ecologically, lactobacilli are associated with fermenting plant materials, milk and milk products, meat and meat products, the mouth, the respiratory, alimentary, and urinary tracts, and the vagina of humans and animals. Essentially, lactobacilli are important in food and feed fermentations, in stabilization of the gut microflora, and prevention of pathogens and spoilage organisms both in foods and the alimentary and genital tracts of humans and animals. The taxon is a heterogeneous group comprised of species with relatively wider phenotypic elasticity where variabilities in physiological properties may be accommodated in a species.

Various methods are used to differentiate the different species and assign new isolates, among which physiological tests, such as carbohydrate fermentation results, serve as a basis to allocate lactobacilli. API 50 CH fermentation system is one such method that enables us to evaluate physiological properties of an organism such as growth and metabolism on individual substrates including carbohydrates, heterosides, polyalcohols, and uronic acids. Therefore, any one of the assimilatory, oxidative, or fermentative pathways could be inferred from growth and color changes in the cupule section of the strip. Since API 50 CH fermentation system accommodates many types of carbohydrates and related compounds, the outcomes are very helpful in evaluating the properties of a strain and elucidating its physiological affiliations. The system also serves as an important tool in lactobacilli identification [11]. Vandamme et al. [13] have pointed out its wider application to taxonomy as an efficient phenotypic procedure able to discriminate inter-strain differences and, even further, going higher up to the family level. Consequently, therefore, different studies have utilized the results as supportive data for lactobacilli taxonomy. Many studies used this kit for identification of lactobacilli, and often incubation is carried out at 30°C [5, 8, 14]. Manufacturers recommend incubation at an optimum temperature for growth of the group under study where 25°C, 30°C, and 37°C are stated as options in the API 50 CH instruction manual (API Systems, BioMéreux, SA, France). API 50 CHL medium (#50 410) is the usual medium employed for inoculation of the

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Table 1. Strains of *Lactobacillus* and related lactic acid bacteria used in the study

Species	Strain	Habitat	Physiol. group[a]	Species	Strain	Habitat	Physiol. group
Atopobium uli[a]	CCUG 31116^T	Human mouth	OHo	*L. intestinalis*	CCUG 30727^T	Murine GIT	FHe
L. acetotolerans	CCUG 32229^T	Plant	FHe	*L. jensenii*	DSM 20557^T	Human vagina	OHo
L. acidophilus	ATCC 5917^T	Animal	OHo	*L. johnsonii*	CCUG 30725^T	Chicken, Pigs	OHo
L. agilis	DSM 20509^T	Unknown, Sewage	FHe	*L. malefermentans*	CCUG 32206^T	Beer	OHe
L. amylophilus	CCUG 30137^T	Plant	OHo	*L. mali*	CCUG 32228^T	Apple juice	OHo
L. amylovorus	DSM 20531^T	Plant	OHo	*L. murinus*	DSM 20452^T	Murine GIT	FHe
L. animalis	NCFB 2425^T	Animal	FHe	*L. oris*	NCFB 2160^T	Human saliva	OHe
L. bifermentans	CCUG 32234^T	Cheese	FHe	*L. parabuchneri*	CCUG 32261^T	Human saliva, Cheese	OHe
L. brevis	DSM 30670^T	Animal	OHe	*L. paracasei* subsp. *paracasei*	NCFB 151^T	Dairy, Humans, Plants	FHe
L. casei subsp. *casei*	ATCC 334	Animal, Plant	FHe	*L. paracasei* subsp. *tolerans*	CCUG 34829^T	Diary, humans, plants	FHe
L. casei subsp. *tolerans*	CCUG 25599^T	Animal, Plant	FHe	*L. paraplantarum*	CCUG 35983^T	Beer, Humans	FHe
L. collinoides	CCUG 32259^T	Cider	OHe	*L. pentosus*	ATCC 8041^T	Silage	FHe
L. coryniformis	CCUG 30666^T	Silage, Cow dung	FHe	*L. plantarum*	ATCC 14917^T	Human, Sourdough	FHe
L. crispatus	DSM 20584^T	Animal	OHe	*L. plantarum*	DSM 9843	Human, Plant	FHe
L. curvatus	CCUG 30669^T	Cow dung, Plant	FHe	*L. plantarum*	ATCC 8014	Sourdough	FHe
L. delbrueckii subsp. *bulgaricus*	DSM 20081^T	Yogurt, Cheese	FHe	*L. reuteri*	DSM 20016^T	Animal, Sourdough	FHe
L. delbrueckii subsp. *delbrueckii*	ATCC 9649	Plant	OHo	*L. rhamnosus*	DSM 21452^T	Animal, Milk, Sewage	OHe
L. farciminis	CCUG 30671^T	Plant, Sausages	OHo	*L. sake*	CCUG 30501^T	Sake beer, Plants	FHe
L. fermentum	ATCC 14931^T	Animal, Plant	OHe	*L. salivarius* subsp. *salivarius*	DSM 20555^T	Human mouth	OHo
L. fructivorans	CCUG 32260^T	Plant	OHe	*L. salivarius* subsp. *salivarius*	CCUG 31453^T	Human mouth, GIT	OHo
L. gasseri	DSM 20243^T	Animal	OHo	*L. suebicus*	CCUG 32233^T	Apple	OHe
L. graminis	CCUG 32238^T	Grass silage	FHe	*L. vaginalis*	CCUG 31452^T	Human vagina	OHe
L. helveticus	ATCC 15009^T	Milk, Cheese	OHo	*Weissella confusa*[b]	CCUG 30113^T	Plants, Milk	OHe
L. hilgardii	CCUG 30140^T	Wine	OHe	*W. kandleri*[b]	CCUG 32237^T	Plants	OHe
				W. minor[b]	CCUG 30668^T	Milk sludge	OHe

[a] Fermentation groups are based on Hammes and Vogel [7], Vandamme, et al. [13] and Stiles and Holzapfel [12]. FHe, facultatively heterofermentative; OHe, Obligately heterofermentative; OHo, Obligately homofermentative.
[b] Previously species of *Lactobacillus.*

washed pure cultures of *Lactobacillus* strains under study into the dried substrates to be investigated.

On the other hand, some studies have indicated that most carbohydrate fermentation ability is plasmid mediated, and lactobacilli are also known to acquire and retain plasmids, though some have been found cryptic [2, 10]. Previous studies by Ahrné and Molin [1] and Johansson et al. [8] have indicated that there have been temperature-dependent variations in the spectrum of carbohydrates metabolized in *Lactobacillus plantarum* and *L. pentosus* strains.

Nevertheless, it has not been indicated elsewhere whether there is an apparent intra-strain variation in other species when incubated at different temperatures. In this paper, therefore, we describe the discrepancy in fermentation patterns observed in type strains of *Lactobacillus* species as affected by the change in the incubation temperature between 30°C and 37°C.

Materials and Methods

Forty-four type and reference strains of *Lactobacillus*, one *Atopobium*, and three *Weissella* listed in Table 1 were secured from different culture collection centers and from our laboratory. After growing the cultures in MRS broth (Oxoid) and further transferring them into *Lactobacillus*-carrying medium, LCM [6] supplemented with 1.5% glucose, they were further transferred into MRS broth. Overnight cultures grown in 10 ml of MRS broth at 30°C were washed twice with sterile physiological saline (0.9% sodium chloride solution), and pellets were suspended in API 50 CHL medium (API systems, BioMéreux, SA, France). Bottom wells were moistened to enhance humidity during incubation. Bacterial suspension was distributed into each one of the 50 cupules according to the manufacturer's instructions and overlaid with sterile paraffin oil (Darmstadt, Germany). One set of strips was incubated at 30°C and another at 37°C. Change in color from violet was monitored and recorded after 1, 2, and 7 days of incubation. Change into dark (black) color for Esculin was taken as a positive reaction. For numerical interpretation in all cases, ''0'' was designated for negative and ''1'' for positive reactions.

Table 2. Type strains of *Lactobacillus* and related organisms showing same API 50 CH fermentation profiles when incubated at 30° and 37°C

Species	Strain	Group[a]	Number of carbohydrates fermented
Atopobium uli	CCUG 31116^T	*Atopobium*	18
L. amylophilus	CCUG 30137^T	Aa	7
L. casei subsp. *casei*	ATCC 334	Bb	22
L. fermentum	ATCC 14931^T	Cb	10
L. graminis	CCUG 32238^T	Bb	15
L. hilgardii	CCUG 30140^T	Cb	9
L. paracasei subsp. *tolerans*	CCUG 34829^T	Bb	6
L. paraplantarum	CCUG 35983^T	Bb	20
L. rhamnosus	CCUG 21452^T	Bb	27
L. pentosus	ATCC 8041^T	Bb	24
L. vaginalis	CCUG 31452^T	Cb	10

[a] Grouping based on Collins et al. [3]. Aa, physiologically an obligate heterofermenter and phylogenetically affiliated to the *Lactobacillus delbrueckii* group; Ab, physiologically an obligate heterofermenter and phylogenetically affiliated to the *L. casei-Pediococcus* group; Bb, physiologically a facultative heterofermenter and phylogenetically related to the *L. casei-Pediococcus* group; Cb, physiologically an obligate heterofermenter and phylogenetically related to the *Leuconostoc* group.

Data analyses. Key characteristics of carbohydrate metabolism from Bergey's manual [9, 7] served for comparison of each strain for its differential physiological traits. Consistency and discrepancy of test scores based on these relationships between the results obtained in the two incubation temperatures were evaluated. The results are presented in Tables 2–4. In order to check the reproducibility of the results, *L. plantarum* and *L. salivarius* subsp. *salivarius* type strains were checked in duplicate at both temperatures.

Results

Ten *Lactobacillus* and one *Atopobium* species have been found to show consistent numbers and types of carbohydrates fermented at 30°C and 37°C as shown in Table 2. Eight of these strains were heterofermentative, while the other two had a homofermentative pathway and all the lactobacilli, except *L. amylophilus*, belonged to the *L. casei-Pediococcus* phylogenetic group of Collins et al. [3].

Table 3 compares 11 intestinal or animal-associated species of *Lactobacillus* and the effect of incubation at the two different temperatures in altering their metabolic patterns. As shown in the table, most of the species have reduced numbers of carbohydrates fermented at 30°C compared with 37°C. Among the 11 strains, *L. jensenii* (7 sugars) showed the highest discrepancy, while *L. oris* (6 sugars), *L. intestinalis* (5 sugars), *L. johnsonii* and *L. crispatus* (4 sugars each) had decreasing orders of magnitude. The least effect was observed in *L. mali*. The results obtained for type strains of *L. plantarum* and *L. salivarius* subsp. *salivarius* from the duplicate experiments were consistent.

Table 4 shows a list of 26 plant- and food-associated *Lactobacillus* and three *Weissella* strains with their fermentation properties. They all showed differences in the numbers and types of carbohydrates they fermented as the incubation temperature was altered. Most have metabolized many of the substrates at 30°C compared with 37°C. *Lactobacillus malefermentans* failed to ferment any of the 49 substrates at 37°C, but fermented seven carbohydrates at 30°C. As shown in the table, the loss or shift of metabolic profile expressed as change in the number and types of carbohydrates fermented with the temperature of incubation from 30°C to 37°C was highest for *L. delbrueckii* subsp. *delbrueckii* (11 sugars), followed by *L. acetotolerans* (9 sugars), *L. helveticus* (8 sugars), *L. sake* and *L. malefermentans* (7 sugars each), *L. collinoides* (6 sugars), and *L. casei* subsp. *tolerans*, *L. delbrueckii* subsp. *bulgaricus*, and *L. parabuchneri* (4 sugars each). Ten of the strains have had a change by one carbohydrate. The variations affected 21 of the strains by altering the differential trait, while five were not affected at all. Nineteen strains were favored to maintain their differential traits at 30°C, while 11 others preferred 37°C. Seven strains did not show temperature preference, still maintaining their distinct traits although their patterns were altered.

Discussion

Among the studied lactobacilli and strains of related taxa regarding their habitats, 34 were known to be associated with plants and foods or ubiquitous and 13 with animals [4, 7]. Grouping of these strains based on the stability of their API 50 CH metabolic outcomes, those strains belonging to *Atopobium uli*, *L. amylophilus*, *L. coryniformis*, *L. graminis*, *L. hilgardii*, *L. paracasei* subsp. *tolerans*, *L. pentosus*, *L. vaginalis*, and *L. rhamnosus* had unaltered patterns despite the change in the incubation temperature between 30°C and 37°C. Except for *Atopobium uli*, *L. rhamnosus*, and *L. vaginalis*, all of these strains are related to plant habitats or both. The other 36 strains showed variable patterns as affected by the change in incubation temperature. This fact demonstrated the presence of a strong intra-strain fluctuation in carbohydrate metabolism patterns in *Lactobacillus* species subject to thermal effects. These fluctuations have also been found to be higher in obligately homofermentative (OHo) species, as observed in *L. delbrueckii* subsp. *delbrueckii* and *L. jensenii*, than any one of the other species. The subspecies of *L. delbrueckii* have shown varying degrees of discrepancy and temperature preference as in the case

Table 3. Type and reference strains of *Lactobacillus* species from intestinal/animal origin and their variable patterns of API 50 CH fermentation profiles between incubation temperatures 37°C and 30°C

Species	Strain	Group[b]	Total no. of CHOs fermented at 30°C	Total no. of CHOs fermented at 37°C	Variability[c]	Trend[d]	Differently fermented substrate[e]	Suitable temp °C[f]
L. acidophilus	ATCC 5917^T	Aa	13	13	2	Replace	Arb, Lac, Mel	30
L. animalis[a]	NCFB 2425^T	Bb	12	14	2	Add	Rib, Esc	37
L. crispatus	DSM 20584^T	Aa	15	15	4	Replace	Amy, DRaf, Glg, Gen	37
L. gasseri	DSM 20243^T	Aa	2	5	3	Add	Amy, Gen, DTag	37
L. intestinalis	CCUG 30727^T	Bb	8	9	5	Add, Replace	Gal, Amy, Esc, Lac, Mel	30
L. jensenii	DSM 20557^T	Aa	20	15	7	Loss, Replace	LAra, Man, Sor, AMDG, Lac, Mel, Amd	37
L. johnsonii	CCUG 30725^T	Aa	5	7	4	Add, Replace	NAG, Cel, Mal, Gen	37
L. mali	CCUG 32228^T	Ab	13	14	1	Add	Mal	30
L. murinus	DSM 20452^T	Bb	21	18	3	Loss	DXyl, βMDX, DArl	37
L. oris	NCFB 2160^T	Cb	17	20	6	Add, Replace	βMDX, DRaf, Gen, DTur, Darl, Gnte	30
L. salivarius subsp. *salivarius*	CCUG 31453^T	Ab	14	16	2	Loss	Rib, DTur	Both

[a] Included in *L. murinus* based on its genetic similarity [13].
[b] Same as in Table 2.
[c] Variability, number of carbohydrates to which the strain showed altered metabolic response by the change in the incubation temperature.
[d] Add, able to catabolize additional carbohydrate; Loss, unable to catabolize a specific carbohydrate degraded at 30°C; Replace, shift to metabolize another carbohydrate in lieu.
[e] Abbreviations to carbohydrates: Gly, Glycerol; DAra, D-Arabinose; LAra, L-Arabinose; Rib, Ribose; DXyl, D-Xylose; LXyl, L-Xylose; βMDX, βMethyl-D-xyloside; Gal, Galactose; Fru, Fructose; Mne, Mannose; Sal, Salicine; Sbe, Sorbose; Su, Sucrose; Rha, Rhamnose; Ino, Inositol; Man, Mannitol; Sor, Sorbitol; AMDM, A-Methyl-D-mannoside; NAG, N-Acetylglucosamine; Amy, Amygdaline; Arb, Arbutin; Esc, Esculin; Cel, Cellobiose; Mal, Maltose; Lac, Lactose; Mel, Melibiose; Tre, Trehalose; Inn, Inulin; Mlz, Melezitose; DRaf, Raffinose; Amd, Starch; Glg, Glycogen; Xlt, Xylitol; Gen, Gentiobiose; DTur, D-Turanose; DLyx, D-Lyxose; DTag, D-Tagatose; DFuc, D-Fucose; DArl, D-Arabitol; Gnte, Gluconate; 2KG, 2-Ketogluconate; 5KG, 5-Ketogluconate.
[f] Suitable temperature, temperature at which the distinctive metabolic properties are retained.

of *L. paracasei* and *L. casei*, implying that these species have different adaptations (Tables 2 and 4). On the other hand, the variation was minimal in the heterofermentative species. An exception from the obligately heterofermentative group was *L. malefermentans*, which showed a typical metabolic restriction to 30°C and failure to utilize any one of the sugars at 37°C, revealing its strong adaptation to the lower temperature. The overall trend of altering metabolic profiles in the plant- and food-associated species when the temperature was shifted from 30°C to 37°C showed better utilization of most substrates at the lower temperature. There was a marked difference between plant- and animal-associated species with regard to temperature preference. Most of the strains from the first group (21 strains) were favored by 30°C or not affected by both, while a small proportion showed preference for 37°C. Conversely, in the case of the animal-associated strains, 7 strains had preference for 37°C or were not affected, while 4 strains strictly needed to be incubated at 30°C.

Further analysis of the suitable temperature that enabled retention of a maximum spectrum of distinctive metabolic features of a species showed no general trend and was rather strain specific. In general, however, 19 strains had consistent results at 30°C, and 11 were strictly preferential for 37°C, while the remaining 4 were equally vigorous without marked deviation at both temperatures, although in all cases there were shifts in the types and numbers of substrates fermented at each temperature. Therefore, the overall observation leads to avoidance of generalization for plant- or animal-associated strains although the majority of the plant-associated and those of ubiquitous nature tend to favor 30°C, in contrast to most of the animal-associated species which tend to stick to 37°C. Specific incubation temperature, therefore, has to be followed, particularly for those strains having shown variations. Nevertheless, for those with stable patterns (Table 2) and those with changing profiles but not affecting their typical key metabolic traits (Tables 3 and 4), results obtained from either of the two temperatures could be dependable.

Another interesting observation was that, except for two species, all 15 strains showing preference for 30°C were heterofermenters. Conversely, 6 of the 11 strains favoring 37°C were homofermenters, while 3 of the 4 strains not drastically affected by the change in the

Table 4. Type and reference strains associated with plants and foods and ubiquitous *Lactobacillus* species between plants and animals and related organisms with different API 50 CH fermentation profiles when incubated at 30°C and 37°C

Species	Strain	Group[a]	Total no. of substrates fermented at		Variability[b]	Trend[c]	Differently fermented substrate[d]	Suitable temp (°C)[e]
			30°C	37°C				
L. acetotolerans	CCUG 32229^T	Ba	21	19	9	Loss, Replace	Rib, Gal, AMDM, Amy, Cel, Mel, Su, Mlz, Gnte	37
L. agilis	DSM 20509^T	Bb	23	25	2	Add	Rha, Amd	30
L. amylovorus	DSM 20531^T	Bb	15	17	2	Add	AMDG, Amy	37
L. bifermentans	CCUG 32234^T	Cb	11	10	1	Loss	DArl	30
L. brevis	DSM 30670^T	Cb	11	11	2	Replace	Lac, Su	30
L. casei subsp. *tolerans*	CCUG 25599	Bb	15	17	4	Add	Arb, Mel, Gnte, Rib	30
L. collinoides	CCUG 32259^T	Aa	17	11	6	Loss	Gal, Man, NAG, Lac, Mel, Gnte	30
L. coryniformis	CCUG 30666^T	Bb	9	10	1	Add	Mal	Both
L. curvatus	CCUG 30669^T	Bb	26	24	2	Loss	DRaf, DArl	37
L. delbrueckii subsp. *bulgaricus*	DSM 20081^T	Aa	7	11	4	Add	Gal, Glu, Man, Mal	30
L. delbrueckii subsp. *delbrueckii*	ATCC 9649	Aa	15	4	11	Loss	LAra, Rib, DXyl, Gal, Rha, Ino, NAG, Esc, Mal, Tre, LFuc	37
L. farciminis	CCUG 30671^T	Cb	17	15	2	Loss	Amy, DTur	30
L. fructivorans	CCUG 32260^T	Aa	6	6	2	Replace	Mne, Man	30
L. helveticus	ATCC 15009^T	Cb	16	8	8	Loss	DXyl, Rha, Man, Arb, Esc, LFuc, Gnte, 5KG	37
L. malefermentans	CCUG 32206^T	Bb	7	None	7	Loss	All	30
L. parabuchneri	CCUG 32261^T	Cb	13	13	4	Replace	Man, Lac, Gen, DTur	Both
L. paracasei subsp. *paracasei*	NCFB 151^T	Bb	21	20	1	Loss	Amy	30
L. plantarum	ATCC 8014	Bb	27	26	1	Loss	Rha	Both
L. plantarum	ATCC 14917^T	Bb	25	24	1	Loss	Rha	Both
L. plantarum	DSM 9843	Bb	26	25	1	Loss	DRaf	Both
L. reuteri	DSM 20016^T	Cb	9	9	2	Replace	Gal, Man	30
L. sake	CCUG 30501^T	Bb	20	13	7	Loss	Rha, Amy, Esc, Sal, Cel, Tre, Glg	30
L. suebicus	CCUG 32233^T	Cb	11	9	2	Loss	βMDX, Gal	37
Weissella confusa	CCUG 30113^T	Cc	14	15	1	Add	Gnte	30
W. kandleri	CCUG 32237^T	Cc	9	8	1	Loss	Gal	30
W. minor	CCUG 30668^T	Cc	12	11	1	Loss	DAral	Both

[a,b,c,d,e] Same as in Tables 1, 2, and 3.

temperature were all heterofermentative species (Tables 3 and 4). This indicates that a heterofermentative type of metabolism seems to have a lower temperature optimum for effective regulation and expression of enzymes. In contrast, higher temperature might narrow the spectrum of enzymatic activity and hence limit an organism to a homofermentative metabolic profile, which also appears not to favor heterofermentation. The results further suggest that in food- and plant-associated lactobacilli, maximum array of metabolic expression might be possible or accelerated at 30°C compared with 37°C, in contrast to those of intestinal and/or animal origin. Similarly, strains with stable patterns would likely have enzymes active at wider temperature ranges and might also have tolerance to stress.

Ahrné and Molin [1] have shown that metabolism of raffinose is changed owing to spontaneous mutations in *L. plantarum* ATCC8014. They paraphrased the mutant phenomenon as a hindrance in using carbohydrate fermentation profiles for *Lactobacillus* identification. The application of API 50 CH fermentation profiles for taxonomic purposes in lactobacilli was thus found appropriate, reproducible, and reliable for those species with stable patterns. Basically, such organisms could be drawn from two sources, namely, from those with totally unaffected patterns by the change in temperature (Table 2) and from those with few or lower levels of alterations where the changes caused no marked difference in the characteristic fermentation pattern for the species [7, 9] (Table 3). The strains *L. amylophilus*, *L. casei* subsp. *casei*, *L. fermentum*, *L. graminis*, *L. hilgardii*, *L. paracasei* subsp. *tolerans*, *L. paraplantarum*, *L. rhamnosus*, *L. pentosus*, and *L. vaginalis* (Table 2) belong to the first group. Those species belonging to *L. coryniformis*, *L. parabuchneri*, *L. plantarum*, *L. salivarius* subsp. *salivarius*, and *W. minor* had a very low level or no metabolic variation,

which does not affect their differential characteristics outlined in Bergey's manual [9] as presented by Hammes and Vogel [7]. Thus, it would seem appropriate to include them with the first group. However, in all the rest of the studied species it appears highly unlikely that one would get reliable results, since the difference is apparently higher.

As outlined by Kandler and Weiss [9], all animal-associated lactobacilli (the homofermenters according to their grouping) do not characteristically ferment gluconate, melezitose, ribose, and xylose, but only the facultative heterofermenters do so. Contrary to this, however, assimilation was observed in plant-associated species owing to the effect of temperature. Such a discrepancy, therefore, poses a serious contradiction to the definitive property of a particular species. From this study it could be inferred that the majority of the plant-related species and some of the animal-associated ones need to be compared at 30°C and most intestinal strains at 37°C. Moreover, it appears very essential to stick to a temperature optimum for a particular species.

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