5:16 # Nutritional requirements of lactobacilli spp. from meat

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Many difficulties have been encountered in identifying and classifying lactobacilli from meat, they mainly belong to the group of streptobacteria and in the past they had been often called "atypical streptobacteria" (Reuter, 1975). Now the physiological and biochemical characteristics of these bacteria are better known following studies of Reuter (1981, Hitchner et al., 1982) and recently Shaw and Harding (1984). Altough the nutritional behaviour within the genus lactobacilli is well documented ; Rogosa et al., 1961 ; Guirard, 1974 ; Ledesma et al., 1978, no studies have been reported for streptobacteria commonly found in meat. So the present paper records the aminoacid and vitamin requirements of this group of bacteria.

Material and methods

- Organisms

The strains used in this study were isolated from vacuum packaged pork or beef that had been stored for two weeks at 4°C (représentative strains : L 345, L 362, L 225, L 304, L 218, L 137), some strains were isolated from dry sausage (L 29, L 32, L 110 and LSO_2 (Sausage starters, lactolabo). Reference strains of Lactobacillus plantarum (lactolabo), L casei, L curvatus and two strains of L sake (gift from Doctor Reuter) were also included in this study.

This collection of lactobacilli was stored à - 20°C in M.R.S. medium with glycerol 50 % (V/V).

Before use these bacteria were transferred daily for three days in M.R.S. medium for activation. An inoculum culture, previously grown on M.R.S. medium for 18 hr at 30°C, was centrifuged and the cells were washed twice with sterile saline water. The density of the suspension was adjusted to turbidity of a Mac Farland n° 2 nephelometric standard. A concentration of 1 % (V/V) of this suspension was added to each experimental culture tube and the assay was incubated at 30°C for 16 hr.

- Media

None of "atypical Streptobacteria" strains grew on the synthetic media described in the litterature. So the medium of Ledesma et al. (1978) was modified to contain glycine (3 g/l) and higher concentration of the following aminoacids ; arginine (3 g/l), glutamate (30 g/l), lysine (11 g/l), proline (15 g/l).

Tests were made in 60 mm calibrated pyrex tubes containing 2,5 ml of medium. Growth response to an aminoacid or a vitamin was determined by omission of each substrat from the medium and was measured photometrically at 660 nm. The results were exprimed as follows : essential (E) (DO less than 0,2, stimulatory (S) (DO between 0,2 and 0,5), non essential (N) (DO up to 0,5).

- Biochemical tests

The fermentation of sugars was tested by using the API 50 CH identification system (API, La Balme les Grottes), according the recommandations given by the manufacturer.

Production of NH_3 from arginine was measured as recommanded by Hitchener et al. (1982).

Results

- Identification

According to their physiological and biochemical characteristics no reported in this study strains isolated in our laboratory share many common characteristics with strains belonging to cluster I and specially cluster II defined by Shaw et al. (1984). On the basis of their sugar fermentation we have tried to divide them in several subgroups with reference to some of the taxons established by Laban et al. (1978) for lactobacilli isolated from dry sausage. These results are given in table 1.

- Vitamin requirements

Like indicated in table 2 all strains required niacin and pantothénate and some of them also riboflavin or and pyridoxal phosphate as well.

- Aminoacid requirements

The results of this experiment are présented in table 3. The failure of the most the strains to growth on Ledesma et al's medium may be explain by their requirement for glycine which is not in this medium. It has been noted that L curvatus grew rather slowly on the modified medium, growth only was measurable after 3 days of incubation.

Conclusion

The vitamin requirement does not seem to be characteristic and specific among species of Streptobacterium

But on the other hand a notable feature common to most of the strains of meat origin is their great requirement for aminoacids : aspartic acid, arginine, histidine, proline are often essential for their growth. These nutrional requirements permit to differentiate clearly these new strains and the typical species of streptobacteria, L plantarum and L casei.

On the basis of their aminoacid requirement it is obvious that "atypical streptobacteria" could not be assigned to these two species even if some strains (322 or 225) may ressemble L plantarum. These nutritional data should be correlate with sugar fermentation and may be helpful to differentiate strains of streptobacteria from meat origin.

L. curvatus appears to constitute a sharply defined species within this group : this is the most fastidious species which has a requirement for all aminoacids except alanine and tryptophane and so it lacks the ability to ferment many sugars compared with the others strains, for example melibiose, saccharose, raffinose, mannitol.

Both strains of L sake exhibit also a specific nutritionnal behaviour different from L curvatus. In fact thréonine, lysine, tyrosine were found to be not essential.

Based on melibiose and raffinose fermentation Laban et al. (1978) defined four groups. In this study nearly all strains from meat products belong to group 2 (Melibiose +, raffinose -) and possesse many characteristics of cluster II defined by Shaw and Harding (1984). All these strains (L 29, L 110, L 304, LSO_2, L 137, L 345, L 218) exhibit a requirement for glycine and proline but neverthless their nutritional requirement is heterogeneous :

- The aminoacid requirement of a strain corresponding to taxon 9 recalls in many respects that of L sake : its fermentation pattern is also very similar and this strain could be included within this species. The similarity between atypical streptobacteria and L sake has been suggested by Reuter (1981) and Shaw and Harding (1984).

- The strains related to other taxons have a different aminoacid requirement from those of L sake thréonine and lysine were found to be essential while cysteine and serine are never required.

It would be interesting to extend this study to strains belonging to clusters I and II established by Shaw and Harding (1984), thus an eventual relationship between aminoacid requirement and carbohydrate fermentation would be more clearly defined.

This study should lead to a better understanding of growth of lactobacilli in meat products : in fact the carbohydrate content of meat is very low and the nitrogenous content is very high, thus many aminoacids are probably available. A question is yet without answer "does an environment such as meat favour the selection of Lactobacilli which require many aminoacids or involve an adaptation of their metabolism ?

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Table 1

Carbohydrate fermentation and arginine degradation by Streptobacterium strains

Strains	Melibiose	Saccharose	Trehalose	Melezitose	α methyl mannoside	Raffinose	Mannitol	Sorbitol	Turanose	Arbutine	Amygdaline	Cellobiose	β methylglucoside	Gluconate	Salicine	Esculine	Maltose	Lactose	Arabinose	Gentiobiose	Arginine 1*	Arginine 2
L Plantarum Lpl L 430	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	-
L casei rhamnosus	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-		-	-
L curvatus R102	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	+	-	-
L sake strain C_1	+	+	+	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-		+	-
L sake strain A_2	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	+	+	-	-
Strain 362	+	+	+	+	-	-	(+)	(+)	-	-	-	+	-	+	+	+	+	+	+	+	-	-
Strain 225	+	+	+	-	-	+	-	-	(+)	-	(+)	+	+	+	+	+	+	+	+	+	-	-
Strain 29 (Taxon 6)**	+	+	+	-	-	-	-	-	-	+	+	+	-	+	+	+	-	+	+	-	+	-
Strain L110 (Taxon 1)**	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-
Strain 304 (Taxon 2)**	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	-
Strain LSO_2 (Taxon 3)**	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-
Strain 137 (Taxon 4)**	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	+	-
Strain 345 (Taxon 3)**	+	+	+	-	-	-	-	-	-	-	(+)	-	-	+	+	+	-	-	-	+	+	-
Strain 218	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	-
Strain 32	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* Arginine 1 NH_3 from arginine (0,05 % glucose)

" 2 NH_3 from arginine (2 % glucose)

- All strains fermented D galactose, D glucose, D fructose, D mannose, N acetylglucosamine, saccharose, ribose
- Negative reactions by all strains from glycerol, erythritol, D arabinose, adonitol, xylose, methyl xyloside, Sorbose, dulcitol, 5 or 2 cetogluconate

** According to taxons of Laban et al. (1978)

Table 2

Vitamin requirements of tested strains

	Pantothenate	Niacin	Pyridoxal	Riboflavin	Thiamin	P aminobenzoic acid	Vitamin B 12	Folic acid	Biotin
L plantarum Lpl L 430	E	E	(E)	E	-	-	-	-	-
L casei rhamnosus	(E)	E	E	-	-	-	-	-	-
L curvatus R 102	E	E	-	-	-	-	-	-	-
L sake C_1	E	E	-	E	-	-	-	-	-
L sake A_2	E	E	-	E	-	-	-	-	-
362	E	E	-	E	-	-	-	-	-
225	E	E	-	-	-	-	-	-	-
29	E	E	-	E	-	-	-	-	-
L110	E	E	E	E	-	-	-	-	-
L304	E	E	E	-	-	-	-	-	-
LSO_2	E	E	E	E	-	(E)	-	-	-
137	E	E	-	-	-	-	-	-	-
345	E	E	(E)	E	-	-	-	-	-
218	E	E	-	E	-	-	-	-	-
32	E	E	-	E	-	-	-	-	-

E : Essential S : Stimulatory N : non essential

Table 3

Aminoacid requirements of tested strains

Strains	Glutamate	Isoleucine	Leucine	Methionine	Valine	Aspartic acid	Glycine	Proline	Histidine	Cystein	Lysine	Arginine	Tyrosine	Tryptophane	Thréonine	Serine	Phenylalanine	Alanine
L plantarum Lpl L 430	E	E	E	E	E	N	S	N	N	N	N	N	N	N	N	N	N	N
L casei rhamnosus	E	E	E	E	E	N	N	N	N	N	N	N	S	S	N	N	N	N
L curvatus R 102	E	E	E	E	E	E	E	E	E	E	E	E	E	S	E	E	E	S
L sake C_1	E	E	E	E	E	E	E	E	E	E	(S)	E	(S)	S	(S)	E	S	S
L sake A_2	E	E	E	E	E	E	E	E	E	(E)	(S)	E	(S)	S	(S)	E	S	E
362	E	E	E	E	E	E	S	E	E	S	S	E	E	S	E	S	S	S
225	E	E	E	E	E	E	S	S	S	S	S	E	S	S	(S)	S	S	E
29	E	E	E	E	E	E	E	E	E	S	S	E	E	S	E	S	S	E
L110	E	E	E	E	E	E	E	E	E	S	E	E	(S)	S	E	S	S	(S)
L304	E	E	E	E	E	E	E	E	E	S	E	E	E	S	E	S	S	E
LSO_2	E	E	E	E	E	E	E	E	E	S	E	E	E	(S)	E	S	S	N
137	E	E	E	E	E	E	E	E	E	S	E	E	E	S	(S)	(E)	S	E
345	E	E	E	E	E	E	E	E	E	S	E	(E)	(E)	(S)	E	S	S	S
218	E	E	E	E	E	E	E	E	E	E	E	E	E	S	E	S	S	E
32	E	E	E	E	E	E	E	E	E	E	E	E	E	(S)	E	E	S	E

E : Essential S : Stimulatory N : Non essential