

The intracellular peptide-hydrolases of *Lactobacillus plantarum*.

Comparison with *Lactobacillus casei*

par

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Summary

Although *Lactobacillus plantarum* has been isolated in large numbers from ripened cheeses, the knowledge of its proteolytic system is very limited. This study was undertaken with the aim of obtaining a clearer picture of the peptide hydrolases of some *L. plantarum* strains. A comparison with the closely related species *L. casei* was also considered.

The intracellular peptide hydrolase system of *L. plantarum* consists of aminopeptidase and dipeptidase activities in addition to a general activity on whole casein and β -lactoglobulin. The electrophoretic pattern showed that it possesses one non-specific aminopeptidase and three dipeptidases. By comparison *L. casei* aminopeptidase was very similar. This latter bacterium showed only one dipeptidase.

Intracellular extracts of *L. plantarum* are able to hydrolyse α_{s1} - and β -casein. The formation of two peptides of higher mobility than casein was detected.

It should be noticed that although similarities were found between the different strains of a some species on the type of enzyme, significant differences in the level of specific activities were detected. In opposite to *L. casei*, *L. plantarum* does not possess carboxypepti-

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dase or aryl-peptidylamidase (endopeptidase like) activity. Specific activity of aminopeptidase and dipeptidase was maximum at the early stationary phase. Caseinolytic activity was maximum during the stationary phase.

Key words:

Lactobacillus plantarum - *Lactobacillus casei* - Peptide-hydrolase - Aminopeptidase - Dipeptidase - Carboxypeptidase - Aryl-peptidyl-amidase - Caseinolytic activity - Stages of growth.

Running title:

The peptide-hydrolases of *L. plantarum*.

Résumé

LES PEPTIDE-HYDROLASES INTRACELLULAIRES DE *LACTOBACILLUS PLANTARUM* COMPARAISON AVEC *LACTOBACILLUS CASEI*

Bien que L. plantarum ait été isolé d'un nombre important de fromages, les connaissances se rapportant à son système protéolytique sont très limitées. Son rôle dans la dégradation des protéines au cours de l'affinage n'a pas été non plus précisé. Cette étude a donc été entreprise dans le but d'avoir une meilleure image du système protéolytique intracellulaire de L. plantarum. Une comparaison avec L. casei (espèce qui lui est très proche) est aussi décrite.

Sur la fraction intracellulaire de L. plantarum il a été mis en évidence des activités aminopeptidasique et dipeptidasique et une activité générale sur la caséine et la β -lactoglobuline. Après dégradation électrophorétique il a été montré que l'aminopeptidase hydrolysait plusieurs acides aminés β -naphtylamides et dipeptides. Trois dipeptidases de spécificités différentes ont aussi été détectées. Par comparaison L. casei ne montrait qu'une aminopeptidase et qu'une dipeptidase.

Le broyat cellulaire de L. plantarum hydrolysait lentement les caséines α_{s1} et β . Deux peptides de forte mobilité étaient alors mis en évidence. Une comparaison effectuée entre différentes souches montre que les mêmes activités y sont retrouvées mais avec une forte variation dans leur niveau d'activités spécifiques. Contrairement à L. casei, L. plantarum ne présente jamais ni d'activité carboxypeptidasique, ni d'activité aryl-peptidyl-amidasique (endopeptidase spécifique).

Chez L. plantarum les activités spécifiques de l'aminopeptidase et de la dipeptidase sont maximums au début de la phase stationnaire de croissance alors que celle de l'activité caséinolytique ne l'est qu'en fin de phase stationnaire.

Mots clés :

Lactobacillus plantarum - *Lactobacillus casei* - Peptide-hydrolase - Aminopeptidase - Dipeptidase - Carboxypeptidase - Aryl-peptidyl-amidase - Activité caséinolytique - Phases de croissance.

Titre abrégé :

Les peptides-hydrolases de *L. plantarum*.

INTRODUCTION

In the *Streptobacterium* group, some attention has been given to the peptide hydrolases of *Lactobacillus casei*. Brandsaeter and Nelson (1956 a) described the caseinolytic activity of *L. casei*, they also reported the presence of a metal activated peptidase system in this organism (Brandsaeter and Nelson, 1956 b). In a comparative study, Tourneur (1972) showed that *L. casei* was less proteolytic than lactobacilli from the *Thermobacterium* group. The fractionation of the crude cell free extract of *L. casei* on D.E.A.E.-cellulose was described by Rymaszewski *et al.* (1974).

A more detailed study was undertaken by El Soda, Desmazeaud and Bergère (1978); these authors isolated, purified and characterized three exopeptidases: an aminopeptidase, a dipeptidase and a carboxypeptidase from *L. casei* NCDO 151. The localization of these exopeptidases in addition to their caseinolytic activity and a very specific endopeptidase (aryl-peptidyl-amidase) were reported (El Soda, Bergère and Desmazeaud, 1978; El Soda and Desmazeaud, 1981).

On the other hand, the knowledge of the proteolytic system of the closely related *L. plantarum* has been very limited, although the organism is isolated in large numbers from ripened cheeses (Davis, 1963; Fryer, 1969). It is likely that in such cheeses the intracellular enzymes act in protein breakdown after the death and lysis of the cells.

Most of the studies of the proteolytic activities of *L. plantarum* were performed with whole cells in milk or cheese systems (Purschel and Pollack, 1972; Miller and Kandler, 1967; Dilanyan and Grushina, 1978). Some interest was also given to the intracellular caseinolytic activity of *L. plantarum* (Tourneur, 1972 and 1974; Ducastelle and Lenoir, 1969).

However, there is no knowledge available concerning the types or number of enzymes constituting the peptide hydrolase system or the production of the enzymes.

A study was therefore undertaken with the aim of obtaining a clearer picture of the peptide hydrolases of some *L. plantarum* strains. A comparative study between this system in *L. plantarum* and the closely related species *L. casei* was also taken into consideration.

METHODS AND MATERIALS

Cultivation of the microorganisms and preparation of the crude cell free extract

The following strains were used for this study: *L. plantarum* CNRZ 73, CNRZ 425, CNRZ 314, CNRZ 174 and DSM 199 and *L. casei* NCDO 1, ATCC 7469, CNRZ 57 and K 7.

The methods described by El Soda, Bergère and Desmazeaud (1978) were used for the cultivation of the cells in MRS medium (Difco) and the preparation of the crude cell free extract.

Electrophoretic fractionation of hydrolyzed casein and of aminopeptidase and dipeptidase activities

The method described by Uriel (1966) and modified by Gripon *et al.* (1975) was used to detect the hydrolysis products obtained by the action of the intracellular enzymes from *L. plantarum* on casein fractions.

Aminopeptidases and dipeptidases were detected after electrophoresis of the cellular extracts. Gels of 7% acrylamide were prepared in 0.1 M Tris-borate buffer pH 7.0. Aminopeptidases were detected on gels with various amino-acid- β -naphthylamides using the method of Miller and McKinnon (1974). Dipeptidases were localized with various dipeptides using the method of Lewis and Harris (1967). These methods were carried out as previously described by El Soda and Desmazeaud (1982).

Enzyme assays and units definition

Aminopeptidase activity was usually determined by measuring the extent of hydrolysis of L-leucine-para-nitroanilide (Sigma). Dipeptidase and carboxypeptidase activities were determined by measuring after ninhydrin reaction, the quantity of α -amino groups liberated by the hydrolysis of, respectively, various dipeptides or various benzyloxycarbonyl-dipeptides. Specific endopeptidase activity was determined by measuring the hydrolysis of N-succinyl-L-phenylalanine-para-nitroanilide. Caseinolytic activity and proteolytic activity on whey proteins were determined by measuring after ninhydrin reaction, the quantity of α -amino groups liberated by the hydrolysis of a 2% protein solution. These methods were carried out as previously described by El Soda and Desmazeaud (1982).

A unit of enzymic activity is defined as that amount of enzyme producing:

- (i) variation of 0.01 unit of absorbance at 410 nm per minute for aminopeptidase or per hour for specific endopeptidase;
- (ii) variation of 0.01 unit of absorbance at 570 nm per 15 minutes for carboxypeptidase and dipeptidase activities or per hour for caseinolytic activity and proteolytic activity on whey proteins.

Specific activity was defined as the number of activity units per mg of protein.

Influence of physiological conditions of cells on the activity of the intracellular peptidase system of *L. plantarum*

For the determination of the effect of cell age on intracellular peptidase activities in *L. plantarum*, cells were harvested by centrifugation at different stages of growth (2 h = beginning of exponential growth; 4 h = exponential growth; 7 h = early stationary phase; 24 h = stationary phase). Cells were washed twice in 0.01 M-K phosphate buffer pH 7.0 and the bacterial suspension was disrupted and enzyme determined according to the procedures described by El Soda and Desmazeaud (1982) and Ezzat *et al.* (1982).

Determination of protein

The protein content of the extracts was determined according to the method of Lowry *et al.* (1951).

TABLE 1 - TABLEAU 1

Detection of intracellular peptide-hydrolase activities
in *Lactobacillus plantarum* CNRZ 73a

*Mise en évidence des activités peptide-hydrolases intracellulaires
chez Lactobacillus plantarum CNRZ 73a*

a = Les résultats sont exprimés en activité spécifique.

Aminopeptidase activity (Substrate L-leucine p-nitroanilide)	8.9
Dipeptidase activity (Substrate Glycyl-L-tyrosine)	2.8
Carboxypeptidase activity (Substrate Zb-Yc)	0.0
Proteolytic activity on milk proteins:	
Whole casein	7.7
β -lactoglobulin	0.4
α -lactalbumin	0.0
Endopeptidase like activity (Substrate SUPHEPA or GLUPHEPA or BAPA)	0.0

a : Results are expressed as specific activity.

b : Z = benzyloxycarbonyl.

c : Y = glycyl-L-arginine, glutamyl-L-tyrosine, glycyl-L-tryptophan, glycyl-L-phenylalanine, glycyl-L-serine, glycyl-L-aspartic acid, glycyl-L-lysine.

SUPHEPA = N-succinyl-L-phenylalanine p-nitroanilide ; GLUPHEPA = glutaryl-L-phenylalanine p-nitroanilide ; BAPA = N α -benzoyl-L-arginine p-nitroanilide.

RESULTS

Description of the intracellular peptide hydrolase system of *L. plantarum*

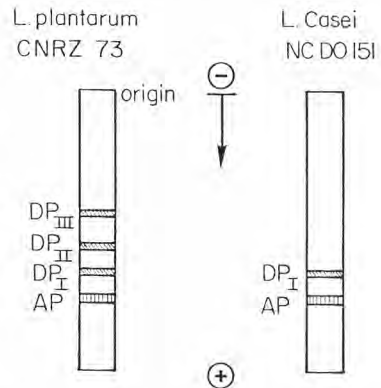
The intracellular peptide hydrolase system of *L. plantarum* consists of aminopeptidase and dipeptidase activities in addition to a general activity on whole casein and β -lactoglobulin. Neither carboxypeptidase nor specific endopeptidase activities on Suphepa, Gluphepa or Bapa substrate were detected (table 1).

The electrophoretic pattern (fig. 1) of the aminopeptidase and dipeptidase activities of *L. plantarum* showed that it possesses one

Fig. 1

Separation of aminopeptidase and dipeptidase by gel electrophoresis from *Lactobacillus plantarum* cell-free extract. Comparison with *Lactobacillus casei*.

Electrophoresis was carried out in 7 % polyacrylamide gel in TRIS-borate buffer at pH 7.0. Aminopeptidase (AP) was localized by gel incubation at 25° C with substrate (amino acid β -naphthylamide) and Fast-Garnet GBC. After 15 min a red ring occurred. Dipeptidase activities (DP) were localized by gel incubation at 25° C with substrate (various dipeptides), L-amino acid oxydase, peroxydase and O-dianisidine. After 10 min a brown ring occurred. Hydrolyzed substrates are indicated in Table 2.



Séparation des activités aminopeptidasique et dipeptidasique des fractions solubles des broyats de L. plantarum et caractérisation des activités après électrophorèse en gel de polyacrylamide. Comparaison avec L. casei.

L'électrophorèse était réalisée dans un gel de polyacrylamide à 7 % en tampon TRIS-borate de pH 7,0. Les aminopeptidasés (AP) étaient localisés en incubant à 25° C les gels, après migration électrophorétique, dans un mélange contenant le substrat (acide aminé- β -naphthylamide) et une solution de Fast-Garnet GBC. Après 15 min, on notait l'apparition d'un anneau de couleur rouge. Les dipeptidasés (DP) étaient localisés en incubant les gels dans un mélange contenant le substrat (dipeptide), de la L-amino acide oxydase, de la peroxydase et une solution aqueuse de O-dianisidine. On notait l'apparition d'un anneau de couleur brune.

Les différents substrats hydrolysés par chaque enzyme sont indiqués au tableau 2.

aminopeptidase hydrolyzing the β -naphthylamides derivatives of lysine, arginine, leucine and alanine in addition to the dipeptides L-leucyl-glycine, L-lysyl-L-tyrosine, L-alanyl-L-histidine, L-methionyl-L-alanine, L-methionyl-glycyl. Three bands of dipeptidase activities were also detected in the crude extract of *L. plantarum* (fig. 1). DP_I and DP_{III} showing a wider range than DP_{II} which cleaved only three dipeptides (table 2).

TABLE 2 - TABLEAU 2

Specificity of aminopeptidases and dipeptidases from *Lactobacillus plantarum*. Comparison with *Lactobacillus casei*
Spécificité des aminopeptidases et des dipeptidases de Lactobacillus plantarum. Comparaison avec Lactobacillus casei

<i>L. plantarum</i>				<i>L. casei</i>	
AP	DP ₁	DP ₁₁	DP ₁₁₁	AP	DP
Arg-βNA ^a	Leu-Gly	Leu-Gly	Leu-Gly	Arg-βNA	Leu-Gly
Ala-βNA	Met-Ala	Met-Ala	Met-Ala	Ala-βNA	Lys-Tyr
Leu-βNA	Met-Gly	Lys-Tyr	Met-Gly	Leu-βNA	Tyr-Leu
Lys-βNA	Ala-His		Ala-His	Lys-βNA	Gly-Tyr
Leu-Gly	Gly-Tyr		Lys-Tyr	Leu-Gly	
Met-Ala			Gly-Tyr	Lys-Tyr	
Met-Gly				Leu-Leu	
Ala-His					
Lys-Tyr					

a : βNA = β-naphthylamide ; AP = aminopeptidase ; DP = dipeptidase ; Arg = L-arginine ; Ala = L-alanine ; Leu = L-leucine ;
 Lys = L-lysine ; Gly = glycine ; Met = L-methionine ; Tyr = L-tyrosine ; His = L-histidine.

The electrophoretic mobilities of the enzymes are indicated in Figure 1.

Chaque enzyme après séparation électrophorétique (selon figure 1) était caractérisée par l'hydrolyse des différents substrats indiqués.

By comparison, it was of interest to notice that the electrophoretic mobility and the specificity of the aminopeptidase of *L. plantarum* and that of *L. casei* were very similar. The mobility of the unique dipeptidase of *L. casei* was also close to that of DP₁ in *L. plantarum* (fig. 1) but, its specificity is different to that of these dipeptidases.

The action of the intracellular extract of *L. plantarum* on caseins showed that the organism is able to hydrolyse both α_{s1} - and β -caseins. After 48 h of incubation, the formation of two peptides of higher electrophoretic mobility than casein from the two fractions was detected (fig. 2) in the pH 4.6 insoluble nitrogen fraction.

Comparative study between the peptide hydrolase system of several strains of *L. plantarum* and *L. casei*

The results (table 3) show that only aminopeptidase, dipeptidase and caseinolytic activities were detected in the different strains of

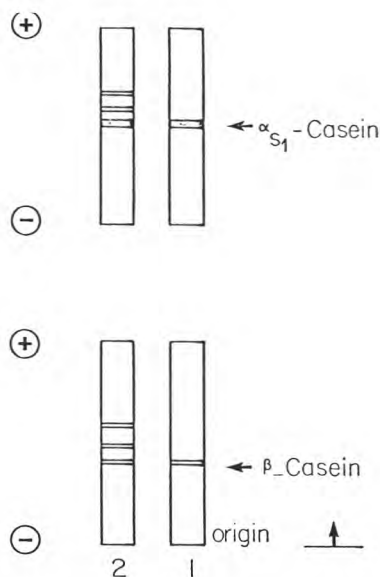
Fig. 2

Hydrolysis of α_{s1} - and β -casein by the crude extract of *Lactobacillus plantarum* CNRZ 73.

After 24 h hydrolysis by crude cell-free extract, α_{s1} - and β -casein degradation was followed with electrophoresis (acrylamide agarose gel with dissociating agents) at 4° C during 3 h with 13 volts/cm tension. Protein and peptides were detected by reaction with Coomassie Blue R 250.

1 = Control with no hydrolyzed α_{s1} - or β -casein.

2 = Hydrolyzed caseins by cell-free extract from *L. plantarum*.



Hydrolyse des caséines α_{s1} et β par l'extrait intracellulaire de *L. plantarum* CNRZ 73.

Après hydrolyse pendant 24 h par l'extrait intracellulaire brut, les mélanges réactionnels contenant la caséine α_{s1} ou β étaient soumis à l'électrophorèse (en présence d'agents dissociants) à 4° C pendant 3 h sous une tension de 13 volts/cm. Les protéines et les peptides étaient ensuite mis en évidence par coloration par le bleu de Coomassie R 250.

1 = Tube témoin (caséine α_{s1} ou β incubée seule).

2 = Caséines hydrolysées par les extraits intracellulaires de *L. plantarum* CNRZ 73.

TABLE 3 - TABLEAU 3

Peptide-hydrolase activities in several *Lactobacillus plantarum*. Comparison with *Lactobacillus casei* strains

Mise en évidence des activités peptide-hydrolases chez plusieurs souches de *Lactobacillus plantarum*.

Comparaison avec *Lactobacillus casei*

Les résultats sont exprimés en % par rapport à la souche la plus active après détermination des activités spécifiques.

	Enzymatic activity (and substrate)				
	Aminopeptidase (Leu Na)	Dipeptidase (Gly-Tyr)	Carboxypeptidase (Z-Gly-Arg)	Endopeptidase (SUPHEPA)	Caseinolytic activity (Whole casein)
A. <i>L. plantarum</i>					
CNRZ 73	31	62	0.0	0.0	72
CNRZ 425	3	54	0.0	0.0	66
CNRZ 314	7	65	0.0	0.0	58
CNRZ 174	18	100	0.0	0.0	42
DSM 199	13	89	0.0	0.0	25
B. <i>L. casei</i>					
NCDO 151	100	91	100	59	3
ATCC 7469	29	20	12	51	5
CNRZ 57	48	25	45	100	100
K 7	14	30	50	78	33

Results are expressed as % of the most active strain, after specific activity determinations.

Leu Na = L-leucine-p-nitroanilide ; Gly-Tyr = glycyl-L-tyrosine ; Z-Gly-Arg = benzyloxycarbonyl-glycyl-L-arginine ; SUPHEPA = N-succinyl-L-phenylalanine p-nitroanilide.

TABLE 4 - TABLEAU 4

Peptidase activities of cells as a function of culture age

*Variation des activités peptidasiques intracellulaires en fonction des stades de croissance**Les résultats sont exprimés en activité spécifique.**Le pH initial du milieu était de 6,5.**Les cellules étaient récoltées après 2, 4, 7 et 24 h de croissance à 30° C.*

Physiological age of the cells	pH of the medium*	Aminopeptidase	Dipeptidase	Caseinolytic activity
Cells at the beginning of exponential growth (2 h) ^a	5.2	2.1	1.9	2.1
Cells in exponential growth (4 h) ^a	4.6	2.2	2.8	2.6
Cells in early stationary phase (7 h) ^a	4.2	2.2	3.1	3.5
Cells in the stationary phase (24 h) ^a	3.9	1.7	2.5	4.5

Results are expressed as specific activity.

* The initial pH of the media was 6.5.

^a (2 h), (4 h), (7 h) and (24 h) = hours of growth at 30° C.

L. plantarum. By comparison, two additional activities were detected in the intracellular extract of all the *L. casei* strains: the carboxypeptidase (hydrolyzing the substrate benzyloxycarbonyl-glycyl-L-arginine) and the endopeptidase like activity (hydrolyzing Suphepa).

It should however be noticed that although similarities were found between the different strains of the same species on the type of enzyme present, significant differences in the level of enzymatic activities were detected.

Influence of cell age on peptidase activities

Table 4 shows the effect of harvesting *L. plantarum* cells at different stages of growth on the levels of peptidase activities. The production of dipeptidase increased gradually at the early stationary phase followed by a significant decrease in the specific activity of enzyme in the late stationary phase.

The trend of the results was different for the caseinolytic activity since the increase in enzymatic activity also occurred during the stationary phase. Aminopeptidase activity was constant during the different stages of the growth. It was decreasing in the stationary phase.

DISCUSSION

The lactic acid bacteria are nutritionally fastidious and require for growth an exogenous supply of preformed amino acids (Law and Sharpe, 1978). Generally, free amino acids initially present in milk are an important nitrogen source only for growth at low cell densities (Mills and Thomas, 1981). After few generations, lactic acid bacteria growth requires casein and peptide breakdown. Thus, *Lactobacillus plantarum* possesses the proteolytic system able to provide amino acids to the cells. However, we have not determined cell-bound extracellular proteinases or peptidases occurring in group N streptococci (Law and Sharpe, 1978) or in *Lactobacillus lactis* (Eggimann and Bachmann, 1980) or *L. bulgaricus* (Argyle *et al.*, 1976).

Although very few differences are detected between *L. casei* and *L. plantarum* as far as fermentation tests are concerned, their peptide hydrolase system is significantly different. In *L. casei* this system consisted mainly of exopeptidases, aminopeptidase, a dipeptidase and a carboxypeptidase (El Soda, Bergère and Desmazeaud, 1978) in addition to an aryl peptidyl amidase (endopeptidase like)

activity (El Soda and Desmazeaud, 1981). On the other hand, the exopeptidase system of *L. plantarum* lacked the carboxypeptidase, and no endopeptidase like activity was detected. This organism was also capable of deeply modifying casein fractions. The *L. plantarum* peptidase system is therefore closer to that of *L. helveticus*, *L. lactis*, *L. bulgaricus* and *L. acidophilus* (El Soda and Desmazeaud, 1982), while the *L. casei* system would be comparable to *L. brevis* and *L. fermentum* (El Soda *et al.*, 1982).

It was of interest to notice that like other lactobacilli (Shankar and Davies, 1978; El Soda and Desmazeaud, 1982, and El Soda *et al.*, 1982) the proteolytic system of *L. plantarum* hydrolyses β -lactoglobulin. This observation will probably be of technological importance because of the growing interest of the dairy industry in manufacturing cheese from ultrafiltrated milk. In other part, *L. plantarum* has been isolated from various ripened cheeses (Davis, 1963; Fryer, 1969). It is likely that in such ripening cheeses the intracellular proteolytic enzymes play a role in protein breakdown after the death and lysis of the cells. The action of *L. plantarum* proteases and peptidases occurring after proteolysis carried up by rennet and starters (Desmazeaud and Gripon, 1977), will participate in the breakdown of residual α_{s1} - and β -caseins and in the production of free amino acids in ripened cheese.

Most *Lactobacillus* species also behave similarly as far as the influence of cell age on peptidase activity is concerned. As a general rule an increase in the level of enzymatic activity is noticed during logarithmic growth and reach its maximum in early stationary phase. A significant decrease in most peptidases is then noticed in the 24 h old cultures. This phenomenon was attributed to the presence of the stationary cells at low pH values for several hours (Simmonds, 1972; Ezzat *et al.*, 1982). Since the production of exopeptidases or caseinolytic activity was only slightly influenced by culture age, it might therefore be concluded that the enzymes of *L. plantarum* are probably constitutive enzymes.

Similar observations were also reported for other species by Simmonds (1970) and Matheson (1963) for *Escherichia coli*, Tokita and Hosono (1976) for *Leuconostoc citrovorum*, and Schmidt *et al.* (1977) for *Streptococcus lactis*.

Considering the results described in this paper it may be concluded that due to the complexity of its exopeptidase system and its ability of modifying caseins *L. plantarum* probably acts during the late stages of ripening of some cheeses where it can be isolated in large numbers. When more informations will be available on the characterization and specificity of the peptidases of this organism the addition of selected combinations of these enzymes to the cheese milk or curd might contribute in accelerating ripening and (or) enhancing flavour development in cheese.

Bibliographie

- ARGYLE (P.), MATHISON (G.) and CHANDAN (R.) (1976). — Production of cell-bound proteinase by *Lactobacillus bulgaricus* and its location in the bacteria cell. *J. Appl. Bacteriol.*, 41, 175-184.
- BRANDSAETER (E.) and NELSON (F. E.) (1956 a). — Proteolysis by *Lactobacillus casei*. I. Proteinase activity. *J. Bacteriol.*, 72, 68-72.
- BRANDSAETER (E.) and NELSON (F. E.) (1956 b). — Proteolysis by *Lactobacillus casei*. II. Peptidase activity. *J. Bacteriol.*, 72, 73-78.
- DAVIS (J.) (1963). — The lactobacilli. II. Applied aspects. *Progress in Industrial Microbiol.*, 4, 95-136.
- DESMAZEAUD (M. J.) and GRIPON (J. C.) (1977). — General mechanism of protein breakdown during cheese ripening. *Milchwissenschaft*, 32, 731-734.
- DILANYAN (Z. H.) et GRUSHINA (E. V.) (1978). — Etude de l'activité protéolytique de quelques souches de bactéries lactiques. 20^e Congrès Int. Laiterie (Paris), 482-483.
- DUCASTELLE (A.) et LENOIR (J.) (1969). — Contribution à l'étude de la flore microbienne du fromage de type Saint-Paulin. III. Son activité protéolytique. *Le Lait*, 49, 615-636.
- EGGIMANN (B.) and BACHMANN (M.) (1980). — Purification and partial characterization of an aminopeptidase from *Lactobacillus lactis*. *Appl. Env. Microbiol.*, 40, 876-882.
- EL SODA (M.), BERGÈRE (J. L.) and DESMAZEAUD (M. J.) (1978). — Detection and localization of peptide hydrolases in *Lactobacillus casei*. *J. Dairy Res.*, 45, 519-524.
- EL SODA (M.), DESMAZEAUD (M. J.) and BERGÈRE (J. L.) (1978). — Peptide hydrolases of *Lactobacillus casei*: Isolation and general properties of various peptidase activities. *J. Dairy Res.*, 45, 445-455.
- EL SODA (M.) and DESMAZEAUD (M. J.) (1981). — General properties of a new ribosomal aryl-peptidyl amidase in *Lactobacillus casei*. *Agr. Biol. Chem.*, 45, 1693-1700.
- EL SODA (M.) et DESMAZEAUD (M. J.) (1982). — Les peptide-hydrolases des lactobacilles du groupe *Thermobacterium*. I. Mise en évidence de ces activités chez *Lactobacillus helveticus*, *L. acidophilus*, *L. lactis* et *L. bulgaricus*. *Can. J. Microbiol.*, 28, 1181-1188.
- EL SODA (M.), ZEYADA (N.), DESMAZEAUD (M. J.), MASHALY (R.) et ISMAIL (A.) (1982). — Les peptide-hydrolases des lactobacilles du groupe *Betabacterium*. Mise en évidence chez *Lactobacillus brevis*, *L. fermentum*, *L. buchneri* et *L. cellobiosus*. *Science des aliments*, 2, 261-273.
- EZZAT (N.), EL SODA (M.), DESMAZEAUD (M. J.) and ISMAIL (A.) (1982). — Peptide hydrolases from the *Thermobacterium* group of lactoacilli. II. Physiological factors and enzyme production. *Milchwissenschaft*, 2, 261-273.
- FRYER (T.) (1969). — Microflora of Cheddar cheese and its influence on cheese flavour. *Dairy Sci. Abstr.*, 31, 471-490.
- GRIPON (J. C.), DESMAZEAUD (M. J.), LE BARS (D.) et BERGÈRE (J. L.) (1975). — Etude du rôle des micro-organismes et des enzymes au cours de la maturation des fromages. II. Influence de la présure commerciale. *Le Lait*, 55, 502-516.
- LAW (B. A.) and SHARPE (M. E.) (1978). — Streptococci in the dairy industry; in *Streptococci*. Skinner F. A. and Quesnel L. B. ed., Academic Press Inc., London.
- LEWIS (W. H. P.) and HARRIS (H.) (1967). — Human red cell peptidases. *Nature*, 215, 351-355.

- LOWRY (O. H.), ROSEBROUGH (N. J.), FARR (A. L.) and RANDALL (R. J.) (1951). — Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193, 265-275.
- MATHESON (A.) (1963). — The localization and properties of aminopeptidase in *Escherichia coli* B. *Can. J. Biochem.*, 43, 323-329.
- MILLER (C. G.) and Mc KINNON (K.) (1974). — Peptidase mutants of *Salmonella typhimurium*. *J. Bacteriol.*, 120, 355-363.
- MILLER (I.) and KANDLER (O.) (1967). — Proteolysis and liberation of free amino acids by lactic acid bacteria in milk. III. Liberation of free amino acids by *Streptobacteria* and *Streptococci*. *Milchwissenschaft*, 22, 608-615.
- MILLS (O. E.) and THOMAS (T. D.) (1981). — Nitrogen sources for growth of lactic streptococci in milk. *N.-Z. J. Dairy Sci. Technol.*, 15, 43-55.
- PURSCHEL (M.) and POLLACK (C.) (1972). — Proteolytic degradation of milk protein by bacteria. II. The action of psychrophilic bacteria and lactobacilli on milk protein. *Die Nahrung*, 16, 451-459.
- RYMASZEWSKI (J.), KORNAKCI (K.), REPS (A.) et PSZCZOLKOWSKA (G.) (1974). — Caractérisation des protéinases intracellulaires des bactéries lactiques. 19^e Congrès Int. Laiterie (New-Dehli), B5, 370-371.
- SCHMIDT (R. H.), MORRIS (H. A.) and Mc KAY (L. L.) (1977). — Cellular location and characteristics of peptidase enzymes in lactic streptococci. *J. Dairy Sci.*, 60, 710-717.
- SHANKAR (P. A.) and DAVIES (F. L.) (1978). — Proteinase and peptidase activities of yoghurt starter bacteria. 20th Int. Dairy Congress, E2, 467-468.
- SIMMONDS (S.) (1970). — Peptidase activity and peptide metabolism in *Escherichia coli* K-12. *Biochemistry*, 9, 1-9.
- SIMMONDS (S.) (1972). — Peptidase activity and peptide metabolism in *Escherichia coli*. In « Peptide transport in bacteria and mammalia gut ». *Ciba Foundation Symposium*, 43-57.
- TOKITA (F.) and HOSONO (A.) (1976). — Production and some properties of the intracellular protease from *Leuconostoc citrovorum*. *Jap. J. Zootech. Sci.*, 47, 272-282.
- TOURNEUR (C.) (1972). — Aptitude à la protéolyse des lactobacilles présents dans les fromages et les lactosérums de fromagerie. *Le Lait*, 52, 149-174.
- TOURNEUR (C.) (1974). — The proteolytic activity of *Lactobacilli*. 19th Int. Dairy Congress, 1E, 366.
- URIEL (J.) (1966). — Méthode d'électrophorèse dans des gels d'acrylamide-agarose. *Bull. Soc. Chim. Biol.*, 48, 969-982.
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