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The esterolytic and lipolytic activities of the Lactobacilli

II. Detection of the esterase system of *Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis* and *Lactobacillus acidophilus*

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Summary

In the present work we report the intracellular esterolytic activities of *Lactobacillus helveticus, L. bulgaricus, L. lactis* and *L. acidophilus* using several nitrophenyl derivatives of fatty acids. All the tested lactobacilli show activities towards derivatives up to five carbons. P-nitrophenyl derivatives were hydrolysed significantly faster than the O-nitrophenyl derivatives. *L. lactis* and *L. acidophilus* strains can be distinguished from the strains of *L. bulgaricus* and *L. helveticus* by their higher esterase activities.

After electrophoretic separation in 7 % acrylamide gels, zymograms showed three main bands distributed from RF 0.5 to RF 0.3 for *L. helveticus*. From *L. bulgaricus*, four bands were distinguished, three of them with electrophoretic mobilities similar to main bands in *L. helveticus*. Some of the *L. acidophilus* and *L. lactis* strains were distinguished by the presence of up to three additional bands of lower electrophoretic mobility.

On the other hand, the specific activity of esterase increased gradually during the growth of *L. helveticus*. The trend was different for the esterase system of *L. bulgaricus* since activity was almost similar during the different stages of growth.

Generally, the optimum temperature for esterase production by the thermophilic lactobacilli was found to be 40-45° C. Little specific activity was detected after cell growth either at 35 or 55° C.

In conclusion, thermophilic lactobacilli possess a complex esterolytic system. It is specific towards short chain fatty acids. Unfortunately, differences between strains are two little for use of these results as a tool for the taxonomy of Lactobacilli. However, this type of enzymatic activities could be used as an indicator for the detection and the control of strains in mixed lactic acid bacteria cultures.

Key words : Esterase - Electrophoretic separation - Physiological age - *Thermobacterium* - Lactobacilli - *Lactobacillus helveticus* - *Lactobacillus bulgaricus* - *Lactobacillus lactis* - *Lactobacillus acidophilus.*

Résumé

Les activités estérolytiques et lipolytiques des lactobacilles

Très peu d'études ayant été consacrées aux systèmes estérasiques et lipolytiques des bactéries lactiques, une étude générale a été entreprise visant à caractériser ce type d'activités chez les lactobacilles. Dans la présente communication, les enzymes intracellulaires ont été caractérisées en utilisant différents dérivés O- et p-nitrophényl d'acides gras. Toutes les souches testées montraient une activité estérolytique envers ces dérivés jusqu'à cinq atomes de carbone au plus. Les dérivés *para* étaient en général hydrolysés plus rapidement que les dérivés *ortho*. Les souches de *L. lactis* et *L. acidophilus* se distinguaient des souches de *L. bulgaricus* et *L. helveticus* par leurs plus fortes activités spécifiques.

Après séparation électrophorétique et analyse colorimétrique de l'hydrolyse de plusieurs substrats, les souches et les espèces révélaient des équipements enzymatiques différents. Chez *L. helveticus,* trois activités principales étaient distribuées de RF 0,5 à RF 0,3. L'une d'elles montrait une large spécificité hydrolysant les dérivés de l'acétate, du propionate et du butyrate. Chez *L. bulgaricus,* sur les quatre bandes mises en évidence, trois coïncidaient avec les activités principales de *L. helveticus*. Chez *L. acidophilus* une seule activité était commune à toutes les souches. D'autre part, chez *L. acidophilus* et *L. lactis* certaines souches se caractérisaient par des activités de faible mobilité électrophorétique.

L'activité spécifique estérasique augmentait graduellement au cours de la croissance de *L. helveticus*. Le maximum de production était observé au début de la phase stationnaire de croissance. La tendance était différente pour *L. bulgaricus* puisque l'activité spécifique estérasique était presque identique pour toutes les phases de croissance. D'autre part, la température optimum de production était 40-45° C. Les activités spécifiques diminuaient notablement si les cellules étaient produites à 35 ou 55° C.

En conclusion, les lactobacilles thermophiles possèdent un riche équipement en activités estérasiques spécifiques des acides gras à courte chaîne. Les différences entre espèces ne sont pas assez marquées pour pouvoir être utilisées dans un but taxonomique. Cependant, la présence de ce type d'activité est intéressante dans une optique de caractérisation précise des souches d'intérêt technologique et pourrait servir de « marqueur » pour suivre la croissance des souches utilisées en mélange, par exemple.

Mots clés : Estérase - Séparation électrophorétique - Age physiologique - *Thermobacterium* - Lactobacilles - *Lactobacillus helveticus* - *Lactobacillus bulgaricus* - *Lactobacillus lactis* - *Lactobacillus acidophilus*.

Introduction

Fat hydrolysis represents with proteolysis and lactose degradation the main biochemical changes occuring during cheese ripening. Lactic acid bacteria which represent the predominant flora of most cheeses are recognized to actively participate in the above mentioned changes. Although, a great deal of attention has been given to their enzymatic activities responsible for lactose, proteins and peptides degradation, very little informations are available as far as lipolytic and esterolytic systems are concerned (LAWRENCE *et al.*, 1976 ; DESMAZEAUD, 1978 ; LAW, 1982 ; DESMAZEAUD, 1983 ; MARSHALL and LAW, 1984).

However, some workers have previously attempted to describe the esterase system of lactic acid bacteria. MORICHI *et al.* (1968) using polyacrylamide gel electrophoresis (PAGE) determined the esterase pattern of several lactic acid bacteria. The number of substrates used during their investigation was however rather limited. Esterase activity of strains of *S. lactis* and *S. cremoris* as well as mixed type cultures was determined using PAGE (HARPER *et al.*, 1969). One esterase with an RF value of o.8 was common to all the strains tested and was the only present for *S. cremoris*. A second band was detected in the other strains. It was also shown by HARPER *et al.* (1980) that glutathione increased the esterolytic activity of *S. lactis*. Detection of the esterolytic activity of *S. lactis, S. cremoris, S. durans* and *L. acidophilus* using high voltage electrophoresis was carried out by SORHAUG and SOLBERG (1970). Two esterase bands were detected in the *streptococci* while only band was found in *L. acidophilus*. Absence of esterolytic activity on O-nitrophenyl butyrate in four strains of lactobacilli was also reported by BRANDL and PFLEGER (1975). Thus, a more general study was therefore undertaken in our laboratories in order to describe the esterolytic and lipolytic activities of most of the *Lactobacillus sp.* involved in the dairy industry. The influence of growth conditions on the previously mentioned activities was also considered in order to reach a better understanding on the enzymatic systems involved in various esters or fat hydrolysis by these organisms. So, in the present communication we report the intracellular esterolytic activities of *Lactobacillus helveticus, L. bulgaricus, L. lactis* and *L. acidophilus* (*Thermobacterium* group) using several O- and P-nitrophenyl derivatives of fatty acids.

I. Materials and methods

Cultivation of the micro-organisms and preparation of the crude cell free extract

The following strains were used during the course of this study *Lactobacillus helveticus* CNRZ 303, CNRZ 223, CNRZ 244, CNRZ 243 and CNRZ 32, *Lactobacillus bulgaricus* CNRZ 369, CNRZ 325, CNRZ 208 and CNRZ 36, *Lactobacillus lactis* CNRZ 250, CNRZ 242 and CNRZ 326 and *Lactobacillus acidophilus* CNRZ 251 and CNRZ 216. The bacteria were maintained by subculturing in sterile skim milk and stored at — 30° C. The methods described by EL SODA *et al.* (1978) were followed for the cultivation of the cells in MRS broth (de MAN *et al.*, 1960) and the preparation of the crude cell free extract with however some modifications : Alumina powder (Sigma type 305) 2 parts to 1 part cells pellet was used instead of the Vibrogen cell mill for the desintegration of the cells. Temperature was kept around 4° C during desintegration and the desintegrated cells were dissolved in 0.01 M phosphate buffer pH 7.0 before centrifugation.

Enzyme assay

The esterase activity of the crude extract was evaluated as described by BRANDL and ZIGER (1973) using several O- and P-nitrophenyl derivatives of fatty acids as listed in Table 1. A unit of enzymatic activity was defined as the variation of 0.1 unit of absorbance at 410 nm in 1 minute. The specific activity was

defined as the number of units per mg of protein. Enzyme assay were carried out at 40° C in 0.01 M phosphate buffer pH 7.5.

Protein determination

The protein concentration of the soluble extract was estimated according to the method of LOWRY *et al.* (1951) using the FOLIN phenol reagent with bovine serum albumin as a standard.

Electrophoretic fractionation of esterase activities

Gels of 7 % acrylamide were prepared in 0.1 M Tris-borate buffer pH 8.5 according to the method of ORNSTEIN (1964) and DAVIS (1964). The enzymatic activities of the soluble extract were revealed on the gels after electrophoresis using various α- and β-naphthyl derivates of fatty acids. The separated esterases were then identified as orange bands using the method described by HARPER *et al.* (1980). The substrate was considered as not hydrolysed if no bands were detected within 8 hrs of incubation at 37° C.

Influence of the physiological age of the cells on esterase production

In order to study the effect of the physiological age of the cells on esterase activity, *L. helveticus* CNRZ 303 and *L. bulgaricus* CNRZ 369 were cultivated in MRS broth at 32° C and harvested by centrifugation at the beginning of the logarithmic phase (I), during the logarithmic phase (II), at the early stationary phase (III) and after 24 hr of incubation (IV). The resulting pellets were then washed twice and the cells disrupted and centrifuged in order to obtain the soluble enzyme fraction. Esterase activity was then determined in the different extracts for each species using P-nitrophenyl-acetate and P-nitrophenyl-butyrate as substrates.

Influence of growth temperature on esterase production

The influence of the growth temperature was evaluated by growing *L. helveticus* CNRZ 303 and *L. bulgaricus* CNRZ 369 in MRS broth at 35, 40, 45, 50 and 55° C. Cells were then harvested at the beginning of the stationary phase by centrifugation. The obtained pellets were then washed, disrupted and the esterase activities on P-nitrophenyl-acetate and P-nitrophenyl-butyrate determined on the resulting extracts.

II. Results and discussion

A. *Detection of the various esterolytic activities*

All the tested lactobacilli show active esterolytic activities towards O- and P-nitrophenyl derivatives of fatty acids up to 5 carbon atoms. None of the strains tested hydrolyzed substrates containing fatty acids of 6, 8, 10, 12 or 14 carbons atoms (table 1). The *Thermobacterium* differ in that respect from *L. casei, L. brevis* and *L. fermentum* which were showed to hydrolyse the O- and P-nitrophenyl derivatives of caproate and caprylate (EL SODA *et al.*, 1986).

TABLE I

The specific activity of the esterase from Lactobacillus *species*

Activité spécifique des estérases de différentes espèces de Lactobacilles

Substrate	L. helveticus					L. bulgaricus				L. lactis			L. acidophilus	
	CNRZ 303	CNRZ 223	CNRZ 244	CNRZ 243	CNRZ 32	CNRZ 369	CNRZ 36	CNRZ 325	CNRZ 208	CNRZ 250	CNRZ 242	CNRZ 326	CNRZ 251	CNRZ 216
P-C_2	1.1	3.7	1.3	3.3	1.7	0.8	2.1	1.6	2.9	1.0	4.6	4.1	5.3	6.1
O-C_2	0.5	0.7	0.5	1.0	0.5	0.5	0.4	0.4	0.4	0.3	1.5	1.3	1.5	1.4
P-C_3	0.9	2.1	1.6	3.5	1.9	1.1	1.3	0.8	1.2	0.6	3.8	2.8	4.0	3.8
P-C_4	0.8	1.2	1.4	4.6	0.7	0.8	1.0	0.7	0.6	1.5	2.9	3.7	2.7	3.1
O-C_4	0.2	0.6	0.2	2.4	0.3	0.4	0.3	0.2	0.2	0.3	0.5	0.6	0.1	0.6
P-C_5	1.7	0.5	1.9	1.4	1.6	1.0	0.7	0.7	1.0	1.3	3.2	2.7	2.5	3.7

Substrates not hydrolysed by any of the strains tested : C_6 - C_8 - C_{10} - C_{12} - C_{14}.

Dérivé non hydrolysé, quelle que soit la souche: C_6 - C_8 - C_{10} - C_{12} - C_{14}.

P-C_2 = P- nitrophenyl-acetate
O-C_2 = O-nitrophenyl-acetate
P-C_3 = P-nitrophenyl-propionate
P-C_4 = P-nitrophenyl-butyrate
O-C_4 = O-nitrophenyl-butyrate
P-C_5 = P-nitrophenyl-valerate
P-C_6 = P-nitrophenyl-caproate
P-C_8 = P-nitrophenyl-caprylate
P-C_{10} = P-nitrophenyl-caprate
P-C_{12} = P-nitrophenyl-laurate
P-C_{14} = P-nitrophenyl-myristate

* These results represent the mean of 3 experiments (variation ± 10 %).
Ces résultats représentent la moyenne de 3 essais (écart maximal ± 10 % de la valeur moyenne).

TABLE II

RF values of the different esterases from Lactobacillus helveticus

Valeurs du RF des différentes estérases de Lactobacillus helveticus

L. helveticus	α-naphthyl acetate							β-naphthyl acetate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
CNRZ 303		0.50							0.50					
223		0.50							0.50					
244		0.50							0.50					
243			0.45							0.45				
32		0.50								0.45				

	α-naphthyl propionate							β-naphthyl propionate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
303		0.50								0.45	0.36			
223		0.50							0.50					
244									0.50		0.36			
243			0.45	0.36		0.14				0.45		0.27		
32		0.50												

	α-naphthyl butyrate							β-naphthyl butyrate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
303		0.50								0.45	0.36			
223		0.50		0.36					0.50					
244			0.45						0.50					
243				0.36		0.14				0.45		0.27		
32		0.50												

	α-naphthyl valerate							β-naphthyl valerate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
303		0.50												
223			0.45											
244			0.45											
243				0.36		0.14				0.45				
32			0.45											

The results in table 1 also demonstrate that as previously found for *Lactobacilli* from *Streptobacterium* and *Betabacterium* (EL SODA *et al.*, 1986), the *Thermobacterium* hydrolysed P-nitrophenyl derivatives of fatty acids significantly faster than the O-nitrophenyl derivatives. The most significant differences were detected in *L. acidophilus* CNRZ 251 where the specific activity for P-nitrophenyl butyrate hydrolysis was 2.7 while it was only 0.1 for the O-derivative.

TABLE III

RF values of the different esterases from Lactobacillus bulgaricus

Valeurs du RF des différentes estérases de Lactobacillus bulgaricus

	α-naphthyl acetate							β-naphthyl acetate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
L.bulgaricus														
CNRZ 369								0.58	0.50					
225	0.58							0.58						
36	0.58							0.58						
208	0.58							0.58						

	α-naphthyl propionate							β-naphthyl propionate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
369		0.50		0.36				0.58	0.50		0.36			
225		0.50		0.36				0.58	0.50		0.36			
36		0.50		0.36				0.58	0.50		0.36			
208		0.50		0.36				0.58	0.50		0.36			

	α-naphthyl butyrate							β-naphthyl butyrate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
369										0.45				
225				0.36						0.45				
36										0.45				
208										0.45				

L. lactis and *L. acidophilus* strains can be distinguished from the strains of *L. bulgaricus* and *L. helveticus* by their higher esterase activities. The specific activity for the hydrolysis of P-nitrophenyl acetate was 5.3 and 6.1 for the strains of *L. acidophilus* while it was 4.1, 4.6 and 1.0 for the strains of *L. lactis*. The results obtained in that respect for the strains of *L. bulgaricus* were 0.8, 2.1, 1.6 and 2.9, while it was 1.1, 3.7, 1.3, 3.3 and 1.7 for the strains of *L. helveticus*. The similar trend of results was also noticed for the hydrolysis of the other substrates. The obtained electrophoretic zymograms of the various strains on the different substrates showed that for the *L. helveticus* strains (table 2) the coloured bands were distributed from RF = 0.50 to RF = 0.14. The major bands E_2, E_3, E_4 were located between RF 0.5 and RF 0.36. Band E_2 showed broad substrate specificity since it hydrolysed the acetate, propionate and butyrate derivates of α- and β-napthylamides in most of the strains. Bands E_3 and E_4 showed narrower specificity, since only α-naphthyl valerate was hydro-

TABLE IV

RF values of the different esterases from Lactobacillus lactis
Valeurs du RF des différentes estérases de Lactobacillus lactis

	α-naphthyl acetate							β-naphthyl acetate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
L. lactis 250		0.50							0.50					
242			0.45							0.45	0.36		0.14	
326			0.45						0.50		0.36			

	α-naphthyl propionate							β-naphthyl propionate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
250				0.36						0.45				
242			0.45	0.36		0.14	0.10			0.45	0.36		0.14	0.10
326				0.36			0.10		0.50	0.45	0.36		0.14	

	α-naphthyl butyrate							β-naphthyl-butyrate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
250				0.36						0.45				
242				0.36			0.10				0.36			0.10
326				0.36							0.36		0.14	

	α-naphthyl valerate							β-naphthyl valerate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
250														
242						0.14						0.14		
326							0.10							0.10

lysed by band E_3 with the exception of *L. helveticus* CNRZ 303 where also β-naphthyl propionate and butyrate were hydrolysed. Band E_4 hydrolysed β-naphthyl propionate and butyrate and ↓α-naphthyl butyrate, while this band was not detected in case of *L. helveticus* CNRZ 32. *L. helveticus* CNRZ 243 was distinguished by the absence of band E_2, and by the presence of two new bands of lower mobility E_5 and E_6 with RF of 0.27 and 0.14 respectively.

TABLE V

RF values of the different esterases from Lactobacillus acidophilus

Valeurs du RF des différentes estérases de Lactobacillus acidophilus

	α-naphthyl acetate							β-naphthyl acetate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
L.acidophilus														
251				0.36	0.27					0.45				
216			0.45									0.27		0.10

	α-naphthyl propionate							β-naphthyl propionate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
251				0.36	0.27		0.10			0.45				
216			0.45		0.27		0.10							0.1C

	α-naphthyl butyrate							β-naphthyl butyrate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
251				0.36	0.27		0.10							0.1C
216			0.45		0.27		0.10					0.27		

	α-naphthyl valerate							β-naphthyl valerate						
	E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_1	E_2	E_3	E_4	E_5	E_6	E_7
251				0.36		0.14	0.10				0.36		0.14	0.10
216			0.45				0.10			0.45				0.10

For *L. bulgaricus* strains (table 3), the obtained zymogram showed the presence of four distinguished bands, three of them with electrophoretic mobilities similar to bands E_2, E_3 and E_4 in *L. helveticus*, while the fourth band E_1 (RF 0.58) migrated faster than E_2 and was specific for α- and β-naphthyl acetate and β-naphthyl propionate. None of the *L. bulgaricus* strains tested

TABLE VI

Effect of the physiological age of the cells on the esterase activity of Lactobacillus species

Influence de l'âge physiologique des cellules sur l'activité estérasique de différentes espèces de Lactobacillus

Substrate	L. helveticus CNRZ 303				L. bulgaricus CNRZ 369			
	I	II	III	IV	I	II	III	IV
P-C_2	0.3	1	1.1	0.8	0.9	0.8	0.8	0.8
P-C_4	0.1	0.8	0.8	0.6	1	1	0.8	0.8

I - Cells harvested during the early logarithmic phase : L. helveticus (3 h), L. bulgaricus (2 h)
II - " " " logarithmic phase : L. helveticus (5 h), L. bulgaricus (4 h)
III - " " " early stationary phase : " (9 h), " (8 h)
IV - " " " end stationary phase : " (24 h), " (24 h)

The starting pH of cells media at zero time of growth was 6.4.
These results are the mean of two experiments

I - Cellules récoltées au début de la phase logarithmique de croissance : L. helveticus (3 h), L. bulgaricus (2 h)
II - Cellules récoltées pendant la phase logarithmique de croissance : " (5 h), " (4 h)
III - " " au début de la phase stationnaire de croissance : " (9 h), " (8 h)
IV - " " en fin de la phase stationnaire de croissance : " (24 h), " (24 h)

Le pH initial du milieu de culture était 6,4.
Les résultats sont la moyenne de deux expériences.

hydrolysed either α or β-naphthyl valerate. In *L. lactis* (table 4), the E_2 and E_4 bands were detected in 2 of the 3 strains tested while E_3 band was found in the 3 strains. For *L. acidophilus* (table 5), the E_3 band was noticed in all the strains tested, and only *L. acidophilus* CNRZ 251 showed the esterase band E_4. On the other hand, *L. acidophilus* and *L. lactis* strains were distinguished by the presence of a new band of lower electrophoretic mobility (E_7).

The results concerning the esterase patterns of the different lactobacilli from the *Thermobacterium* group are comparable to the work reported by MORICHI *et al.* (1968). They showed that the different species gave consistent characteristic patterns which were different for each species. On the other hand, differences in the number of esterase bands for the different species can be noticed. They also reported the presence of 4 esterase bands in *L. helveticus*.

B. *Effect of the physiological age of the cells on enzyme production*

The specific activity of esterase increased gradually during the growth of *L. helveticus* (table 6). The maximum production was observed at the early stationary phase followed by significant decrease in the specific activity of esterase in the late stationary phase. Similar observations were previously described for the esterase system of *L. casei, L. plantarum, L. brevis* and *L. fermentum* (EL SODA *et al.*, 1986). The decrease in enzyme activity at the end of the stationary phase was reported to be probably due to the presence of the cells exposed to lower pH value for several hours during the stationary phase. The trend of the results was different for the esterase system of *L. bulgaricus* since the esterase activity was almost similar during the different stages of growth of this micro-organism with little decrease in activity in the late stages of the growth cycle (table 6).

C. *Influence of incubation temperature of the cells on esterase production*

The optimum temperature from esterase production by the *Thermobacterium* was found to be 40-45° C (fig. 1). Little specific activity was detected after cell growth either at 35 or 55° C. Thus, growth temperature is a factor controlling esterase production by thermophilic lactobacilli. The present results agree with those of HEMME *et al.* (1980), who showed that pyruvate-kinase and 3-phospho-glyceraldehyde dehydrogenase were inactivated when cells were incubated at temperature greater than the optimum temperature for growth. Our results are also comparable to the work of EZZAT *et al.* (1982) who demonstrated that the optimum temperature for peptide hydrolase production by the *Thermobacterium* was 40° C. Certain interspecies differences were also detected for this enzymatic system. For instance maximal dipeptidase production by *L. helveticus* occurred at 40° C, by *L. bulgaricus* at 35° C and by *L. lactis* at 25° C.

In conclusion, this work revealed the presence of several esterases in the crude intracellular extract of lactobacilli from the *Thermobacterium* group. It is of importance to mention here that these activities do not correspond to residual peptide-hydrolase activities (results not shown) which were previously described (EL SODA and DESMAZEAUD, 1982). The specificity of the esterase system of the

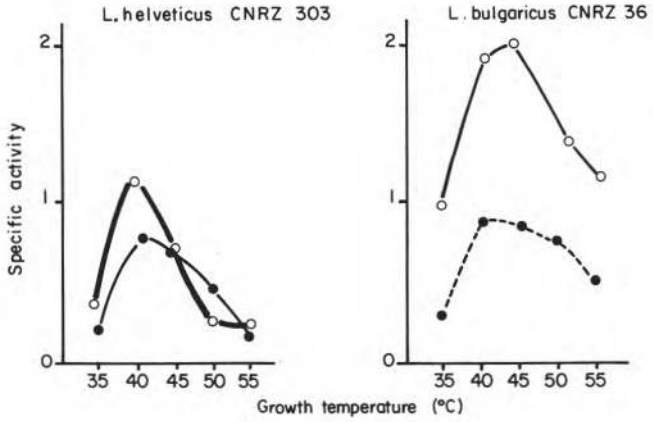

Fig. 1

Specific activity of ester hydrolases from two Lactobacillus *as a function of growth temperature in MRS medium.*

Activité spécifique du système estérasique de deux Lactobacillus *en fonction de la température de croissance dans le milieu MRS.*

○——○ = p-*nitrophényl acetate substrate.*
substrat p-*nitrophényl acétate.*

●——● = p-*nitrophenyl butyrate substrate.*
substrat p-*nitrophényl butyrate.*

lactobacilli towards short chain fatty acids would suggest a potential role of these enzymes in the enhancement of cheese flavour during ripening.

The use of esterase profiles as a tool for the taxonomy of *Lactobacillus acidophilus, L. bulgaricus, L. lactis* and *L. helveticus* as previously reported for *Escherichia coli* (GOULLET, 1973) or *Serratia* (GOULLET, 1978 et 1981) is not so clear than in the case of *L. casei* and *L. plantarum* as suggested by EL SODA *et al.* (1986), since differences between thermophilic strains are too little. However, we think that this type of enzymatic activities could be used as an indicator for the detection and the control of strains in mixed lactic acid bacteria cultures, if a precise choice of these strains in the mixtures was made, according to their enzyme profiles.

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