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Effect of bile on the β-galactosidase activity of dairy propionibacteria

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Abstract — In the present study, the effect of bile on the growth, cell permeabilization and β-galactosidase activity of dairy propionibacteria was determined. Two groups of strains with different behavior in media with oxgall (dehydrated fresh bile) could be observed, and were named bile-tolerant and non bile-tolerant groups. The β-galactosidase activity of bile-tolerant strains increased during the growth in media containing 0.15 and 0.30% oxgall. The incubation of cell suspensions in phosphate buffer with oxgall increased the β-galactosidase activity of all the studied strains. They were permeabilized by bile, permitting more substrate to enter the cells to be hydrolyzed by β-galactosidase. A higher specific activity was observed in cell-free extracts obtained from cultures in media with oxgall, indicating increased enzyme synthesis in the presence of bile. The enzyme activity was also increased by sodium taurocholate and sodium chloride. These results suggest that bile-tolerant strains of propionibacteria can provide meaningful β-galactosidase activity by permeabilization of the cells and increased enzyme synthesis during growth in the gut. The non bile-tolerant strains did not grow in media with bile. They were permeabilized, but their β-galactosidase was inactivated when they were exposed for a long time to bile in the growth media. Thus, their contribution to the β-galactosidase activity in the gut does not appear to be significant.

***Propionibacteria* / bile tolerance / β-galactosidase**

Résumé — **Effet de la bile sur l'activité β-galactosidase de bactéries propioniques laitières.** Dans la présente étude, l'effet de la bile sur la croissance, la perméabilisation cellulaire et l'activité β-galactosidase de bactéries propioniques laitières a été déterminé. Deux groupes de souches ayant des comportements différents dans des milieux supplémentés en oxgall (bile fraîche déshydratée) pouvaient être observés et ont été appelés l'un bile-tolérant et l'autre bile non-tolérant. L'activité

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β-galactosidase des souches bile-tolérantes augmentait au cours de la croissance dans les milieux contenant 0,15 et 0,30 % d'oxgall. L'incubation des suspensions cellulaires dans un tampon phosphate avec oxgall augmentait l'activité β-galactosidase pour toutes les souches étudiées. Elles étaient perméabilisées par la bile, permettant à plus de substrat d'entrer dans les cellules et d'être hydrolysé par la β-galactosidase. Une activité spécifique plus élevée était observée dans les extraits intracellulaires obtenus à partir de cultures dans les milieux supplémentés en oxgall, indiquant une synthèse enzymatique accrue en présence de bile. L'activité enzymatique était également augmentée par le taurocholate de sodium et le chlorure de sodium. Ces résultats suggèrent que les souches bile-tolérantes de bactéries propioniques peuvent produire une activité significative de β-galactosidase par perméabilisation des cellules et synthèse accrue d'enzyme pendant la croissance dans l'intestin. Les souches bile non-tolérantes ne croissaient pas dans les milieux avec bile. Elles étaient perméabilisées, mais leur β-galactosidase était inactivée quand elles étaient exposées pendant longtemps à la bile dans les milieux de croissance. Leur contribution à l'activité β-galactosidase dans l'intestin pourrait donc être négligeable.

***Propionibacterium* / tolérance à la bile / β-galactosidase**

1. INTRODUCTION

Lactose contained in the milk is hydrolyzed into the monosaccharides glucose and galactose in the intestine by the enzyme lactase. The enzyme concentrations in the intestine are highest immediately after birth, but later fall to very low values in adult mammals [9]. Lactose maldigestion is a phenomenon which is relatively common in many populations, caused by a decrease in the intestinal lactase activity. Abdominal pain, flatulence or diarrhea after milk consumption, the intestinal symptoms of lactose maldigestion, are caused by fermentation of the undigested lactose by colonic bacteria that produce high quantities of CH_4, H_2 and CO_2 [16].

A possibility for improving the utilization of lactose in persons that suffer from this disorder is via the incorporation of viable microorganisms in a dairy product as a source of the enzyme. Different types of microorganisms possess the β-D-galactosidase enzyme, and could be considered as a dietary supplement in lactose-maldigesting individuals. In this sense, yogurt and milk fermented with *Lactobacillus acidophilus* are widely recommended for dietary management of lactose maldigestion [8, 15]. Many people, however, do not consume these products due to their acid flavor. The use of non-fermented milk containing the bacteria of yogurt or *L. acidophilus* seems to be an acceptable alternative [10]. Nevertheless, other dairy products could be used with the same objective if the starter bacteria were able to provide β-galactosidase activity in the intestinal environment.

The dairy propionibacteria can be isolated from soil, vegetable, silage, raw milk, and dairy products such as kefir and Swiss-type cheeses. The presence of these bacteria in products that are frequently consumed by man as well as animals has led to an analysis of their possible role in human and animal health [11]. In spite of the frequency with which the propionibacteria enter the human intestine via a dairy product, at present there is no information available on their contribution to the β-galactosidase activity in the intestine, even though most species of dairy propionibacteria possess β-galactosidase activity.

It is considered that to produce beneficial effects in the host the bacteria used should be able to survive and grow in the gastrointestinal tract, resisting the gastric acids of the stomach and bile salts of the intestine [5, 13]. Studies carried out by Noh and Gilliland [12] have shown that bile salts

increase the cellular permeability of lactobacilli, thereby allowing a greater availability of β-galactosidase in the intestine.

In our laboratory we have demonstrated that certain strains of propionibacteria possess the ability to survive in intestinal conditions [14]. However, little is known about the bile tolerance of this genus. β-Galactosidase activity has been characterized in *Propionibacterium shermanii* [6], but the effect of bile on the enzyme has not been described for the genus *Propionibacterium*. Therefore, the objective of the present work was to evaluate the effect of the bile on growth and β-galactosidase activity of different strains of propionibacteria.

2. MATERIALS AND METHODS

2.1. Microorganisms and culture media

The microorganisms used in this study were the following: *P. freudenreichii* CRL 757, TL 503, TL 502, TL 253, Pe, Ce and Reβ; CNRZ 727; *P. jensenii* TL 494 and TL 219; *P. acidipropionici* CRL 1198 and Q4, (CRL: Centro de Referencia para Lactobacilos; TL: Laboratoire de technologie laitière; CNRZ: Centre national de recherche zootechnique). The strains Ce, Pe, Reβ, isolated in our laboratory from raw milk, and strain Q4 from a commercial Swiss-type cheese, were identified according to the criteria of Bergey's Manual of Systematic Bacteriology [2].

All the strains were stored at –20 °C in 10% (w/v) reconstituted skim milk containing 0.5% yeast extract. Before use they were activated by 3 successive transfers each 24 h at 35 °C in a lactose broth of the following composition: 1% Tryptone, 1% yeast extract, 0.05% cysteine, 0.05% Tween 80, 0.025% K_2HPO_4, 0.005% $MnSO_4$. The pH of the medium was adjusted to 6.8. The broth was sterilized at 121 °C for 15 min and then supplemented with 1% filter-sterilized lactose.

2.2. Bile tolerance

In order to determine the behavior of the strains in the presence of bile, the culture medium was supplemented with dehydrated fresh bile (oxgall, Difco) in 3 different final concentrations: 0.15, 0.30 and 0.50% (w/v). This provided bile salts in the concentration range found in the gastrointestinal tract of man [4]. Active cultures of each strain were centrifuged (5 000 *g*, 10 min, 4 °C) and the pellets suspended in an adequate volume of 0.1% peptone-water to obtain 5-fold concentrated suspensions. Each suspension was used to inoculate lactose broth with or without oxgall to an initial absorbance at 560 nm of approximately 0.1. The cultures were incubated at 35 °C, a temperature close to that of the human body, and growth was followed by observing changes in absorbance over time.

2.3. Effect of bile on growth and β-galactosidase activity

Three bile-tolerant strains and a non bile-tolerant strain were selected for further study. They were cultured in lactose broth with or without 0.15 and 0.30% oxgall. Growth was followed over time by counts of viable cell number in lactose broth with 1.5% agar incubated for 5 d at 35 °C in an anaerobic chamber (Form Scientific Anaerobic System, model 1024) with a mixture of N_2/CO_2, 90:10%.

To evaluate the effect of bile on enzymatic activity, 30 mL of each culture were collected at different times during growth until reaching the stationary phase. Cells were harvested (10 000 *g*, 10 min, 4 °C), washed with phosphate buffer 0.05 mol·L^{-1}, pH 7, suspended in the same buffer to adjust the absorbance at 560 nm in 0.5 units and finally concentrated 20 times. The enzymatic reaction was carried out with 200 µL of cell suspensions from culture controls and oxgall respectively; 0.836 mmol·L^{-1} *o*-nitrophenyl β-D-galactopyranoside (ONPG) and phosphate buffer 0.05 mmol·L^{-1}, pH 7, in a final volume of 1 mL.

Cell-free supernatants of each culture were recovered to assay β-galactosidase activity. The reaction mixture in this case contained 200 μL of supernatant, and oxgall was not included.

The reaction mixtures were incubated for 15 min at 37 °C and the reactions stopped with 0.5 mol·L^{-1} Na_2CO_3. When the samples contained cells, these cells were removed by centrifugation (5 000 *g*, 10 min) before determining the absorbance at 440 nm. The standard curve was obtained with *o*-nitrophenyl (ONP). The enzymatic unit was defined as nmol of ONP released per mL and per min of reaction.

2.4. Effect of bile on cellular permeability

Cell suspensions of each strain were obtained from cultures in the media without oxgall. They were incubated in phosphate buffer with and without 0.15 and 0.30% oxgall for 15 min at 37 °C, before beginning the enzymatic reaction by adding the substrate. The reactions were stopped after 15 min, and the activity determined as above mentioned.

Cell suspensions were prepared as indicated from a 24-h culture of *P. acidipropionici* Q4 developed in lactose broth without oxgall. One mL of suspension was added to 4 mL of phosphate buffer with or without oxgall (final concentration of 0.3%), and incubated for 15 min at 37 °C. Cell-free supernatants were obtained from each mixture by centrifugation at 10 000 *g* for 15 min at 4 °C, and the absorbance at 280 and 260 nm was determined. Protein contents of these supernatants were calculated as described by Warburg and Christian [18].

2.5. β-Galactosidase activity in cell-free extracts

Cell-free extracts of *P. acidipropionici* Q4 were prepared from 1 L of cultures in lactose broth with or without 0.15% oxgall, and incubated at 35 °C for 24 h. Cells were harvested by centrifugation at 10 000 *g* for 10 min at 4 °C, washed twice in phosphate buffer 0.05 mol·L^{-1}, pH 7, diluted in the same buffer up to 25% (w/v) and disrupted mechanically with a French press. Cell debris was removed by centrifugation, 2 cycles of 20 min per cycle, at 15 000 *g* and 4 °C.

The enzymatic activity was determined in each extract with 0.836 mmol·L^{-1} ONPG in phosphate buffer 0.05 mol·L^{-1}, pH 7 as above mentioned. The protein content was determined according to Bradford [1] using serum albumin (Sigma) as standard and the specific activity was expressed as nmol of ONP released per min per mg of protein.

In order to determine the effect of bile, sodium taurocholate and sodium chloride on enzymatic activity, 0.15 and 0.30% oxgall, 0.045, 0.09 and 0.30% sodium taurocholate or 0.5 to 5.0 mmol·L^{-1} sodium chloride were included in the reaction mixtures.

3. RESULTS

3.1. Growth in the presence of bile

The behavior of strains of propionibacteria in the presence of bile was determined by measuring changes in the absorbance at 560 nm over time for cultures in lactose broth with or without oxgall (dehydrated fresh bile) in 3 different concentrations (0.15, 0.30 and 0.50%). All strains reached the stationary phase of growth with a lower cellular density in media containing oxgall than in the control cultures (Fig. 1). In most cases, the inhibitory effect was greater when the concentration of oxgall increased in the medium, but differed depending on the studied strain. Of the 11 tested strains, 6 reached absorbances of over 0.5 in media with 0.30 and 0.50% oxgall, and were arbitrarily classified as bile-tolerant.

Three bile-tolerant strains (*P. freudenreichii* CRL 757, *P. acidipropionici* CRL 1198

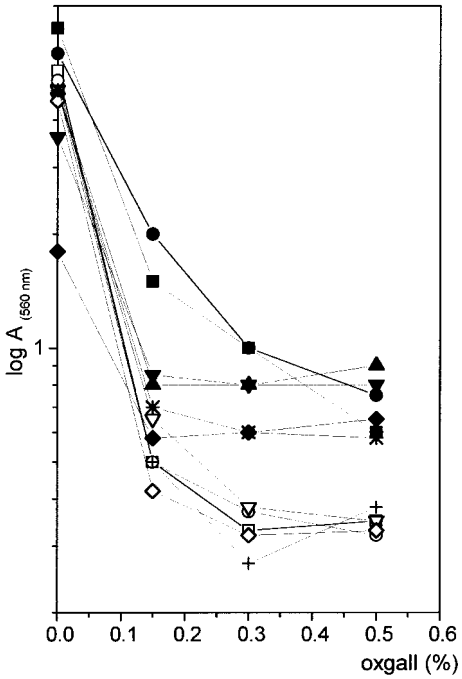

Figure 1. Effect of oxgall at different concentrations on the growth of *P. freudenreichii* Pe (▲), Ce (▼), Re (◆), CRL 757 (❋), TL 503 (□), TL 253 (+) and CNRZ 727 (◇); *P. acidipropionici* CRL 1198 (●) and Q4 (■); *P. jensenii* TL 494 (○) and TL 219 (▽). The log of absorbance at 560 nm reached at the stationary phase of growth was plotted against the corresponding concentration of oxgall in the medium.

Figure 1. Effet de l'oxgall à différentes concentrations sur la croissance de *P. freudenreichii* Pe (▲), Ce (▼), Re (◆), CRL 757 (❋), TL 503 (□), TL 253 (+) et CNRZ 727 (◇) ; *P. acidipropionici* CRL 1198 (●) et Q4 (■) ; *P. jensenii* TL 494 (○) et TL 219 (▽). Les log de l'absorbance à 560 nm atteints au cours de la phase stationnaire de croissance ont été rapportés aux concentrations correspondantes en oxgall dans le milieu de culture.

and *P. acidipropionici* Q4) and 1 non-tolerant strain (*P. freudenreichii* TL 253) were selected for further study. The development of these strains was measured by counts of viable cells. As shown in Figures 2a to 5a, growth rates were lower in the presence of oxgall than in its absence.

3.2. Effect of bile on the activity of cell suspensions

As shown in Figures 2 to 4, the highest β-galactosidase activity in control cultures of bile-tolerant strains was observed at the exponential phase of growth. This activity remained constant until the end of the assay except for *P. acidipropionici* CRL1198, in which a decrease of the activity was observed after 12 h of incubation (Figs. 2a and 2b). When oxgall was added to the

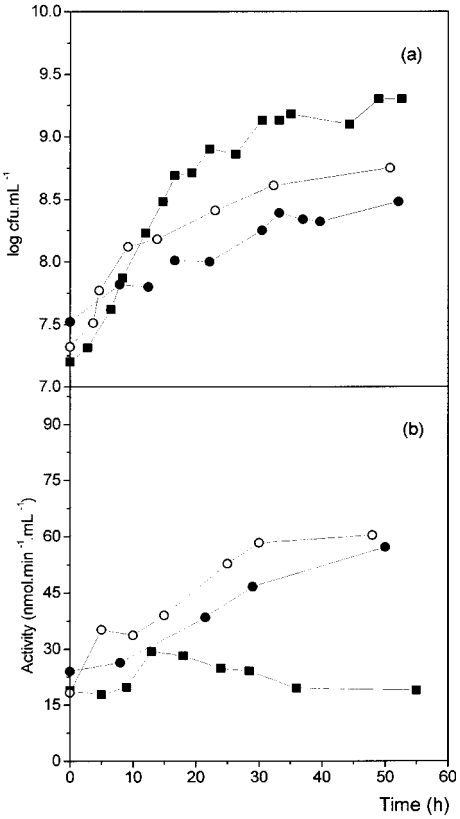

Figure 2. a: Growth and **b:** β-galactosidase activity of *P. acidipropionici* CRL 1198 in lactose broth with 0.15% (○) and 0.30% (●) oxgall and without bile (■).

Figure 2. a : Croissance et **b :** activité β-galactosidase de *P. acidipropionici* CRL 1198 dans le bouillon lactosé avec 0,15 % (○), 0,30 % (●) et sans (■) oxgall.

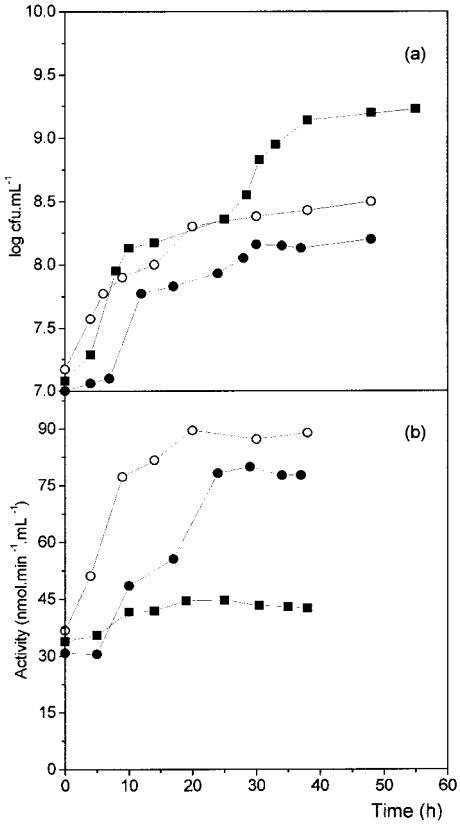

Figure 3. a: Growth and **b:** β-galactosidase activity of *P. acidipropionici* Q4 in lactose broth with 0.15% (○) and 0.30% (●) oxgall and without bile (■).

Figure 3. a : Croissance et **b :** activité β-galactosidase de *P. acidipropionici* Q4 dans le bouillon lactosé avec 0,15 % (○), 0,30 % (●) et sans (■) oxgall.

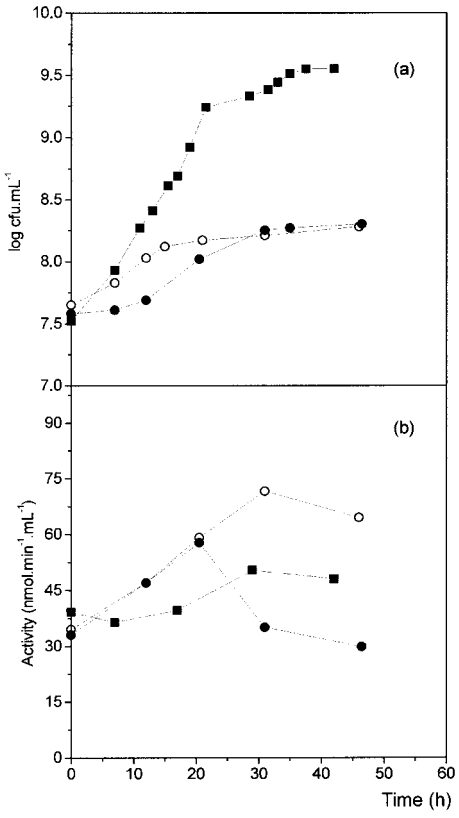

Figure 4. a: Growth and **b:** β-galactosidase activity of *P. freudenreichii* CRL 757 in lactose broth with 0.15% (○) and 0.30% (●) oxgall and without bile (■).

Figure 4. a : Croissance et **b :** activité β-galactosidase de *P. freudenreichii* CRL 757 dans le bouillon lactosé avec 0,15 % (○), 0,30 % (●) et sans (■) oxgall.

media, the activity was greater than in the control cultures and reached higher values with 0.15% than with 0.30% oxgall (Figs. 2 to 4).

The non bile-tolerant strain (*P. freudenreichii* TL 253) showed low β-galactosidase activity, both in the absence and in the presence of oxgall (Fig. 5b). None of the studied cultures showed activity in cell-free supernatants.

Cell suspensions obtained from control cultures were also preincubated in buffer with 0.15 and 0.30% oxgall for 15 min at 37 °C before beginning the enzymatic reaction. As shown in Table I, preincubation with bile increased the enzymatic activity in a more noticeable way with 0.15 than with 0.30% bile. The effect was similar in all the strains studied, even in the non bile-tolerant strain.

P. acidipropionici Q4 had the highest enzymatic activity in all the trials with oxgall. A cell suspension of this strain was

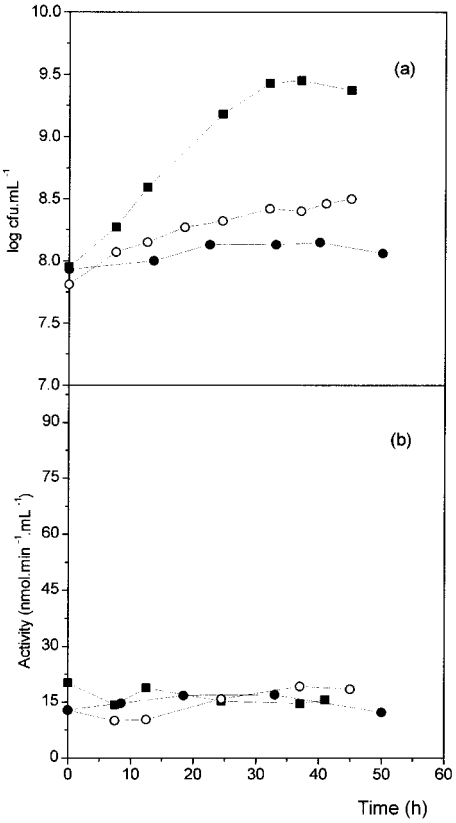

Figure 5. a: Growth and **b:** β-galactosidase activity of *P. freudenreichii* TL 253 in lactose broth with 0.15% (○) and 0.30% (●) oxgall and without bile (■).

Figure 5. a : Croissance et **b :** activité β-galactosidase de *P. freudenreichii* TL 253 dans le bouillon lactosé avec 0,15 % (○), 0,30 % (●) et sans (■) oxgall.

diluted in buffer with 0.3% oxgall and incubated for 15 min at 37 °C. The supernatant obtained after centrifuging the mixture showed a greater increase in absorbance at 260 nm (A_{260} = 0.248) than when the experiment was carried out in buffer without oxgall (A_{260} = 0.070). Absorbances were also determined at 280 nm (0.177 and 0.085 respectively); protein contents, calculated as described by Warburg and Christian [18], were 0.080 and 0.075 mg·mL^{-1} for the supernatants obtained in the presence and in the absence of oxgall respectively. These cell-free supernatants did not show β-galactosidase activity, indicating that in the presence of oxgall there was a release of intracellular material that did not include the enzyme. In spite of this permeabilization the cells remained viable, as shown in Figures 2a to 5a.

3.3. Effect of bile on the activity of cell-free extracts

Cell-free extracts of *P. acidipropionici* Q4 obtained from cultures with oxgall and without oxgall (control culture) were dialyzed against phosphate buffer and assayed for β-galactosidase activity in buffer containing oxgall, sodium taurocholate or sodium chloride.

When the extract obtained from the control culture was analyzed, greater activity was detected in reaction mixtures containing bile salts or sodium salts than in the mixture without any addition, indicating a direct stimulating effect of some component of the bile, probably a sodium salt, on the enzymatic activity (Tab. II).

When the strain was grown in a culture medium containing 0.15% oxgall, a higher specific activity compared with that of the control culture was observed. This result suggested that the enzyme could be synthesized in a greater amount in the presence of bile. In this case, incubating the extract with oxgall or another supplement (Tab. II) did not increase the enzymatic activity.

4. DISCUSSION

Resistance to bile is an important property in probiotic strains [7]. In the present study, we have determined the resistance of 11 strains of propionibacteria to bile salts by including oxgall in the growth medium. Two groups with different behavior could be observed, and were named bile-tolerant

Table I. Effect of pretreatment with bile on β-galactosidase activity by cell suspensions grown in the absence of oxgall.

Tableau I. Effet du prétraitement par la bile sur l'activité β-galactosidase de suspensions de cellules cultivées en absence d'oxgall.

Oxgall (%)	β-Galactosidase activity ($nmol \cdot mL^{-1} \cdot min^{-1}$)			
	P. acidipropionici CRL1198	*P. acidipropionici* Q4	*P. freudenreichii* CRL 757	*P. freudenreichii* TL 253
–	20.06 ± 1.83	45.26 ± 2.53	32.38 ± 3.44	23.80 ± 0.64
0.15	32.41 ± 6.56*	103.48 ± 3.93*	70.70 ± 7.63*	44.03 ± 2.34*
0.30	29.33 ± 5.10*	84.40 ± 9.30*	62.38 ± 3.46*	38.19 ± 7.94

* Cells were harvested from media without bile salts at the logarithmic phase of growth, when the cultures showed the highest enzymatic activity. The enzymatic activity was determined with cell suspensions, as indicated in Material and Methods. The results were statistically analyzed by ANOVA. Results are given as means ± SD. An asterisk indicates that the values are significantly different compared with their corresponding control ($P < 0.05$).

* Les cellules ont été récoltées à partir des milieux sans sels biliaires au cours de la phase logarithmique de croissance, lorsque les cultures atteignaient leur activité β-galactosidase maximale. L'activité β-galactosidase était déterminée comme indiqué dans Matériel et méthodes. Les résultats représentent la moyenne ± écart-type. Ils ont été analysés statistiquement par le test ANOVA. L'astérisque indique que les valeurs sont significativement différentes par rapport au contrôle correspondant ($P < 0,05$).

and non bile-tolerant groups. The resistance to bile did not seem to be related to the species but to the strains, as observed in *P. freudenreichii* which was present in both groups. Similar results have been obtained by Chateau et al. [3] in lactobacilli isolated from commercial probiotic products.

Six of our strains could be considered as bile-tolerant and could be able to survive and grow in the intestinal environment. However, non bile-tolerant strains could equally represent an important source of enzyme if they could be permeabilized by bile, considering the high proportions of propionibacteria found in Swiss-type cheeses.

β-Galactosidase activity in the tolerant strains reached its maximum value in the late exponential phase of growth, as previously observed for this genus [6]. Even when the cultures were inhibited in the presence of oxgall, the activity was greater if the bile salts were included in the medium, reaching the highest value with 0.15% oxgall. One of the tolerant strains, *P. freudenreichii* CRL 757, showed a loss of β-galactosidase activity at the stationary phase of the culture with 0.3% oxgall. In this case, the enzyme would have been damaged in a medium with a high oxgall concentration. The low enzymatic activity of the non-tolerant strain was not modified in the presence of bile.

The strains studied were permeabilized by bile, permitting more substrate to enter the cells to be hydrolyzed by β-galactosidase. These observations were consistent with the findings of other authors for lactobacilli species [12, 17]. An excessive bile concentration in the reaction mixture reduced the activity, which, however, was maintained above the activity of the control. The bile salts also permeabilized the cells of the non bile-tolerant strain, and this probably reduced cell viability and β-galactosidase activity after their long exposure to oxgall in the culture medium.

Some component of oxgall induces activation of the β-galactosidase in bile-tolerant

Table II. Specific activity of cell extracts of *P. acidipropionici* Q4 incubated with oxgall, sodium chloride or sodium taurocholate.

Tableau II. Activité spécifique des extraits cellulaires de *P. acidipropionici* Q4 incubés avec oxgall, le chlorure de sodium ou le taurocholate de sodium.

Treatment		Specific activity ($nmol \cdot min^{-1} \cdot mg^{-1}$)	
		Control culture	Culture with oxgall
	–	68.97 ± 7.66	96.05 ± 4.78[b]
Oxgall (%)	0.15	86.78 ± 9.68[a]	100.55 ± 4.52[c]
	0.30	86.93 ± 11.75[a]	104.78 ± 7.30[c]
	0.045	81.71 ± 2.82[a]	104.84 ± 0.06[c]
Sodium taurocholate (%)	0.090	89.27 ± 7.81[a]	109.72 ± 4.26[c]
	0.300	84.10 ± 3.10[a]	104.37 ± 0.28[c]
	0.5	84.51 ± 3.45[a]	92.12 ± 4.92[b]
NaCl	2.5	87.87 ± 4.86[a]	98.76 ± 5.32[b]
($mmol \cdot L^{-1}$)	5.0	77.46 ± 3.74[a]	101.94 ± 3.68[b]

Cells harvested from cultures in the presence and absence of oxgall were disrupted mechanically with a French press and dialyzed against phosphate buffer. Cell-free extracts were assayed for β-galactosidase activity as described in Material and Methods. Activity was expressed as nmol ONP released per min per mg of protein in the extract. The results were statistically analyzed by ANOVA. Results are given as means ± SD. Different characters in superscript indicate that the values were significantly different compared with the untreated control culture ([a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.005$).

Les cellules récoltées de cultures en présence ou en absence d'oxgall étaient éclatées mécaniquement avec une presse de French et dialysées contre un tampon phosphate. Les extraits intracellulaires étaient testés pour l'activité β-galactosidase comme indiqué dans Matériel et méthodes. Les activités étaient exprimées en nmol d'ONP libérées par min et par mg de protéine dans l'extrait. Les résultats représentent la moyenne ± écart-type. Ils ont été analysés statistiquement par le test ANOVA. Des caractères différents en indice indiquent que les valeurs sont significativement différentes par rapport à l'extrait contrôle sans traitement ([a] $P < 0{,}05$; [b] $P < 0{,}01$; [c] $P < 0{,}005$).

strains of propionibacteria. This component could be a sodium salt, since both sodium taurocholate and sodium chloride produced a similar effect to that of complete bile. Moreover, both Na^+ and K^+ ions have been reported to stimulate the β-galactosidase activity of *P. shermanii* [6].

Our results suggest that bile-tolerant strains of propionibacteria ingested in high amounts can provide meaningful β-galactosidase activity in the intestinal environment.

The permeabilization of cells would increase the availability of enzyme; the presence of bile would to increase its specific activity either by synthesis or by activation of the enzyme; and finally, the bile tolerance of the cells would permit the microorganisms to grow and be present in high concentrations in the intestine.

In strains with moderate specific activity or that are less sensitive to permeabilization, such as *P. acidipropionici* CRL 1198, the ability to grow in the intestine classifies them among those strains able to provide meaningful activity in the intestinal environment.

The non bile-tolerant strains, unable to grow in the presence of bile, could be more rapidly eliminated by the continuous renewal

of the intestinal content, and their contribution to the β-galactosidase activity does not appear to be significant.

In conclusion, an adequate selection of strains based on bile tolerance and its influence on β-galactosidase activity will permit the use of propionibacteria in dairy products that improve the tolerance to lactose maldigestion in certain individuals.

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