

## The probiotic properties of propionibacteria

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**Abstract** — The beneficial properties of lactic acid bacteria have been extensively studied and there is scientific evidence that support their use as food microbial supplements. Some potential probiotic properties have been clearly demonstrated for lactobacilli and other genera but not for propionibacteria (PAB). This article reports the effect of feeding propionibacteria on the composition and the metabolic activity of the intestinal microflora related to faecal enzymes involved in the release of carcinogenic compounds. The studies were carried out with four strains of *Propionibacterium*. The effect of feeding with PAB was evaluated in Balb/c mice receiving conventional solid food or food supplemented with meat over varying time periods. The results indicate that this feeding influences the composition of the intestinal microflora, specially modifying the number of anaerobes and coliforms in the caecal content. The metabolic activity of the resident microflora was also modified by feeding with propionibacteria. The greatest effect was observed on  $\beta$ -glucuronidase activity. However, some differences were also observed on azoreductase and nitroreductase activities. The data suggest that PAB may have a role in the prevention of colon cancer. © Inra/Elsevier, Paris.

### *Propionibacterium* / probiotic / carcinogenesis / $\beta$ -glucuronidase

**Résumé** — **Propriétés probiotiques des bactéries propioniques.** Les propriétés bénéfiques des bactéries lactiques ont été étudiées en détail et leur emploi comme additif alimentaire est scientifiquement reconnu. Des potentialités probiotiques ont été clairement démontrées pour les lactobacilles et d'autres genres, mais n'ont pas encore été très étudiées pour les bactéries propioniques laitières. Cet article montre les effets d'une alimentation avec des bactéries propioniques sur la composition et l'activité métabolique de la microflora intestinale en relation avec les enzymes impliquées dans la libération des carcinogènes. Les travaux ont été réalisés avec quatre souches de *Propionibacterium*. Un régime conventionnel et un régime supplémenté en viande, avec ou sans bactéries propioniques, étaient évalués sur des souris sur différentes durées. Les résultats montrent que les bactéries propioniques laitières influencent la composition de la flore intestinale, en particulier en modifiant le nombre d'anaérobies et de coliformes. L'activité métabolique était également modifiée, principalement celle de la  $\beta$ -glucuronidase. Néanmoins, des différences étaient aussi observées concernant les activités azoré-

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ductase et nitroréductase. Ces données laissent penser que les bactéries propioniques pourraient jouer un rôle dans la prévention du cancer du colon. © Inra/Elsevier, Paris.

## *Propionibacterium* / probiotique / carcinogène / $\beta$ -glucuronidase

### 1. INTRODUCTION

Probiotics are live microbial supplements that may exert beneficial effects on the health status of the host by improving the properties of the indigenous microflora of the gastrointestinal tract. Some of the ways in which probiotics influence the intestinal ecosystem include: 1) direct antagonism toward specific groups of microorganisms through the production of antimicrobial compounds; 2) competition for nutrients; or 3) competition for adhesion sites on the intestinal epithelial surface. In these ways, probiotics may help to prevent the establishment of potentially pathogenic microorganisms in the gut [6]. Probiotics may also exert beneficial effects on the host by modifying metabolic processes that occur in the gut. In some cases, their effects are mediated by stimulation of mammalian enzymes, such as intestinal lactase, while in others, the beneficial effects are derived from modifications of the intestinal microflora [18].

The indigenous microflora is a rich and complex ecosystem that has an intimate interaction with its host. The ability of the colonic microflora to generate mutagens, carcinogens and tumour promoters from dietary and endogenously produced precursors is well-documented [18]. The bacterial enzymes involved in these harmful processes include azoreductase, nitroreductase, nitrate reductase,  $\beta$ -glucuronidase and  $\beta$ -glycosidase.

Azo dye compounds represent a large group of chemicals extensively used in the textile, pharmaceutical, food and cosmetic industries. Even when these are not mutagenic in the standard Ames plate assay, they

are reduced to aromatic amines, that are known carcinogens, by azoreductases from intestinal bacteria [2]. Nitroreductases participate in the conversion of aromatic nitro compounds such as dinitrotoluene and nitrobenzenes to toxic or carcinogenic amines [18]. Nitrate reductase activity of the intestinal bacteria is related to methaemoglobinaemia produced after nitrate administration and the formation of N-nitroso compounds with mutagenic activities [4].

Steroids and some carcinogenic compounds are metabolised in the liver and conjugated with glucuronic acid before secretion via bile into the small intestine. Bacterial  $\beta$ -glucuronidases can hydrolyse the glucuronide releasing the parent compounds [5, 7, 13].

A variety of glycosidic compounds present in fruits, vegetables, and beverages such as tea and wine are poorly absorbed in the small intestine. When these compounds reach the colon they are hydrolysed by  $\beta$ -glycosidases associated with the resident flora, which may result in the release of toxic or mutagenic aglycones [3].

Certain probiotic strains of *Lactobacillus* and *Bifidobacterium* sp. have been shown to decrease the faecal enzyme activities that have the capacity to convert procarcinogens to carcinogens in the colon [3, 8, 9]. Probiotics may exert their action either by reducing the number of some intestinal bacteria with high faecal enzyme activities, or by modulating the expression of these enzymes [16].

Dairy propionibacteria (PAB) have been successfully used as animal growth promoters [12], and have also been used in combination with lactic acid bacteria for the

treatment of human intestinal disorders [19]. Nevertheless, some potential probiotic properties that have been demonstrated for lactic acid bacteria have not yet been studied in PAB [14].

The aim of this study was to determine the role of dietary supplementation of mice with PAB in inducing changes in the metabolic activities of the resident flora related to faecal enzyme activities which release carcinogenic compounds in the colon.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial strains and culture conditions

*Propionibacterium freudenreichii* CRL 757 and SG1, and *Propionibacterium acidipropionici* CRL 1198 and Q4, from CRL, Collection of Centro de Referencia para Lactobacilos, Argentina, were used. Strains SG1 and Q4 were isolated from Swiss-type cheeses. All strains were stored at  $-20^{\circ}\text{C}$  in 10 % (w/v) reconstituted non-fat milk (NFM) containing 0.5 % yeast extract. Before use, they were activated by three successive transfers in lactose broth of the following composition: 1 % (w/v) Tryptone, 1 % (w/v) yeast extract, 0.05 % (w/v) cysteine, 0.05 % Tween 80, 0.025 % (w/v)  $\text{K}_2\text{HPO}_4$ , 0.005 % (w/v)  $\text{MnSO}_4$ , pH 6.8 for 24 h at  $37^{\circ}\text{C}$ . The broth was sterilised at  $121^{\circ}\text{C}$  for 15 min and then supplemented with 1 % filter-sterilised lactose.

### 2.2. Animals and feeding procedures

The experimental model was the BALB/c male albino mouse with an average initial weight of 24 g. Mice were randomly divided into 5 treatment groups each containing 10 animals. Each group received a conventional balanced diet (CARGILL, Molinos, Entre Ríos, Argentina) with or without (control) different strains of PAB. An additional group of five animals was employed as a control before feeding.

Twenty-four h cultures of each PAB strain grown in lactose broth was harvested by centrifugation, washed in sterile saline solution and suspended in sterile NFM ( $10\text{ g}\cdot 100\text{ mL}^{-1}$ ) to the desired concentration ( $10^8\text{ cells}\cdot\text{mL}^{-1}$ ). The sus-

pensions were provided in the drinking bottles to test groups of mice while the control group was given sterile NFM. The mice received the diet and their assigned drink 'ad libitum' for 7 d. They were then returned to a normal diet of solid food plus water for a further 7 d.

A second trial was performed with 2 groups of mice each containing 5 animals fed a diet including cooked red meat. The conventional solid food was blended with minced red meat (1:1), molded again and cooked. In addition, one group received sterile NFM and the other NFM supplemented with *P. acidipropionici* CRL 1198 prepared as indicated above. Following 14 d of dietary treatments, the mice were fed a conventional diet and water for a further 7 d.

Finally, a third trial was performed with 3 groups of mice each containing 10 animals. Two groups received the same dietary treatments indicated in the second experiment and the third group received the same solid diet but sterile water in the drinking bottles during all the trial.

### 2.3. Enzyme activities

In the first and third trial, five mice per cage were sacrificed at the end of each treatment and after 7 d post treatment. The caeca were bound at both extremes with sterile thread, cut 2 cm from each tie and introduced into an anaerobic chamber (Forma Scientific Anaerobic System, model 1024) with an atmosphere of 85 %  $\text{N}_2$ , 5 %  $\text{CO}_2$  and 10 %  $\text{H}_2$ . The caecal contents were removed, weighed and diluted 10-fold in pre-reduced  $0.1\text{ mol}\cdot\text{L}^{-1}$  potassium phosphate buffer pH 7.4 containing 0.9 % (w/v) NaCl. They were immediately assayed for enzymatic activities.

The mice faeces of the second trial were collected by squeezing the rectal area of the animals. Faeces from each group were pooled, weighed and 10 % (w/v) suspensions in  $0.1\text{ mol}\cdot\text{L}^{-1}$  potassium phosphate buffer pH 7.4 containing 0.9 % (w/v) NaCl prepared. They were immediately assayed for  $\beta$ -glucuronidase activity.

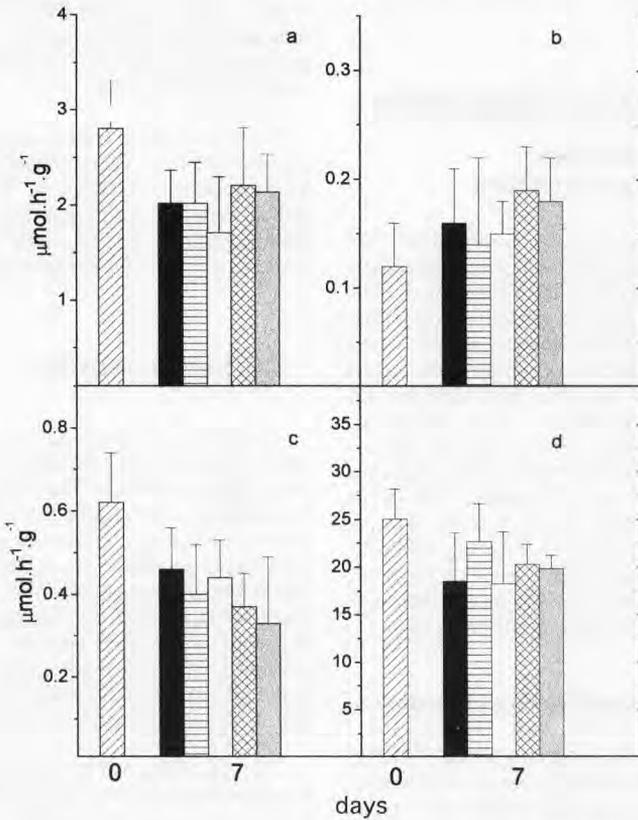
Azoreductase, nitroreductase and nitrate reductase activities were determined under anaerobic conditions as indicated by Wise et al. [20].  $\beta$ -Glucuronidase and  $\beta$ -glucosidase activities were aerobically assayed as indicated by Rowland et al. [17].

2.4. Bacterial counts

Serial 10-fold dilutions of the caecal homogenates were made in pre-reduced 0.1 % (w/v) peptone in the anaerobic chamber. Total anaerobes were enumerated on BHI blood agar supplemented with vitamin K and hemin, incubated at 37 °C for 96 h. MacConkey agar incubated aerobically for 24 h at 37 °C was used to enumerate coliforms.

3. RESULTS

There were no significant differences between treatment groups in the activities of azoreductase, nitroreductase, nitrate reductase or  $\beta$ -glucosidase in the caecal contents of mice on the 7<sup>th</sup> day of feeding with a conventional solid food and NFM or NFM containing PAB (figure 1). Similar results were obtained on the 7<sup>th</sup> day post treatment (data



**Figure 1.** (a) Azoreductase, (b) nitrate reductase, (c) nitroreductase and (d)  $\beta$ -glucosidase activities in the caecal content of mice on the 7<sup>th</sup> day of feeding with experimental diets containing skim milk or skim milk and different strains of *Propionibacterium*. Symbols: ▨ control before feeding; ■ skim milk (control); ▤ *P. freudenreichii* CRL 757; ▥ *P. freudenreichii* SG1; ▦ *P. acidipropionici* Q4; and ▧ *P. acidipropionici* CRL1198.

**Figure 1.** (a) Azoreductase, (b) nitrate reductase, (c) nitroreductase et (d)  $\beta$ -glucosidase dans le contenu du caecum des souris le 7<sup>e</sup> j d'alimentation avec un régime expérimental contenant du lait écrémé ou lait écrémé avec différentes souches de *Propionibacterium*. Symboles : ▨ avant alimentation ; ■ lait écrémé (contrôle) ; ▤ *P. freudenreichii* CRL 757 ; ▥ *P. freudenreichii* SG1 ; ▦ *P. acidipropionici* Q4 et ▧ *P. acidipropionici* CRL1198.

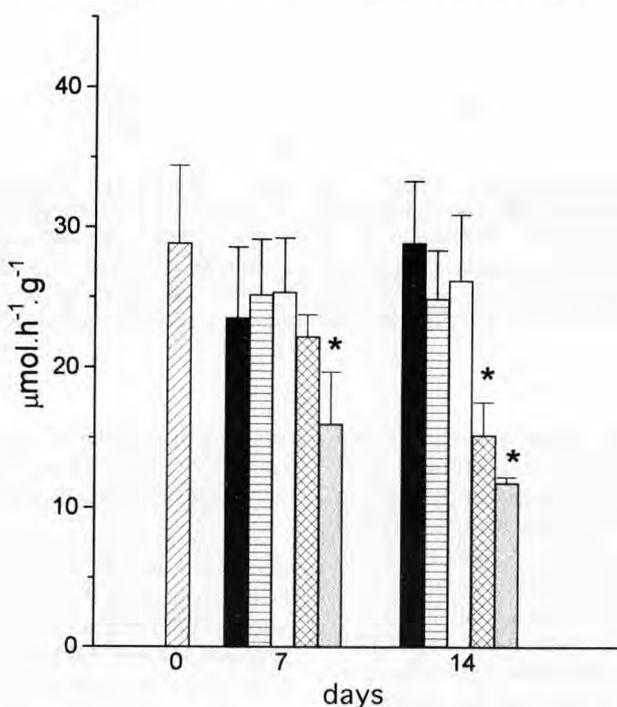
not shown). A mean consumption of  $5 \times 10^8$  cells per day of each PAB strain was estimated from the volume of NFM containing PAB consumed by each group.

$\beta$ -Glucuronidase activity was slightly reduced in all treatment groups on the 7<sup>th</sup> day of feeding compared to the basal level (figure 2). However, this activity was significantly lower ( $P < 0.05$ ) in mice fed with *P. acidipropionici* CRL 1198 while in the other groups, the level of  $\beta$ -glucuronidase activity was not significantly different. A week after cessation of feeding PAB,  $\beta$ -glu-

curonidase activity still remained significantly lower ( $P < 0.05$ ) in both groups fed with two strains of *P. acidipropionici*.

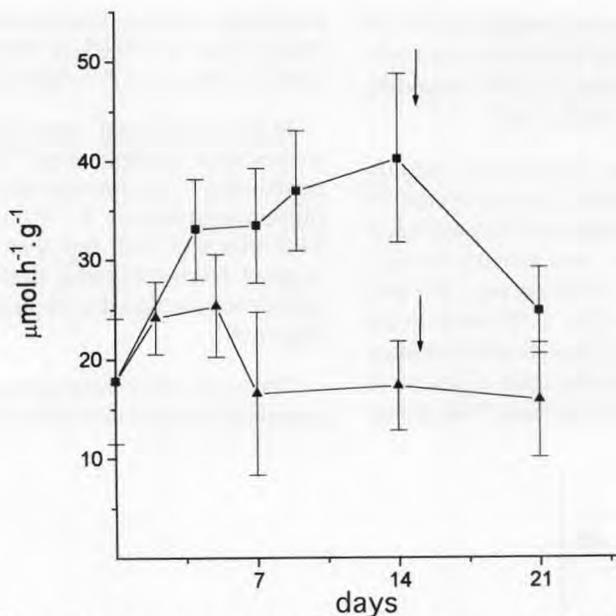
In the second trial, mice received a red cooked meat supplement and NFM or NFM containing *P. acidipropionici* CRL 1198 (mean consumption:  $5 \times 10^8$  cells·d<sup>-1</sup>) for 14 d after which all supplementation was stopped. During this trial,  $\beta$ -glucuronidase activity was measured in the faeces over 21 d (figure 3).

The results showed an increase in  $\beta$ -glucuronidase activity in the faeces of mice fed



**Figure 2.**  $\beta$ -Glucuronidase activity in the caecal content of mice on the 7<sup>th</sup> and 14<sup>th</sup> d of feeding with experimental diets containing skim milk or skim milk and different strains of *Propionibacterium*. Symbols: ▨ control before feeding; ■ skim milk (control); □ *P. freudenreichii* CRL 757; □ *P. freudenreichii* SG1; ▩ *P. acidipropionici* Q4; and ▤ *P. acidipropionici* CRL 1198. Activities that are significantly different ( $P < 0.05$ ) to the control before feeding are shown as asterisks.

**Figure 2.** Activité  $\beta$ -glucuronidase dans le contenu du caecum des souris le 7<sup>e</sup> et 14<sup>e</sup> j d'alimentation avec un régime expérimental contenant du lait écrémé ou lait écrémé avec différentes souches de *Propionibacterium*. Symboles : ▨ avant alimentation ; ■ lait écrémé (contrôle) ; □ *P. freudenreichii* CRL 757 ; □ *P. freudenreichii* SG1 ; ▩ *P. acidipropionici* Q4 et ▤ *P. acidipropionici* CRL 1198. Les différences significatives ( $p < 0,05$ ) sont indiquées par une astérisque.



**Figure 3.**  $\beta$ -Glucuronidase activity in faeces of mice fed a conventional diet with meat supplement. Mice received skim milk (■) or skim milk with *Propionibacterium acidipropionici* CRL1198 (▲) in the drinking bottles. Arrow indicates the day that feeding supplemented diets ceased.

**Figure 3.** Activité  $\beta$ -glucuronidase dans les fèces des souris nourries avec un régime conventionnel supplémenté en viande. Les souris recevaient du lait écrémé (■) ou du lait écrémé avec *Propionibacterium acidipropionici* CRL1198 (▲) dans la boisson. La flèche indique le jour de cessation du régime.

with sterile NFM supplement throughout the 14 d of feeding which returned to its basal level 7 d after the end of the feeding period. On day 14, the activity had increased 2.2-fold compared to the value at day 0 in the sterile milk supplemented group. In contrast, the  $\beta$ -glucuronidase activity in the faeces of PAB supplemented mice increased to a much lesser extent than the former during the first 5 d of the trial after which the enzyme activity showed a reduction, which was not statistically significant, and remained at the basal level from day 7 to day 21 of the experiment.

The levels of azoreductase, nitroreductase and  $\beta$ -glucuronidase activities were also determined in the caecal contents of mice on day 14 and 21 (table I). Another group of mice received water in addition to the meat

supplement in order to assess the enzyme levels that would be found during treatment with meat supplementation only.

The group which received meat-supplement plus water showed higher activities of the enzymes studied at days 14 and 21 compared to the basal levels before treatment. Supplementation of the meat diet with milk did not have any lowering effect on  $\beta$ -glucuronidase activity throughout the experiment but it prevented the increase in azoreductase and nitroreductase activities observed in the group receiving water.

In the PAB-fed group, there was a significant ( $P < 0.05$ ) reduction in  $\beta$ -glucuronidase activity on day 14 compared with the level found in the sterile milk and water treatments. In contrast, feeding with PAB resulted only in a slight reduction in azore-

**Table I.** Enzymes activities ( $\mu\text{mol}$  substrate transformed  $\text{h}^{-1}\cdot\text{g}^{-1}$  of caecal content) from the caeca of mice fed with the meat supplement and water, sterile milk or milk plus *P. acidipropionici* CRL 1198.

**Tableau I.** Activités enzymatiques ( $\mu\text{mol}$  substrat transformé  $\text{h}^{-1}\cdot\text{g}^{-1}$  de contenu du caecum) dans les caecum des souris nourries avec un aliment conventionnel additionné de viande et d'eau, de lait ou de lait contenant *P. acidipropionici* CRL 1198.

	Day 0	Day 14	Day 21
<b>Azoreductase</b>			
Basal level	2.80 $\pm$ 0.60		
Meat diet and water		5.27 $\pm$ 0.54 <sup>a</sup>	3.84 $\pm$ 0.52
Meat diet and milk		2.76 $\pm$ 0.67	2.51 $\pm$ 0.58
Meat diet and milk plus CRL 1198		1.84 $\pm$ 0.40	2.11 $\pm$ 0.63
<b>Nitroreductase</b>			
Basal level	0.80 $\pm$ 0.22		
Meat diet and water		1.44 $\pm$ 0.24 <sup>b</sup>	1.04 $\pm$ 0.40
Meat diet and milk		0.86 $\pm$ 0.10	0.92 $\pm$ 0.24
Meat diet and milk plus CRL 1198		0.60 $\pm$ 0.15	0.78 $\pm$ 0.23
<b><math>\beta</math>-Glucuronidase</b>			
Basal level	23.65 $\pm$ 5.60		
Meat diet and water		35.98 $\pm$ 3.82 <sup>c</sup>	30.90 $\pm$ 5.54 <sup>c</sup>
Meat diet and milk		35.39 $\pm$ 6.21 <sup>c</sup>	21.09 $\pm$ 5.14
Meat diet and milk plus CRL 1198		16.50 $\pm$ 9.36 <sup>d</sup>	12.10 $\pm$ 4.75 <sup>d</sup>

The different groups were fed a solid conventional diet supplemented with red cooked meat (50 % of the solid diet) and drank either water, milk or milk plus PAB ( $10^8$  cfu·mL<sup>-1</sup>). The enzyme activities were determined in the mice caecal contents at the end of 14 d of treatment and a week after the supplemented diets were stopped. The results were statistically analysed by ANOVA. Results are means  $\pm$  SD ( $n = 5$ ). Different superscripts in values of the same enzyme indicate significant differences between treatments ( $P < 0.05$ ).

Les différents groupes des souris étaient nourris avec un régime conventionnel supplémenté avec de la viande rouge cuisinée (50 % des solides dans le régime) et de l'eau, du lait ou du lait contenant PAB ( $10^8$  bactéries mL<sup>-1</sup>). Les activités enzymatiques étaient déterminées dans le contenu du caecum à la fin d'un traitement de 14 j et une semaine après la fin de la supplémentation. Les données étaient analysées par la méthode statistique ANOVA. Les données sont la moyenne  $\pm$  erreur standard ( $n = 5$ ). Les différents caractères dans des données pour la même enzyme indiquent les différences significatives entre groupes ( $p < 0,05$ ).

ductase and nitroreductase activities compared with the groups receiving sterile milk or water during the supplemental period.

Total anaerobes and coliform populations were evaluated in caecal contents of mice in order to determine if the population levels and enzymes activities were related. The number of coliforms increased in all groups on day 14 of the treatments (figure 4) but was only significantly different in the milk-fed group. A week after all groups were

shifted to non-supplemented diets, coliform counts were reduced to the level prior to supplementation. They were lowest in the group supplemented with PAB but the result was not statistically significant.

When total anaerobe counts were analysed, no significant differences between the treatment groups were found on day 14. However, all groups showed significantly ( $P < 0.05$ ) higher number of anaerobes compared with the control before feeding. On

day 21, the number of anaerobes was only statistically significant in the water-fed group. However within the treatments, the number of anaerobes in the milk-fed group showed a significant reduction compared with the value on day 14.

#### 4. DISCUSSION

Effective probiotic bacteria must be capable of maintaining their viability in the gastrointestinal tract, despite the numerous adverse factors that may affect them, including acids and enzymes in the stomach, bile salts and enzymes in the intestine and antagonistic bacterial interactions [10]. The behaviour of PAB in the presence of bile salts was studied in our laboratory by growing a number of strains in lactose broth in the presence of different oxgall concentrations [21]. Some strains maintained viability when they were suspended in milk and then diluted in an artificially developed gastric content at pH 2 [unpublished data]. The present work was performed with two strains of *P. freudenreichii* and two strains of *P. acidipropionici* selected on the basis of these studies.

The results showed that the feeding of mice with PAB did not induce changes in the azoreductase, nitroreductase, nitrate reductase or  $\beta$ -glucosidase activities of the intestinal microflora (figure 1).

Nitrate reductase activity is a characteristic of *P. acidipropionici*; however, nitrite formation by nitrate reductase activity was similar in all treatment groups, indicating that the contribution of strains of *P. acidipropionici* to the intestinal nitrite concentration was negligible. A similar result has been reported by Cole et al. [4], who did not find nitrite formation in caecal contents of rats monoassociated with *Clostridium*, even when the strain assayed actively reduced nitrate to nitrite after in vitro cultivation.

It has been shown that PAB isolated from human faeces hydrolyse synthetic  $\beta$ -glucuronides and may contribute to the release

of steroids or carcinogenic compounds conjugated with glucuronic acid [13]. In the present study, the  $\beta$ -glucuronidase activity did not increase when mice were fed with dairy PAB indicating that these bacteria have a different behaviour from those of intestinal origin. Moreover, this activity was lower in the group of mice given *P. acidipropionici* CRL 1198 than in the other groups. Therefore, this strain was selected for the further studies.

Faecal bacteria have both inducible and repressible enzymes. The ability to hydrolyse glucuronides and to reduce azo and nitro compounds may be induced by a diet with a high content of beef and fat [11]. In order to determine whether dairy PAB influenced the induction of enzyme activities, mice were fed with a red cooked meat supplement (50 % of the solid diet) and received either water, skim milk or skim milk containing *P. acidipropionici* CRL 1198 for 14 d. The azoreductase, nitroreductase and  $\beta$ -glucuronidase activities were induced by the meat diet, but different results were obtained in mice receiving sterile milk or milk containing *P. acidipropionici* CRL 1198 in addition to red meat. Milk feeding did not have any protective effect on the induction of  $\beta$ -glucuronidase activity by the meat diet. In contrast, PAB supplementation prevented the increase of the activity.

In a previous study, we determined the numbers of PAB excreted during feeding of mice with milk containing  $10^8$  *P. acidipropionici* CRL 1198 per millilitre [15]. The highest number of PAB in that study was  $7 \times 10^9$  cfu per gram of faeces on the 7<sup>th</sup> day of feeding. The lowest level of  $\beta$ -glucuronidase activity in the present study was also obtained on the 7<sup>th</sup> day of feeding, suggesting that a high number of viable *P. acidipropionici* CRL 1198 is required to significantly reduce  $\beta$ -glucuronidase activity.

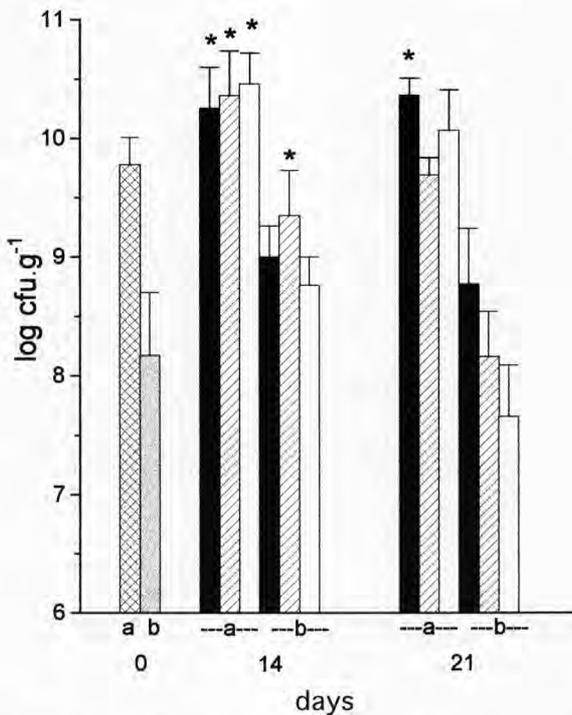
It is believed that strict anaerobic bacteria are the major organisms responsible for the azoreductase and nitroreductase activities [2]. It has been demonstrated in new-

born rats that these activities developed in the caecum concurrently with colonisation by obligated anaerobes [1]. Anaerobes and *E. coli* also have high  $\beta$ -glucuronidase activity [5, 7]. In contrast, *E. coli* has a very low nitroreductase activity, and it would not contribute to the whole nitroreductase activity within the intestine [1].

The contribution of each microbial group to total faecal enzyme activities depends both on the level of each population and on their metabolic activities in the intestinal ecosystem. In our study, changes on the coliform population were closely related to

those of  $\beta$ -glucuronidase activity. However, the metabolic activity of the intestinal microflora was modified faster than the microbial populations. In PAB-fed mice, the number of coliforms was close to control before feeding, after 14 d of the treatment (figure 4) and the  $\beta$ -glucuronidase activity was at a lower level than the control on the same day (table 1).

Feeding with PAB produced only a slight reduction in the azoreductase and nitroreductase activities compared with the control group receiving sterile milk during meat supplementation. Our results suggested that



**Figure 4.** Counts of anaerobic total flora (–a–) and coliforms (–b–) in the caecal content of mice fed with meat supplement. Mice received water (■), skim milk (▨), or skim milk with *Propionibacterium acidipropionici* CRL1198 (□) in their drinking bottles. Counts of anaerobes (⊠) and coliforms (□) before feeding are indicated as controls. Asterisks indicate significant ( $P < 0.05$ ) differences between treatments and the numbers before supplementation.

**Figure 4.** Flore totale anaérobie (–a–) et coliformes (–b–) dans le contenu du caecum des souris nourries avec un régime supplémenté en viande. Les souris recevaient de l'eau (■) du lait écrémé (▨) ou du lait écrémé avec *Propionibacterium acidipropionici* CRL1198 (□) dans la boisson. Anaérobies (⊠) et coliformes (□) avant l'alimentation sont indiqués comme témoins.

these enzymatic activities were not related to reductions in coliform numbers.

The azoreductase and nitroreductase activities were lower in the groups receiving sterile milk or milk supplemented with PAB than in the group receiving water during meat supplementation but the total number of anaerobes was similar in all treatments. Changes in the metabolic activity of the intestinal microflora may be produced without appreciable changes in the number of microorganisms.

Total anaerobic counts evaluate all genera that can grow under strictly anaerobic conditions. Therefore, we could only conclude that different anaerobic populations could be stimulated in mice receiving supplemented diets depending on the supplement employed. In the two groups that received milk during meat supplementation, some populations of anaerobic bacteria that do not have azoreductase or nitroreductase activities could have been stimulated faster than other bacteria possessing these activities. However, it is important to note that in the group treated with PAB, anaerobe counts remained at a high level even 7 d following the cessation of supplementation and that azoreductase and nitroreductase activities in that treatment group were the lowest of all groups. Apparently, there was an additional effect by feeding with dairy PAB. We are conducting further experiments to determine the composition of the anaerobic flora in mice fed with dairy PAB.

In conclusion, the present study indicates that PAB may influence the composition and metabolic activities of the intestinal microflora of mice. The greatest effect was observed on the  $\beta$ -glucuronidase activity in the caecal content of mice during supplementation with *P. acidipropionici* CRL 1198. However, this PAB also exerted influences on the nitro- and azoreductases in a diet which otherwise favoured the increase of their activities. These data suggest that PAB may have a role in preventing colon cancer.

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