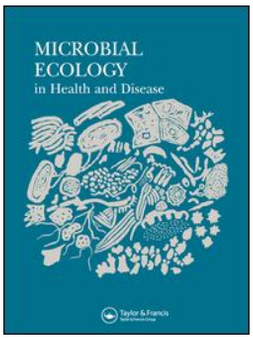

Microbial Ecology in Health and Disease

ISSN: (Print) 1651-2235 (Online) Journal homepage: informahealthcare.com/journals/zmeh20

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To cite this article: M. A. McConnell & G. W. Tannock (1993) Lactobacilli Do Not Influence Enzyme Activities of Duodenal Enterocytes of Mice, Microbial Ecology in Health and Disease, 6:6, 315-318, DOI: 10.3109/08910609309141341

To link to this article: https://doi.org/10.3109/08910609309141341

Published online: 11 Jul 2009.

MICROBIAL ECOLOGY IN HEALTH AND DISEASE VOL. 6: 315–318 (1993)

Short Communication

Lactobacilli Do Not Influence Enzyme Activities of Duodenal Enterocytes of Mice

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Received 21 June 1993; revised 26 July 1993

Alkaline phosphatase and phosphodiesterase I activities of duodenal enterocytes harvested from mice with or without lactobacilli as intestinal inhabitants were determined. The presence of lactobacilli as members of the digestive tract microbiota did not influence the two enzyme activities.

KEY WORDS—Lactobacilli; Duodenum; Enterocytes; Enzymes; Alkaline phosphatase; Phosphodiesterase.

INTRODUCTION

Lactobacilli inhabit the digestive tract of many mammalian species including mice, rats, pigs and fowl.[7] In the case of these four animal species, lactobacilli are numerous in proximal regions of the digestive tract because certain strains can adhere to, and form layers on, the surface of stratified, squamous epithelia lining parts of the tract.[8] Lactobacilli shed from these layers provide a constant source of inocula resulting in the presence of these bacteria throughout the digesta in the gastrointestinal tract of the animal host.[9] *Lactobacillus* species of digestive tract origin are commonly used as ingredients in preparations of living microbes fed to animals to promote health and weight gain (probiotics).[1] The mechanism of action of such probiotics is controversial, but it is clear that lactobacilli when inhabiting the digestive tract, influence the biochemistry of the intestinal milieu of mice.[4,5,11]

The activities of enzymes in the duodenal epithelium of germ-free mice are higher than those of conventional rodents.[2] Several studies have shown that association of germ-free rodents with bacteria of intestinal origin decreases these enzyme activities and suggest that lactobacilli may be mediators of the effect.[2,12,13] However, inconsistent alkaline phosphatase results using lactobacilli as microbial associates have been obtained, perhaps due in part to the relatively simple bacterial community established in the ex-germ-free animals that were used.[2,14,16,17] We have compared two of the enzyme activities investigated by other workers, alkaline phosphatase (EC 3.1.3.1) and phosphodiesterase I (EC 3.1.4.1), in duodenal enterocytes from mice harbouring a complex normal microbiota that included lactobacilli and from animals whose microbiota differed only in that lactobacilli were absent. The comparisons were made using enterocytes harvested from the villous tips where most alkaline phosphatase and phosphodiesterase is detected.[14]

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0891–060X/93/060315–04 $07.00

MATERIALS AND METHODS

Mice

Reconstituted lactobacillus-free mice (RLF) were from our colony derived and maintained in isolators by gnotobiotic methodology as described previously.[10] The animals harbour a gastrointestinal microbiota functionally equivalent, on the basis of 26 microbiota-associated characteristics, to that of conventional mice but lactobacilli are absent. Reconstituted lactobacillus-free mice intentionally colonised with a lactobacillus microbiota (RLFL) were derived by inoculating RLF mice with cultures of all three *Lactobacillus* strains comprising the lactobacillus microflora of conventional mice in our

Table 1. Comparison of duodenal enterocyte enzyme activities and protein concentration

Test	RLF (10 groups)*	RLFL (11 groups)	*P*
Alkaline phosphatase			
Total* (μmol product/ml/min)	38·76 (5·40)	42·99 (8·13)	0·999
Specific (μmol product/min/mg protein)	3·67 (0·45)	3·49 (0·75)	0·999
Phosphodiesterase			
Total* (μmol product/ml/min)	0·51 (0·14)	0·45 (0·06)	0·999
Specific (μmol product/min/mg protein)	0·055 (0·007)	0·035 (0·005)	0·074
Protein (mg/ml)	10·57 (1·42)	12·90 (1·29)	0·468

*Results given are means with SEM in parentheses.
*Five mice per group. Combined data from males and females are recorded since initial analysis did not show a difference in values between sexes within RLF and RLFL groups.

facility: *L. delbrueckii* strains 18 and 21, and *L. fermentum* strain 20 as described previously.[11] RLFL animals used in the study were the progeny of lactobacillus-colonised mice and had therefore been in contact with lactobacilli throughout life. RLF mice used in this study were drawn from nine litters and RLFL mice from 11 litters.

Preparation of enterocytes

The method of Whitt and Savage[14] was followed, all specimens being collected between 10 and 11 a.m. Mice aged 6 weeks were killed by carbon dioxide anaesthesia followed by cervical dislocation, and a 3 cm length of duodenum, measured from the pyloric sphincter, was removed from each animal. A sterile glass rod was inserted at the proximal end of the duodenum and the specimen tied firmly to the rod using fine cotton. The duodenum was then everted on the glass rod, rinsed with cold (4°C) 0·9 per cent sodium chloride solution, blotted gently with filter paper and placed in 6 ml of Solution A (1·5 mM potassium chloride, 96 mM sodium chloride, 27 mM sodium citrate, 8 mM potassium dihydrogen phosphate, 5·6 mM disodium hydrogen phosphate, pH 7·3) for 30 min at 37°C. The specimen was then transferred to a tube containing 6 ml of Solution B (5 mM EDTA, 0·23 M sucrose) and incubated at 37°C for 10 min. Enterocytes were dislodged from the tissue into the solution by rotating the glass rod backwards and forwards 15 times. Enterocyte suspensions from five mice of the same sex were pooled and placed in a preweighed centrifuge tube. The enterocytes were harvested by centrifugation at 500 *g* for 10 min at 4°C. The supernatant was discarded and the weight of the drained pellet obtained. The pelleted enterocytes were suspended in 1 ml of a sucrose buffer solution (0·23 M sucrose, 0·5 M Tris hydrochloride, 5 mM potassium chloride, 3 mM β-mercaptoethanol, pH 8·2).

Enzyme assays

Alkaline phosphatase and phosphodiesterase I activities of duodenal enterocytes were determined by the methods described by Whitt and Savage.[14] Enterocyte suspensions were diluted in 10-fold steps to 10^{-4} in 0·9 per cent sodium chloride solution and 0·1 ml volumes of the dilutions were transferred to test tubes. For alkaline phosphatase assay, 1 ml of reaction mixture (1·0 M Tris hydrochloride, 0·1 M sodium chloride, 1·0 mM magnesium chloride, 16 mM *p*-nitrophenylphosphate, pH 9·5) was added to each tube and the solutions were incubated at 37°C for 30 min. The reaction was stopped by the addition of 10 ml of 0·02 M sodium hydroxide solution. The absorbance of the preparations at 420 nm wavelength was determined and the total activity calculated by reference to a standard curve constructed using *p*-nitrophenol. For phosphodiesterase assay, 1 ml of reaction mixture (0·1 M Tris acetate, 3 mM zinc chloride, 1·0 mM

4-nitrophenylphenylphosphonate, pH 8·0) was added to tubes containing 0·1 ml volumes of diluted enterocyte suspension and incubated at 37°C for 30 min. The reaction was stopped by the addition of 3 ml of 0·02 M sodium hydroxide. The absorbance of the preparations was measured at 400 nm wavelength and the total activity calculated by reference to a *p*-nitrophenol standard curve. The protein concentration of the enterocyte suspensions was determined by the method of Lowry *et al.*[3] using bovine serum albumin as the standard. Student's *t*-test was used for the statistical evaluation of data.

RESULTS

Alkaline phosphatase and phosphodiesterase activities and protein content of the duodenal enterocytes did not differ between mice with (RLFL), or without (RLF), lactobacilli as inhabitants of the digestive tract (Table 1).

DISCUSSION

Lactobacilli inhabiting the proximal gastrointestinal tract of mice might influence enzyme activities in the duodenal epithelium because they inhabit the gastric region of the digestive tract in large numbers, which is upstream from the duodenum. Hence lactobacillus metabolites present in the digesta might influence host biochemical activities in more distal regions of the tract. The normal microbiota of the digestive tract of mice is known to influence the rate of migration of enterocytes from crypt to villous tip, as well as enzyme activities of the enterocytes.[6,12,13,15] The specific microbes responsible for these influences have not, however, been identified. Comparisons of enzymatic activity in the intestinal contents of RLF and RLFL mice have demonstrated that lactobacilli affect bile salt hydrolase, azoreductase and β-glucuronidase activities.[4,5,11] We used these animals, therefore, to investigate the possible involvement of lactobacilli in microbiota-mediated influences on duodenal enterocytes. Unlike animals used in previous studies of enterocyte enzymes, our mice harboured a complex microbiota that differed between animal groups only in the presence or absence of lactobacilli. Our results support those of Whitt and Savage[14] and clearly demonstrate that lactobacilli inhabiting the digestive tract of mice do not influence alkaline phosphatase and phosphodiesterase activities associated with duodenal enterocytes.

ACKNOWLEDGEMENTS

The support of the New Zealand Dairy Research Institute, and technical advice from Dr Dixie Whitt, University of Illinois, is gratefully acknowledged.

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