Onderstepoort J. vet. Res., 54, 581–583 (1987)

FREQUENCY AND OCCURRENCE OF *LACTOBACILLUS ACIDOPHILUS* IN THE GUT OF THE PIG, AS INDICATED BY ITS PRESENCE IN THE FAECES

MONIKA H. M. VON AULOCK[1] and W. H. HOLZAPFEL, Department of Microbiology, University of Pretoria, Pretoria 0002

ABSTRACT

VON AULOCK, MONIKA H. M. & HOLZAPFEL, W. H., 1987. Frequency and occurrence of *Lactobacillus acidophilus* in the gut of the pig, as indicated by its presence in the faeces. *Onderstepoort Journal of Veterinary Research*, 54, 581–583 (1987).

Faecal samples of 2 pigs, kept on a constant diet, were analysed for *Lactobacillus acidophilus* and other lactic acid bacteria at 1 week intervals over a period of 3 months. Enumeration of *L. acidophilus* by selective methods was verified by phenotypic identification of representative isolates. The total lactic acid bacteria (LAB) population ranged between 10^8 and 10^{10} colony-forming units per gram of faeces over the 3 months period. *L. acidophilus* contributed on average a relatively constant figure of 10 % to the total LAB population.

INTRODUCTION

Systematic investigation of the intestinal microbes of humans and warm-blooded animals dates back to the end of the previous century (Mitsuoka, Sega & Yamamoto, 1964). After the initial description of *Bacillus acidophilus* by Moro in 1900 (Johnson, Phelps, Cummins, London & Gasser, 1980) *Lactobacillus acidophilus* and other lactobacilli received special attention as beneficial intestinal and caecal organisms.

The ability to inhabit the small and large intestines and to produce beneficial effects is due to characteristics, such as adherence to columnar epithelial cells (Gilliland, 1979; Barrow, Brooker, Fuller & Newport, 1980), resistance to bile salts (Johnson *et al.*, 1980; Mäyrä-Mäkinen, Manninen & Gyllenberg, 1983) and lysozyme (Johnson, Ray & Speck, 1984), as well as certain antimicrobial properties (Shahani, 1977; Barefoot & Klaenhammer, 1983). Although several bacterial groups, including lactobacilli, are regularly isolated from faecal samples of pigs, their relative numbers (and proportions in the total population) may vary considerably (Vervaeke & Van Nevel, 1972; Vervaeke, Van Nevel, Decuypere & Van Asshe, 1973; Salanitro, Blake & Muirhead, 1977; Robinson, Allison & Buchlin, 1981). According to Salanitro *et al.* (1977), the type of intestinal bacteria isolated depends on factors such as (i) the media and methods employed, (ii) the type and amount of feed consumed by the pigs, (iii) environmental conditions (housing and hygiene), (iv) sex and age of the animals, and (v) microbial variation among the animals.

Lactobacilli are reportedly the most important microbial group in pig faeces (Russell, 1979). However, all reports on this group, and specifically *L. acidophilus*, have thus far been based on circumstantial observations, usually after single sampling. This study was therefore undertaken to determine whether the *L. acidophilus* and total *Lactobacillus* populations in the faeces remain constant over a given period under a more or less fixed set of conditions. Two pigs from the same farrow were used for this purpose.

MATERIALS AND METHODS

Experimental animals: Two male pigs (*Sus scrofa domestica*), A and B, were used in this study. They originated from the same farrow and received a constant standard diet. Faecal samples were collected weekly over a period of 3 months from the age of 5 weeks. Both animals were castrated at 11 weeks of age, *i.e.* 6 weeks after commencement of the experiment. The samples were collected aseptically in plastic bags and analysed within 1–2 h of collection.

Isolation of lactic acid bacteria: A dilution series was prepared in quarter strength sterile Ringer's buffer and surface-plated onto both Rogosa agar (Rogosa, Mitchell & Wiseman., 1951) and Briggs agar (Briggs, 1953) (5 plates per dilution factor). The inoculated plates were incubated at 37 °C for 3 days under anaerobic conditions (Gas-Pak; BBL). The total number of lactic acid bacteria per gram of faeces was estimated for both types of agar used. Representative colonies were isolated onto MRS agar (De Man, Rogosa & Sharpe, 1960) and incubated at 37 °C for 48 h under anaerobic conditions.

After purification and phase contrast microscopy, all catalase-negative strains were transferred into test tubes containing MRS-broth with 0,02 % (w/v) cysteine hydrochloride, and Durham tubes, followed by incubation at 37 °C for 3 days. All homofermentative strains were subjected to a series of physiological tests (Reuter, 1964; Mitsuoka, 1969; Lauer, Helming & Kandler, 1980). Special care was taken to determine whether typical "β-colonies" on Briggs' agar were indeed *L. acidophilus* (Mitsuoka, 1969). In addition to a typical carbohydrate fermentation pattern, the production of a racemic mixture of lactic acid, as well as the ability to grow at 45 °C but not at 19 °C, served as key characteristics for designation of a strain to a hitherto heterogeneous taxon *L. acidophilus* (Lauer *et al.*, 1980).

RESULTS AND DISCUSSION

Based on observations by Ochi & Mitsuoka (1958), β-colonies on Briggs agar plates were regarded as being representative of *L. acidophilus*. For verification 170 strains were isolated at weekly intervals over a 3 months' period and identified as *L. acidophilus* (Von Aulock & Holzapfel, in preparation 1987).

The highest total lactic acid bacterial (LAB) population numbers were found on Rogosa agar, and these results (an average of 2 determinations) are given in Table 1. The average numbers of β-colonies (indicative of *L. acidophilus*) are also given in Table 1, and are based on 5 parallel determinations per dilution factor.

The total LAB population ranged between *ca.* 10^8 and 10^{10}/g for both pigs over the experimental period (Fig. 1 & 2). For both animals the highest LAB and *L. acidophilus* numbers were reached after 9 weeks. The *L. acidophilus* population (as β-colonies) ranged between 10^7 and 10^9/g (Fig. 3 & 4), and averaged *ca.* 10 % of the total LAB population. Although this was especially true for pig B, higher ratios of up to 40 % (after 10 weeks) were found for pig A. The relative contribution of *L.*

[1] Present address: Pretoria College of Pharmacy, University of Pretoria, Brooklyn, Pretoria 0002

Received 18 August 1987—Editor

TABLE 1 Total lactic acid bacterial (LAB) population compared with typical *L. acidophilus* colonies in the faeces of 2 experimental pigs. Counts given are the mean of 2 to 5 determinations for LAB population and *L. acidophilus* respectively

Time in weeks	Pig A		Pig B	
	Total LAB population/g	*L. acidophilus* /g	Total LAB population/g	*L. acidophilus* /g
0	$1,5 \times 10^9$	$1,18 \times 10^8$	$5,0 \times 10^8$	$1,1 \times 10^7$
1	$3,5 \times 10^8$	$6,8 \times 10^7$	$2,36 \times 10^8$	$2,0 \times 10^7$
2	$3,13 \times 10^8$	$1,95 \times 10^7$	$2,93 \times 10^8$	$8,5 \times 10^6$
3	$2,8 \times 10^8$	$2,05 \times 10^7$	$3,32 \times 10^8$	$1,52 \times 10^7$
4	$3,9 \times 10^8$	$4,0 \times 10^9$	$1,0 \times 10^9$	$1,08 \times 10^8$
5	$2,27 \times 10^8$	$2,9 \times 10^7$	—	—
6	$8,38 \times 10^8$	$3,4 \times 10^7$	$5,83 \times 10^8$	$2,4 \times 10^7$
7	$3,8 \times 10^8$	$5,47 \times 10^7$	$6,05 \times 10^8$	$2,05 \times 10^7$
8	$2,56 \times 10^9$	$3,5 \times 10^8$	$5,08 \times 10^9$	$5,97 \times 10^8$
9	$4,38 \times 10^9$	$5,05 \times 10^8$	$1,15 \times 10^{10}$	$1,63 \times 10^9$
10	$1,0 \times 10^9$	$4,02 \times 10^8$	$2,56 \times 10^8$	$1,2 \times 10^7$
11	$1,99 \times 10^9$	$7,0 \times 10^7$	$2,13 \times 10^8$	$2,0 \times 10^7$

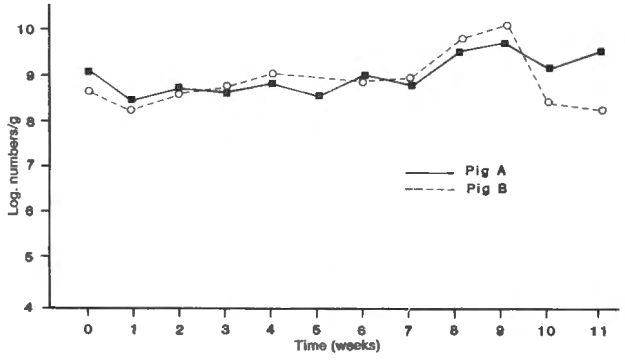

FIG. 1 Change in the total lactic acid bacterial population in the faeces of 2 experimental pigs (A and B) kept on a constant diet over 11 weeks

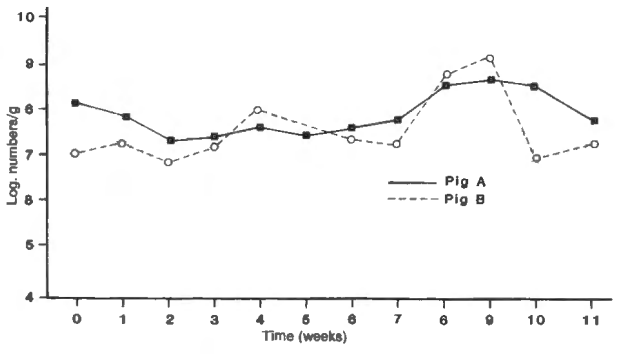

FIG. 2 Change in the *Lactobacillus acidophilus* population in the faeces of 2 experimental pigs (A and B) kept on a constant diet over 11 weeks

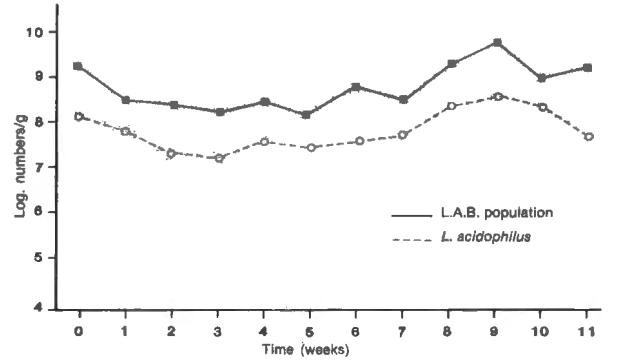

FIG. 3 Relationship between the total lactic acid bacterial population (LAB) and *Lactobacillus acidophilus* in the faeces of experimental pig A, kept on a constant diet over 11 weeks

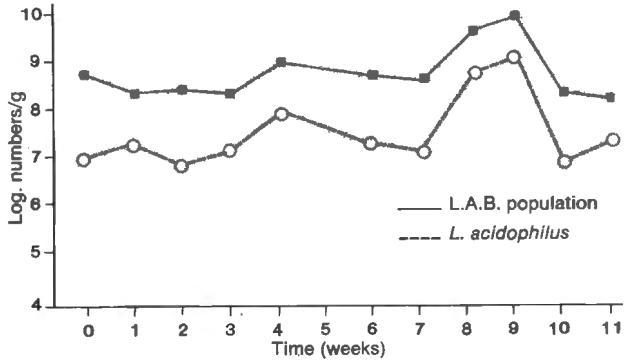

FIG. 4 Relationship between the total lactic acid bacterial population (LAB) and *Lactobacillus acidophilus* in the faeces of experimental pig B, kept on a constant diet over 11 weeks

acidophilus to the total number of LAB in the intestines, therefore, appears to vary to some extent, even under practically constant external conditions. Large fluctuations of up to fifteenfold were found in the total LAB population over the experimental period (Fig. 1 & 2). For both pigs, the LAB and *L. acidophilus* (Fig. 3 & 4) populations showed a steady decline during the first 3 weeks, and thereafter a gradual increase up to the 9th week, when the highest populations were recorded. In spite of the fact that the overall tendencies found for pigs A and B were comparable, exact similarities in population shifts could not be established.

These findings have definite implications for the application of *L. acidophilus* biotherapy to pigs and other animals. Despite definite proofs of beneficial actions *in vivo* (Gilliland & Speck, 1977; Pollmann, Danielson & Peo, 1980; Rigby & Petit, 1980; Soerjadi, Rufner, Snoeyenbos, Weinack & Smyser, 1981; Klupsch, 1985; Moyen, Bonneville & Fauchére, 1986) some controversy still exists. The mechanism of action and colonisation by these strains is not yet completely understood (Lauer *et al.*, 1980). Assuming the intestinal tract to be a chemostat, it is evident that, amongst other factors, the volume and type of diet will determine the composition and numbers of the microbial population.

These studies have emphasized the complexity of the digestive tract as an ecosystem. Before biotherapy can be scientifically applied, more studies will be necessary on intestinal bacteria. Special attention should be given to their *in vivo* survival, colonisation and implicit relationships in the intestinal tract.

ACKNOWLEDGEMENT

Financial support for this project by the CSIR and University of Pretoria is gratefully acknowledged.

REFERENCES

BAREFOOT, T. & KLAENHAMMER, T., 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. *Applied and Environmental Microbiology*, 45, 1808–1815.

BARROW, P. A., BROOKER, B. E., FULLER, R. & NEWPORT, J. J., 1980. The attachment of bacteria to the gastric epithelium of the pig and its importance in the microbiology of the intestine. *Journal of Applied Bacteriology*, 48, 147–154.

BRIGGS, M., 1953. The classification of lactobacilli by means of physiological tests. *Journal of General Microbiology*, 9, 234–248.

DE MAN, J. D., ROGOSA, M. & SHARPE, M. E., 1960. A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology*, 23, 130–135.

GILLILAND, S. E., 1979. Beneficial interrelationships between certain microorganisms and humans: candidate micro-organisms for use as dietary adjuncts. *Journal of Food Protection*, 42, 164–167.

GILLILAND, S. E. & SPECK, M. L., 1977. Antagonistic action of *Lactobacillus acidophilus* towards intestinal and food-borne phatogens in associative cultures. *Journal of Food Protection*, 40, 820–823.

JOHNSON, J. L., PHELPS, C. F., CUMMINS, C. S., LONDON, J. & GASSER, F., 1980. The taxonomy of the *Lactobacillus acidophilus* group. *International Journal of Systematic Bacteriology*, 30, 53–68.

JOHNSON, M., RAY, B. & SPECK, M. L., 1984. Freeze-injury of cell wall and its repair in *Lactobacillus acidophilus*. *Cryoletters*, 5, 171–176.

KLUPSCH, H. J., 1985. Man and microflora. *South African Journal of Dairy Technology*, 17, 153–156.

LAUER, E., HELMING, C. & KANDLER, O., 1980. Heterogeneity of the species *Lactobacillus acidophilus* (Moro) Hansen & Moquot as revealed by biochemical characteristics and DNA-DNA hybridization. *Zentralblatt für Bakteriologie und Hygiene. I. Abteilung Originale*, C 1, 150–168.

MÄYRÄ-MÄKINEN, A., MANNINEN, M. & GYLLENBERG, H., 1983. The adherence of lactic acid bacteria to the columnar epithelial cells of pigs and calves. *Journal for Applied Bacteriology*, 55, 241–245.

MITSUOKA, T., 1969. Vergleichende Untersuchungen über die Laktobazillen aus den Faeces von Menschen, Schweinen und Hühnern. *Zentralblatt für Bakteriologie und Hygiene. I. Abteilung Originale*, 210, 32–51.

MITSUOKA, T., SEGA, T. & YAMAMOTO, S., 1964. Eine verbesserte Methodik der qualitativen und quantitativen Analyse der Darmflora von Menschen und Tieren. *Zentralblatt für Bakteriologie und Hygiene. I. Abteilung Originale*, 195, 455.

MOYEN, E. N., BONNEVILLE, F. & FAUCHÈRE, J. L., 1986. Modification par l'erythromycine et un extrait de *Lactobacillus acidophilus* de la colonisation de l'intestin et de la translocation de *Campylobacter jejuni* chez la sours axenique. *Annales Institut Pasteur/Microbiologie*, V1/137A, 199–207.

OCHI, Y. & MITSUOKA, T., 1958. Studies on lactobacilli. *Japanese Journal of Veterinary Science*, 20, 71.

POLLMANN, D. S., DANIELSON, D. M. & PEO, E. R. Jr., 1980. Effects of microbial feed additives on performance of starter and growing finishing pigs. *Journal of Animal Science*, 51, 577–581.

REUTER, G., 1964. Vergleichsuntersuchungen an 90 thermophilen Laktobazillen-Stämmen verschiedener Herkunft. *Zentralblatt für Bakteriologie und Hygiene. I. Abteilung Originale*, 193, 454.

RIGBY, C. E. & PETIT, J. R., 1980. Observations on competitive exclusion for preventing *Salmonella typhimurium* infection of broiler chickens. *Avian Diseases*, 24, 604–615.

ROBINSON, I. M., ALLISON, M. J. & BUCKLIN, J. A., 1981. Characterization of the fecal bacteria of normal pigs. *Applied and Environmental Microbiology*, 41, 950–955.

ROGOSA, M., MITCHELL, J. A. & WISEMAN, R. F., 1951. A selective medium for the isolation of oral and fecal lactobacilli. *Journal of Bacteriology*, 62, 132.

RUSSELL, E. G., 1979. Types and distribution of anaerobic bacteria in the large intestine of pigs. *Applied and Environmental Microbiology*, 37, 187–193.

SALANITRO, J. P., BLAKE, I. G. & MUIRHEAD, P. A., 1977. Isolation and identification of fecal bacteria from adult swine. *Applied and Environmental Microbiology*, 33, 79–84.

SOERJADI, A. S., RUFNER, R., SNOEYENBOS, G. H., WEINACK, O. M. & SMYSER, C. F., 1981. The influence of lactobacilli on the competitive exclusion of paratyphoid salmonellae in chickens. *Avian Diseases*, 25, 1027–1033.

SHAHANI, K. M., 1977. Natural antibiotic activity of *Lactobacillus acidophilus* and *L. bulgaricus*. II. Isolation of acidophilin from *L. acidophilus*. *Journal of Cultured Dairy Products*, 12, 8.

VERVAEKE, I. J. & VAN NEVEL, C. J., 1972. Comparison of three techniques for the total count of anaerobes from intestinal contents of pigs. *Applied Microbiology*, 24, 513–515.

VERVAEKE, I. J., VAN NEVEL, C. J., DECUYPERE, J. A. & VAN ASSHE, P. F., 1973. A comparison of two methods for obtaining anaerobic counts in different segments of the gastrointestinal tract of piglets. *Journal of Applied Bacteriology*, 36, 397–405.