

Lactobacilli and Bacteremia in Southern Finland, 1989–1992

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In order to assess the potential of lactobacilli to cause serious infections, we studied the prevalence of bacteremia due to *Lactobacillus* species during a 4-year period (1989–1992) in southern Finland, which has a population of about 2.5 million. Among 3,317 blood culture isolates, lactobacilli were identified in eight patients, five of whom had a severe disease predisposing to bacteremic complications. The eight strains isolated were identified to the species level and typed by carbohydrate fermentation tests and by direct sequencing of enzymatically amplified 16S rRNA. The results did not provide evidence that any particular species or subspecies of *Lactobacillus* was the cause of the infections; no infections caused by isolates similar to the recently introduced dairy probiotic strain, *Lactobacillus* GG (ATCC 53103), were observed. The data show an infrequent association of lactobacilli with bacteremic infections in spite of the ubiquitous presence of these organisms in the gastrointestinal tract and their widespread consumption in fermented milks; thus, there is strong evidence that their pathogenic potential is very low.

Lactic acid bacteria are generally considered commensal microorganisms with no pathogenic potential. Their ubiquitous presence in the gastrointestinal tract and their traditional use in fermented food and dairy products, without significant problems, attest to their lack of pathogenicity. Recent reviews and case reports [1, 2] have called attention to episodes of human bacteremic infections that have been associated with lactic acid bacteria. The fact that these infections were usually seen in patients with underlying diseases known to predispose to bacteremic complications underscores the low virulence of these bacteria. Furthermore, it is believed that these infections were derived from the patients' own gastrointestinal microflora rather than from food and dairy products, but direct proof is lacking.

Lactobacilli are consumed in large amounts in fermented dairy products and cheese. The use of these items is especially widespread in Finland, where the annual per capita consumption of fermented milk products is ~36 kg. *Lactobacillus* strain GG, which is of human origin, is a relatively new probiotic strain intended for use in dairy foods and special dietary products. To assess the safety of its use, we have carried out a series of experiments in animal models [3–5]. No cases of bacteremia caused by lactobacilli were reported in a study with lethally irradiated mice, although several other microorganisms from the intestinal tract caused bacteremia and, subsequently,

death [4]. Clinical studies of oral intake of freeze-dried *Lactobacillus* GG or *Lactobacillus* GG-fermented dairy products, which involved both adults and infants who had diarrhea, Crohn's disease, or rheumatoid arthritis or were immunocompromised, have been conducted; no adverse effects were noted [6, 7]. Indeed, these studies indicated that oral intake of *Lactobacillus* strain GG, either in the form of fermented dairy products or as a freeze-dried powder at doses of 10^{10} or 10^{11} cfu/d was both safe and beneficial.

The ability of *Lactobacillus* GG to survive in the intestinal canal and to colonize the gastrointestinal tract has been shown for both adults [8, 9] and children [7]. On the basis of demonstrated safety, *Lactobacillus* GG dairy products were placed on the market in Finland in 1990, and the amount consumed annually rapidly rose to ~3 million kg. This trend has provided an opportunity to obtain further data on the safety of *Lactobacillus* GG through epidemiological surveillance.

Cases and Isolates

All blood culture isolates from southern Finland, which has a population of ~2.5 million, are collected at the Department of Bacteriology and Immunology, University of Helsinki (Helsinki). During the years 1989–1992, 3,317 isolates associated with bacteremia were detected. Strains initially identified as lactobacilli on the basis of typical morphology on gram staining, a negative catalase reaction, and frequent resistance to vancomycin were isolated from 11 patients. These strains were stored at -70°C until 1993, when they were recoded and blinded, cultured on MRS agar (LAB M, Bury, UK), and characterized by their carbohydrate fermentation pattern (API 50 CHL; bioMérieux, Marcy l'Etoile, France).

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Table 1. Cases of bacteremic lactobacillus infections in southern Finland, 1989–1992, based on detailed identification of the isolated strains.

Isolate no.	Year of isolation	Sex/age (y) of patient	Diagnosis	Strain identification by API 50 CHL*	16S rRNA sequence clustering†
1	1990	M/76	Infection of aortic aneurysm graft	<i>L. rhamnosus</i> [‡]	<i>L. rhamnosus</i> (ATCC 7469 ^T)
2	1992	M/78	Infection of abdominal aortic graft	<i>L. rhamnosus</i> [‡]	<i>L. casei</i> (ATCC 393 ^T)
3	1990	M/63	Carcinoma with liver metastasis [§]	<i>L. rhamnosus</i> [‡]	<i>L. rhamnosus</i> (ATCC 7469 ^T)
4	1992	M/59	NA [§]	<i>L. paracasei paracasei</i>	<i>L. paracasei</i> subspecies <i>paracasei</i> (ATCC 4646)
5	1990	M/70	Fever of unknown origin, urinary complaints	<i>L. rhamnosus</i> [‡]	<i>L. rhamnosus</i> (ATCC 7469 ^T)
6	1992	M/57	Respiratory infection (kidney transplant recipient)	<i>L. paracasei paracasei</i>	<i>L. paracasei</i> subspecies <i>paracasei</i> (ATCC 4646)
7	1989	F/33	Salpingitis	<i>L. curvatus</i>	<i>L. casei</i> (ATCC 393 ^T)
8	1991	M/55	Pneumonia (liver transplant recipient)	<i>L. fermentum</i>	Not in <i>L. casei</i> , <i>L. paracasei</i> , or <i>L. rhamnosus</i> cluster

NOTE. ATCC = American Type Culture Collection; NA = not available; ^T = type strain.

* Interpreted according to APILAB Plus V 4.0 (bioMérieux, Marcy l'Etoile, France).

† V1 and V2 region sequences; there was 99% similarity within a cluster.

‡ Equivocal identification (unacceptable or doubtful profile according to APILAB Plus V 4.0).

§ Patient died.

|| Unequivocal identification (good, very good, or excellent profile according to APILAB Plus V 4.0).

One of the strains did not grow on MRS agar and was thus not identified as *Lactobacillus*. One strain was identified as *Leuconostoc mesenteroides* on the basis of its carbohydrate fermentation pattern, and another strain was identified as *Carnobacterium divergens*. Thus, our analysis is based on eight confirmed cases of lactobacillus bacteremia. Three of the patients were older than 65 years, and five had a severe underlying condition (organ transplant with immunosuppressive treatment or metastatic cancer) (table 1).

Results

In order to identify the lactobacilli to the species level, their ability to ferment 50 carbohydrate substrates was tested. The fermentation pattern of each strain was unique, indicating that the infections were not related to a common source. One of the strains was unequivocally identified as *Lactobacillus curvatus* (strain 7), and the other was identified as *Lactobacillus fermentum* (strain 8), whereas the fermentation patterns of the remaining six strains (all apparently belonging to species of *Lactobacillus casei*, *Lactobacillus paracasei*, or *Lactobacillus rhamnosus*) differed from each other and differed in terms of the typical characteristics of the species (table 1).

Lactobacillus strain GG (ATCC [American Type Culture Collection] 53103) was analyzed together with the clinical isolates. The organism's fermentation pattern resulted in an unsatisfactory identification as *L. rhamnosus*. It differed from most

of the clinical isolates on the basis of several reactions, while two clinical isolates (strains 3 and 5) were closer in relatedness, differing only in one or two reactions.

As a further test of relatedness between the clinical isolates and *Lactobacillus* strain GG, the 16S rRNA was analyzed by determining the nucleotide sequence of its variable regions V1 and V2 after amplification of the 1.5-kb fragment of DNA by means of PCR [10, 11]. As a reference, type strains from the species *L. casei*, *L. rhamnosus*, and one strain of *L. paracasei* subspecies *paracasei* were included in the analysis. Three clusters were found among the eight clinical strains, with a 99%–100% similarity within each cluster; seven of the strains clustered with one of the three reference strains (table 1). Thus, isolates 1, 3, and 5, as well as *Lactobacillus* strain GG, clustered with *L. rhamnosus* (ATCC 7469^T); isolates 2 and 7 clustered with *L. casei* (ATCC 393^T); and isolates 4 and 6 clustered with *L. paracasei* subspecies *paracasei* (ATCC 4646). It is of interest that strain 7, identified as *L. curvatus* by the API system, was found in this analysis to be a member of the *L. casei* (ATCC 393^T) cluster. The remaining isolate (strain 8), identified by the API system as *L. fermentum*, differed from all three clusters in terms of its 16S rRNA gene sequences.

Conclusion

The published cases of bacteremia and endocarditis caused by lactic acid bacteria have been thoroughly reviewed by Gas-

ser [2]. In 89% of the cases of endocarditis due to *Lactobacillus* species, the patient had a serious underlying structural heart condition (rheumatic valvular disease, a valvular prosthesis or other cardiac surgical procedures, a congenital malformation, or prior episodes of endocarditis due to other bacteria). The mean frequency with which lactobacilli were isolated from patients with bloodstream infections in France was 0.101% during the years 1988–1990 [2], which is a little lower than our finding of 0.241% but underscores the low virulence of lactobacilli.

Annually, the amount of fermented milk products (buttermilk fermented with *Lactococcus* and *Leuconostoc* strains and yogurt fermented with *Streptococcus thermophilus* and *Lactobacillus bulgaricus* strains) consumed in Finland is about 180 million kg. The amount of *Lactobacillus* GG-containing products consumed was ~3 million kg in 1992, resulting in an estimate (based on daily consumption of the products) that a minimum of 40,000 persons had ingested the strain.

The data we have presented identify *Lactobacillus* strain GG as a strain of *L. rhamnosus* despite its atypical fermentation pattern. During the 4 years covered by the study, which involved 3,317 blood culture isolates, *L. rhamnosus* strains were found in only three patients, and none of these strains was identical to *Lactobacillus* strain GG. In the subsequent years 1993 and 1994, six of 1,875 blood culture isolates studied in the Department of Bacteriology were found to be *Lactobacillus*. As noted previously, most of the patients had severe underlying conditions. Detailed identification by API 50 CHL was obtained for only two strains; these organisms were identified as *Lactobacillus acidophilus* and *Lactococcus raffinolactis*. None of the isolates was similar to *Lactobacillus* strain GG. By this time, the amount of *Lactobacillus* strain GG dairy products

consumed had risen to >3 million kg per year. The lack of bacteremic infections associated with this strain, in addition to the fact that no increase in lactobacillus bacteremia was observed from 1989 to 1994, provides strong evidence of the safety of *Lactobacillus* strain GG products.

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