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Inhibition of *Staphylococcus aureus* by the commensal bacteria of human milk

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ABSTRACT

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Aims: To study the bacterial diversity in expressed human milk with a focus on detecting bacteria with an antimicrobial activity against *Staphylococcus aureus*, known as a causative agent of maternal breast infections and neonatal infections.

Methods and Results: Random isolates ($n = 509$) were collected from breast milk samples ($n = 40$) of healthy lactating women, genotypically identified, and tested for antimicrobial activity against *Staph. aureus*. Commensal staphylococci (64%) and oral streptococci (30%), with *Staph. epidermidis*, *Strep. salivarius*, and *Strep. mitis* as the most frequent isolates, were the predominant bacterial species in breast milk. One-fifth of *Staph. epidermidis* and half of *Strep. salivarius* isolates suppressed growth of *Staph. aureus*. Enterococci (*Ent. faecalis*), isolated from 7·5% of samples, and lactic acid bacteria (LAB) (*Lactobacillus rhamnosus*, *Lact. crispatus*, *Lactococcus lactis*, *Leuconoctoc mesenteroides*), isolated from 12·5% of samples, were also effective against *Staph. aureus*. One *L. lactis* isolate was shown to produce nisin, a bacteriocin used in food industry to prevent bacterial pathogens and spoilage.

Conclusions: Expressed breast milk contains commensal bacteria, which inhibit *Staph. aureus*.

Significance and Impact of the Study: The strains inhibitory against the pathogen *Staph. aureus* have potential use as bacteriotherapeutic agents in preventing neonatal and maternal breast infections caused by this bacterium.

Keywords: commensal bacteria, human milk, *Staphylococcus aureus*, antimicrobial activity.

INTRODUCTION

Human milk is generally accepted to be the best nutrition for neonates. Breast milk contains the needed nutrients and antibacterial and antiviral factors protecting the infant against infections (May 1994). Expressed human milk contains commensal bacteria, mainly non-pathogenic coagulase-negative staphylococci from the bacterial flora of the maternal skin (Caroll *et al.* 1979; Eidelman and Szilagyi 1979; El-Mohandes *et al.* 1993b). However, transmission of pathogens, such as *Staphylococcus aureus* and *Streptococcus agalactiae*, has also been reported (Bingen *et al.* 1992; Novak *et al.* 2000; Le Thomas *et al.* 2001). The published reports on the bacteria in human milk have so far focused on pathogenic bacteria. These are important for hospitals gathering breast milk for milk banks. The knowledge of human milk commensal bacteria is limited.

Recently, the interest in human normal bacterial flora has resumed. Bacterial interference, 'bacteriotheraphy', where commensal bacteria are used to prevent colonization of the host by pathogens, offers a promising alternative for combating infections. It has proven to be useful in preventing recurrent streptococcal tonsillitis in children, otitis media, ulcerative colitis, urinary tract and gastrointestinal infections, respiratory infections and diarrhoea (Strauss 2000; Huovinen 2001; Reid *et al.* 2001). Commensal bacteria compete for nutrients and host-cell binding sites and kill pathogens by producing acid, hydrogen peroxide or bacteriocins (Huovinen 2001). Bacteriocins are bacterially produced antimicrobial peptides, which are being researched

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for their potential use as food preservatives (Cleveland *et al.* 2001). Nisin, a bacteriocin produced by *Lactococcus lactis*, is approved as a food preservative (E234) in over 50 countries (Delves-Broughton *et al.* 1996).

Staph. aureus is known as a food-poisoning agent as well as a common cause of infections, such as serious antibiotic-resistant hospital infections (Farr *et al.* 2001). New evidence indicates the involvement of *Staph. aureus* in sudden infant death syndrome (SIDS) (Zorgani *et al.* 1999), which is the predominant cause of death between 1 week and 1 year of life in industrialized countries (Blackwell and Weir 1999). *Staph. aureus* is also the main causative agent in infectious mastitis affecting 20–30% of lactating women (Jonsson and Pulkkinen 1994; Semba *et al.* 1999).

We explored the commensal bacterial composition of expressed breast milk with an emphasis on finding bacterial strains having antimicrobial activity against *Staph. aureus*.

METHODS

Collection of samples

Breast milk samples ($n = 40$) from healthy lactating volunteers were collected via two child welfare clinics in Finland. Thirty samples (75%) were taken within 90 days of delivery and 10 (25%) samples after 90–421 days. The donors were requested to clean their hands and the breasts with warm water, and then collect milk into sterile test tubes by manual expression or using a clean breast pump. The samples were stored in domestic freezers until collected.

Bacterial strains

A total of 509 isolates were obtained by plating 100 μl of each sample on non-selective MRS agar plates (Becton Dickinson Microbiology Systems, Cockeysville, MD) and incubating anaerobically at 37°C for 24–48 h. Anaerobic conditions and MRS medium were used to support growth of lactic acid bacteria (LAB), which are nutritionally fastidious anaerobes. Colonies (10–21) were randomly picked from each plate and purified on MRS, Todd-Hewitt (Oxoid Ltd., Basingstoke, England) or M17 agar plates (Oxoid Ltd., Basingstoke, England) containing 0·1% (w/v) of glucose. M17G or Todd-Hewitt generally produced better growth of the isolates of streptococci, lactococci, and enterococci than MRS. Isolates of lactobacilli and staphylococci grew well on MRS agar plates. Samples were plated on lactobacilli-selective LBS agar plates (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and grown at 37°C under anaerobic conditions for 48 h. The strains were stored at −70°C in the respective growth media supplemented with 30% (v/v) of glycerol (Merck KgaA, Darmstadt, Germany).

The reference strains *Staphylococcus aureus* subsp. *aureus* HAMBI 66, equal to ATCC 12600 (type strain), and *Listeria innocua* HAMBI 2316, equal to ATCC 51742, were obtained from the HAMBI collection (Department of Applied Chemistry and Microbiology, University of Helsinki, Helsinki, Finland), *Micrococcus luteus* AL NCIMB 8166, equal to ATCC 10240 from the National Collection of Industrial and Marine Bacteria (Aberdeen, UK), and *Lactobacillus rhamnosus* GG from Valio Ltd (Helsinki, Finland).

Identification of bacteria

Staphylococci-specific PCR amplification of glyceraldehyde-3-phosphate dehydrogenase (*gap*)-encoding gene followed by restriction fragment length polymorphism (RFLP) analysis was applied to distinguish and identify staphylococci from other catalase positive cocci (Yugueros *et al.* 2000, 2001). Catalase positive cocci, which gave no product in staphylococci-specific *gap*-PCR or had an unclear RFLP pattern, and all catalase negative isolates were identified by partial sequencing of 16S ribosomal RNA gene as described by Edwards *et al.* (1989). A 920 bp fragment of 16S RNA gene was amplified using primers pA 5′ AGA GTT TGA TCC TGG CTC AG 3′ and pE′ 5′ CCG TCA ATT CCT TTG AGT TT 3′. Standard procedures were used for extracting chromosomal DNA, for agarose gel electrophoresis, and for PCR amplification of DNA fragments (Sambrook *et al.* 1989). PCR fragments were sequenced at the sequencing service of the Helsinki University Institute of Biotechnology using pA forward primer. BLAST sequence alignment program of National Center for Biotechnology Information was used for the identification of bacterial species (http://www.ncbi.nlm.nih.gov/BLAST/). The isolates were named according to the species producing the best significant alignment scores. *Lact. rhamnosus* strains were profiled by a random amplified polymorphic DNA (RAPD) analysis (Williams *et al.* 1990) using oligonucleotide primers able to distinguish *Lact. rhamnosus* GG from other *Lact. rhamnosus* strains (T. Halme, A. Suoniemi and S. Tynkkynen, unpublished).

Detection of antimicrobial activity

Antimicrobial activity of breast milk colonies against *Staph. aureus* was detected by the modified sandwich overlay method as described (Rodriguez *et al.* 2000). Appropriate dilutions of milk samples were plated on MRS medium (Becton Dickinson Microbiology Systems) and incubated at 37°C in anaerobic conditions. After 48 h, the colonies on the plates were counted and overlaid with 5 ml of Luria Bertani (LB) 0·75% agar medium seeded with 0·2 ml of an overnight culture of *Staph. aureus* ATCC 12600. After overnight incubation at 37°C, the colonies surrounded by

inhibition zones were counted. Colonies with largest inhibition zones were isolated.

Antimicrobial activity of isolated bacterial strains was evaluated by modification of the 'spot on the lawn' method described by Navarro *et al.* (2000). MRS, Todd-Hewitt (Oxoid LTD., Basingstoke, England), or M17G agar plates were spotted with 2 μl of an overnight culture of the tested isolate, grown aerobically or anaerobically at 37°C for 24–48 h and then overlaid with the indicator organism (*Staph. aureus* ATCC 12600). Colonies surrounded by inhibition zones were observed after overnight incubation at 37°C.

Nisin bioassay

Nisin production of *L. lactis* isolates was detected by a green fluorescent protein (GFP)-based microplate bioassay (Reunanen, J.O.K. and Saris, P.E.J., unpublished). The method was modified from that of Wahlström and Saris (1999) by using a red-shifted P11 mutant of the *gfp* gene (Heim *et al.* 1994), derived from plasmid pKPSPgfp (*Sac* I) (Scott *et al.* 1998) as a reporter gene instead of the bioluminescence *luxHb* gene. Both assays are based on nisin induction of the *nisF* promoter connected to a reporter gene. Nisin concentration is signaled via the two-component regulatory system composed of NisK (histidine kinase sensing nisin) and NisR (transcriptional activator). Specificity of the assay is based on the specificity of the NisK to nisin; even the structurally most similar peptide subtilin is not sensed by NisK (Wahlström and Saris, 1999).

For the nisin bioassay, 50 ml of M17G medium was inoculated with an overnight culture of a human milk isolate and allowed to grow to the late exponential growth phase ($O.D._{600\ nm}$ was 0·5). Pasteurized culture supernatant was diluted (1 : 1, 1 : 2, 1 : 4,…, 1 : 256) with 0·1% Tween 80 (adjusted to pH 2·5 with HCl) and dilutions were mixed with indicator bacteria carrying the plasmid where the *gfp* gene is placed under control of the P_{nisF} promoter (Reunanen, J.O.K. and Saris, P.E.J., unpublished). Different dilutions were divided (several replicates) to a microtitre plate and allowed to grow for overnight at 30°C without aeration. The fluorescence (excitation 485 nm, emission 538 nm) was detected as relative fluorescence units (RFUs) by using Fluoroskan Ascent 374 scanning fluorometer (Labsystems, Helsinki, Finland) connected to a computer using Ascent version 1·2 software (Labsystems).

RESULTS

Diversity of bacteria in expressed human milk

A total of 509 anaerobic or facultatively anaerobic random colonies were isolated from 40 samples of breast milk (10–21 isolates per sample) plated on MRS agar and grown under anaerobic conditions. The isolates were identified by partial sequencing of the 16S rRNA gene or by *gap*-RFLP method (Table 1).

The most abundant bacteria, staphylococci, were obtained from 39 milk samples representing 64% of the total colonies on MRS agar (Table 1). In seven samples, staphylococci were the only bacteria found. *Staph. epidermidis* was isolated from 39 samples. Other coagulase-negative staphylococci, such as *Staph. hominis*, *Staph. capitis* and *Staph. lugdunens*, were occasionally isolated (Table 1). Coagulase-positive *Staph. aureus* was isolated from five samples.

Streptococci were the second most abundant, found in 29 samples, representing 30% of the total bacteria (Table 1). Oral streptococci, *Strep. salivarius* and *Strep. mitis* were the most frequent isolates, obtained from 18 and 11 samples, respectively. Other oral species, *Strep. parasanguis*, *Strep. peroris*, and *Strep. oralis* were occasionally found (Table 1). The partial sequences of 16S rRNA gene used for identification of the isolates were 500–800 bp in length. We assumed that strains having minimum 97% similarity in the sequence represented the same species (Zoetendal *et al.* 1998; Favier *et al.* 2002). Isolates with less than 97% similarity to sequences in the database were named to genus level only. Sequences of 28 isolates derived from nine samples were identical to unidentified oral streptococcal isolates in the database. These are named as *Streptococcus* sp. (oral) in Table 1. *Strep. agalactiae*, a pathogenic β-haemolytic streptococcus, was isolated from one sample, where it represented 43% of all isolates.

LAB were present in five samples (Table 1). *Lact. crispatus* was isolated from a sample collected 14 days after delivery and *Lact. rhamnosus* samples taken after 96 and 188 days of delivery. *Lact. rhamnosus* isolates were analysed by RAPD profiling and found identical to the commercial dairy strain *Lact. rhamnosus* GG (data not shown). The other LAB isolates, *L. lactis* and *Leuc. mesenteroides*, originating from milk samples of 76 and 34 days after delivery, respectively, were isolated from MRS based on their antagonistic effect against *Staph. aureus*.

Ent. faecalis, found in three samples was the predominant bacterial species in two of them, representing 60 and 93% of all isolates.

Inhibitory activity of the breast milk colonies against *Staph. aureus*

The inhibitory activity of breast milk (anaerobic or facultatively anaerobic) bacterial colonies against *Staph. aureus* was tested on MRS agar plates. The anaerobic colony count in the sampled breast milk was generally less than 1×10^4 ml^{-1}. The median colony count was $5{\cdot}6 \times 10^2$ CFU ml^{-1} and 55% of the samples had colony

Table 1 Identification of isolates from samples ($n = 40$) of expressed human milk

Species*	Number of isolates (%)	Occurrence in milk samples
Staphylococcus		
Staph. aureus	9 (1·8)	5
Staph. capitis	15 (2·9)	8
Staph. epidermidis	255 (50)	39
Staph. hominis	16 (3·1)	11
Staph. lugdunens	2 (0·4)	2
Staphylococcus sp.	30 (5·9)	19
Total	327 (64)	39
Streptococcus		
Strep. agalactiae	6 (1·2)	1
Strep. mitis	30 (5·9)	11
Strep. oralis	1 (0·2)	1
Strep. parasanguis	21 (4·1)	5
Strep. peroris	3 (0·6)	3
Strep. salivarius	62 (12)	18
Streptococcus sp. (oral)	28 (5·5)	9
Total	151 (30)	29
Lactic acid bacteria		
Lactobacillus rhamnosus	5 (0·9)	2
Lactobacillus. crispatus†	2 (–)	1
Lactococcus lactis‡	1 (–)	1
Leuconostoc mesenteroides‡	1 (–)	1
Total	9	5
Enterococcus		
Ent. faecalis	21 (4·1)	3
Enterococcus sp.	1 (0·2)	1
Total	22 (4·3)	3
Other		
Actinomyces odontolyticus	1 (0·2)	1
Rothia mucilaginosus	1 (0·2)	1
Yeasts	2 (0·4)	1
Total	509	40

*Isolated from MRS unless otherwise indicated.
†Isolated from LBS.
‡Selected by detected inhibitory activity against *Staph. aureus* ATCC12600.

counts between 1×10^2 and 1×10^3 CFU ml^{-1}. After incubation, plates were overlaid with the test organism.

Colonies exhibiting inhibitory activity against *Staph. aureus* were found in 36 samples out of 40 (Table 2). Most of the inhibitory colonies could only partly inhibit the growth of *Staph. aureus* and produced opaque zones of inhibition (Fig. 1). These were found in 35 samples and they represented 9·3–100% of all colonies obtained from one sample (Table 2). In addition, 16 milk samples exhibited colonies producing clear zones of inhibition (divided in categories <1, 1–2 or >2 mm in Table 2) by completely preventing the growth of *Staph. aureus* near the colony (Fig. 1). Strains surrounded by largest clear inhibition zones were isolated and identified as *L. lactis*, *Leuc. mesenteroides*, *Lact. rhamnosus*, *Ent. faecalis*, *Strep. salivarius*, and *Staph. epidermidis* species.

Inhibitory activity of the bacterial isolates against *Staph. aureus*

Seven percent of the tested isolates ($n = 443$) produced a clear zone of inhibition against *Staph. aureus*, whereas 22% of the isolates had a weak antimicrobial activity, visible as opaque zones of inhibition (Table 3).

All enterococcal isolates ($n = 22$) inhibited growth of *Staph. aureus* and 18% produced a clear zone. About 41% of *Strep. salivarius* isolates ($n = 48$) and 23% of the other streptococci ($n = 57$) were effective against *Staph. aureus*. Of the *Staph. epidermidis* isolates ($n = 255$), 23% inhibited the growth of *Staph. aureus*, but only 4% exhibited a clear zone of inhibition. *Lact. rhamnosus* isolates ($n = 5$) and *Lact. crispatus* ($n = 2$) isolates were all active against *Staph. aureus*.

Detection of nisin production by a *L. lactis* isolate

One *L. lactis* strain, referred to LACMH27, originating from MRS plates, showed strong inhibitory activity against *Staph. aureus* (ATCC 12600) and exhibited also clear inhibition zones with indicator strains of *L. innocua* (ATCC

Table 2 Inhibitory activity of human milk bacterial colonies against *S. aureus* ATCC 12600

Intensity of growth inhibition against *Staph. aureus**	Occurrence of inhibitory colonies in human milk samples ($n = 40$)	Percent inhibitory colonies of all colonies obtained from one sample; average (range)
Opaque	35	39 (9·3–100)
Width of the clear zone around the colony		
<1 mm	15	6·7 (0·6–22)
1–2 mm	2	3·6 (3·5–3·6)
≥2 mm	1	40
No inhibition	4	

*Shown in Fig. 1.

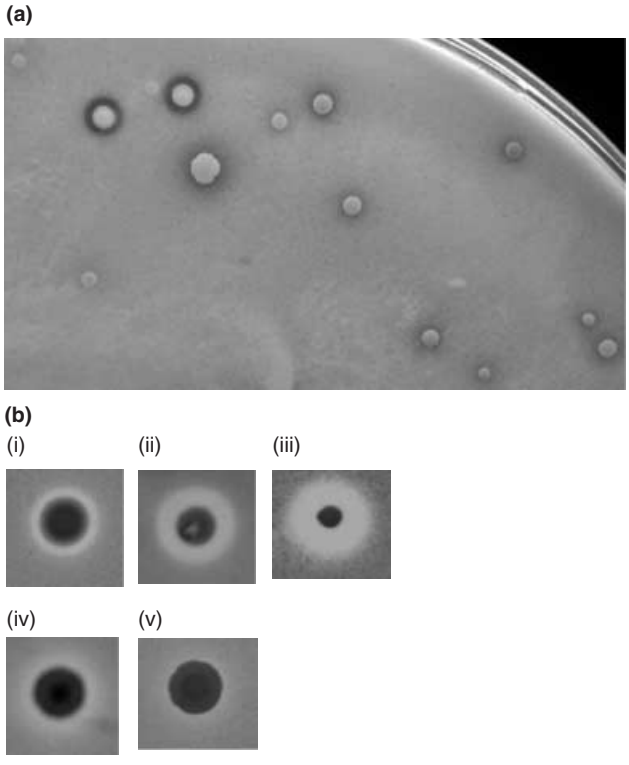

Fig. 1 (a) Breast milk colonies displaying inhibitory activity against the test organism *Staph. aureus* grown as a lawn on MRS agar. (b) Colonies representing clear zones (i) <1 mm; (ii) 1–2 mm; (iii) ≥2 mm and (iv) an opaque zone of inhibition and (v) no inhibition against *Staph. aureus*

Table 3 Inhibitory activity of breast milk isolates against *Staph. aureus* ATCC 12600

		Zone of inhibition, *n* (%)	
Isolates* identified as	*n*	Opaque	Clear
Staph. epidermidis	255	47 (18)	11 (4·3)
Other staphylococci	54	9 (17)	None
Strep. salivarius	48	16 (33)	4 (8·3)
Other streptococci	57	9 (16)	4 (7)
Enterococci	22	18 (82)	4 (18)
Lact. rhamnosus	5	None	5 (100)
Lact. crispatus†	2	None	2 (100)
Total	443	99 (22)	30 (7)

*Isolated from MRS unless otherwise indicated.
†Isolated from LBS.

51742) and *M. luteus* (ATCC 10240). As many *L. lactis* strains produce the broad spectrum bacteriocin nisin (Hurst 1981) strain LACMH27 was tested for potential nisin production using the nisin-specific GFP-based microplate bioassay. The culture supernatant diluted 1 : 128 of strain LACMH27 yielded 31·5 RFU, 5 IU ml^{-1} of nisin gave rise to 34·4 RFU and the background without nisin resulted in 19 RFU. The results represent the mean of two to four parallel samples. As the RFU of the culture supernatant of strain LACMH27 was higher than the background value, it was concluded that the strain had produced nisin into its growth medium, as only nisin can induce the indicator strain to produce GFP.

DISCUSSION

The published studies of breast milk microbiota are focused on pathogenic bacteria, as possible sources of infection (Caroll *et al.* 1979; Eidelman and Szilagyi 1979; Law *et al.* 1989; Bingen *et al.* 1992; El-Mohandes *et al.* 1993a; Wright and Feeny 1998; Novak *et al.* 2000; Le Thomas *et al.* 2001). The species diversity and the importance of the normal bacterial flora have received little attention so far. We explored the anaerobic and facultatively anaerobic bacterial diversity in expressed human milk of healthy lactating women and the ability of these commensal bacteria to interfere with the growth of *Staph. aureus*. The inhibitory activity of the breast milk isolates against *Staph. aureus* shown in this study is a novel finding. Four percent of the *Staph. epidermidis* isolates suppressed and 18% clearly diminished its growth. In addition, 41% of the *Strep. salivarius* isolates and 23% of the other representatives of viridans streptococci also showed anti-*Staph. aureus* activity. In a recent study, viridans group streptococci have been reported to inhibit colonization by methicillin-resistant *Staph. aureus* (MRSA) of oral cavities in infants (Uehara *et al.* 2001). The commensal bacteria in human milk may thus have a role in protecting the infant and mother against *Staph. aureus* infections.

Our results support the view that commensal staphylococci and streptococci are predominant bacterial species in breast milk regardless of whether cultured aerobically (Caroll *et al.* 1979; Eidelman and Szilagyi 1979; West *et al.* 1979) or anaerobically (this study). Staphylococci and streptococci represented 64 and 30% of the total bacterial isolates, respectively. Coagulase-negative staphylococci, of which *Staph. epidermidis* was the predominant species, have been reported frequent also in previous studies (Caroll *et al.* 1979; Eidelman and Szilagyi 1979; West *et al.* 1979; Pittard *et al.* 1985; Law *et al.* 1989; El-Mohandes *et al.* 1993b). These may have originated from the maternal skin during breastfeeding (West *et al.* 1979). In addition to previously reported *Staph. epidermidis* and *Staph. aureus*, we detected *Staph. hominis* and *Staph. capitis* as breast milk contaminants by using the recently described gap-RFLP method (Yugueros *et al.* 2000, 2001), and *Staph. lugdunens* by 16S rRNA sequencing.

Our study confirms the reports of viridans streptococci or α-haemolytic streptococci as common contaminants in

breast milk (Eidelman and Szilagyi 1979; West *et al.* 1979). These species may have transferred from infant's mouth to the breast and from there to the milk as they are not known as residents of human skin (West *et al.* 1979). There are no earlier reports on the diversity of oral species in breast milk. *Strep. salivarius* and *Strep. mitis*, shown to be frequent in breast milk in this work, are also known as prevalent species in the oral cavity of infants (Könönen 2000). *Strep. parasanguis*, *Strep. oralis* and *Strep. peroris* occasionally found in breast milk are also known as oral species (Kawamura *et al.* 1998). Interestingly, the prevalence of viridans streptococci in the intestine was reported as unique to healthy infants compared to atopic infants (Kirjavainen *et al.* 2001). *Actinomyces odontolyticus* and *Rothia* (*Stomatococcus*) spp., which we isolated from human milk, are known oral species in infant's mouths in age between 0 and 6 months (Könönen 2000).

Most of the detected staphylococci and streptococci are known as residents of the human normal bacterial flora, but also pathogenic species, *Staph. aureus* and *Strep. agalactiae* were isolated. *Staph. aureus*, found in five milk samples, has been reported as a rare contaminant in breast milk of healthy lactating women also in previous studies (Caroll *et al.* 1979; West *et al.* 1979; Law *et al.* 1989; El-Mohandes *et al.* 1993a). Our study confirms the previous reports, that contamination of breast milk with *Strep. agalactiae* may occur in a non-symptomatic mother (Atkins *et al.* 1998; Olver *et al.* 2000).

Consistent with earlier reports, enterococci were occasionally found in breast milk (Eidelman and Szilagyi 1979; Wright and Feeny 1998). We isolated *Ent. faecalis* from three milk samples out of 40, in two of which it was the predominant bacterial species. All enterococcal isolates ($n = 22$) inhibited, and 18% suppressed the growth of *Staph. aureus*.

Lact. plantarum has been isolated from breast milk (West *et al.* 1979) but the occurrence of other LAB seems not to have been explored. We found LAB in about 10% of breast milk samples. Lactobacilli, *Lact. crispatus* and *Lact. rhamnosus*, were the predominant LAB species, but *Lact. lactis* and *Leuc. mesenteroides* were also found.

Lact. crispatus was obtained from breast milk sampled 14 days after delivery. This species is the predominant vaginal lactobacillus in healthy women (Song *et al.* 1999). The infant may therefore have derived it from the vagina during the delivery and then transmitted it to the maternal breast skin during nursing. This kind of transmission cycle of group B streptococci (GBS) has been reported (Atkins *et al.* 1998). *Lact. rhamnosus* isolates from two samples had RAPD profile identical to the commercial strain *Lact. rhamnosus* GG, which is a commonly used probiotic strain in milk products in Finland.

One of the colonies having the largest zones of *Staph. aureus* inhibition was identified as *Lact. lactis*, and shown to produce a bacteriocin, nisin. This indicates that nisin type bacteriocins may play a role in the bacteriological homeostatis of breast milk. Also the breast milk isolates of *Lact. crispatus* and *Lact. rhamnosus* suppressed the growth of *Staph. aureus*. Since *Lact. rhamnosus* GG is known as a producer of antimicrobial substances (Silva *et al.* 1987), it may explain the observed antimicrobial activity of the *Lact. rhamnosus* isolates having an identical RAPD profile.

The numerical data in this study leads to the conclusion that an infant consuming about 800 ml breast milk per day will ingest about 8×10^4–8×10^6 commensal bacteria while suckling. Staphylococci and oral streptococci, especially *Staph. epidermidis* and *Strep. salivarius*, which were the predominant bacterial species in breast milk, have also been identified from stool samples of breast-fed infants (Millar *et al.* 1996; Kirjavainen *et al.* 2001; Favier *et al.* 2002). Thus, the bacterial composition of the infant faecal flora seems to reflect the bacterial composition of breast milk. Our results indicate that the commensal bacteria of breast milk may have a role in preventing growth of pathogens.

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