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Distribution of Genes Encoding the Trypsin-Dependent Lantibiotic Ruminococcin A among Bacteria Isolated from Human Fecal Microbiota

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Fourteen bacterial strains capable of producing a trypsin-dependent antimicrobial substance active against *Clostridium perfringens* were isolated from human fecal samples of various origins (from healthy adults and children, as well as from adults with chronic pouchitis). Identification of these strains showed that they belonged to *Ruminococcus gnavus*, *Clostridium nexile*, and *Ruminococcus hansenii* species or to new operational taxonomic units, all from the *Clostridium coccoides* phylogenetic group. In hybridization experiments with a probe specific for the structural gene encoding the trypsin-dependent lantibiotic ruminococcin A (RumA) produced by *R. gnavus*, seven strains gave a positive response. All of them harbored three highly conserved copies of *rumA*-like genes. The deduced peptide sequence was identical to or showed one amino acid difference from the hypothetical precursor of RumA. Our results indicate that the *rumA*-like genes have been disseminated among *R. gnavus* and phylogenetically related strains that can make up a significant part of the human fecal microbiota.

Up to 10^{14} bacteria are present in the human intestinal tract. The microbiota that reside in the colon are essentially anaerobic: *Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Fusobacterium*, *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus* have been reported to be the predominant genera of the large bowel (34). The involvement of these microbiota in the salvage of energy from nondigested dietary compounds can lead to both toxification and detoxification of metabolic compounds (13). These microbes also affect the immune system of the host (7). Another role is the protection of the host against invasion by potentially pathogenic microorganisms (5), referred to as colonization resistance. Numerous interbacterial antagonisms taking place within the gut have been reported. However, very few have been elucidated thus far at the molecular level.

The production of toxic metabolites, such as bacteriocins, has been shown to be involved in the colonization process of complex ecosystems and in colonization resistance phenomena. Many lactic acid bacteria can produce such compounds (16, 19), and it is well established that they play a crucial protective role in the dairy industry (39). The rat urinary tract (2) or nasopharyngeal region (21) and the human oral cavity (12) are other ecosystems in which bacteriocins could protect against bacterial infections.

In the recent years, a large number of molecules belonging to the different families of bacteriocins have been well characterized genetically and biochemically. Numerous bacterial strains capable of producing bacteriocins were isolated from mammalian digestive ecosystems (1, 3, 10, 17, 18, 20, 33). Thus, it has been suggested that the role of bacteriocins in the gut could be similar to the one they play in other complex ecosystems (28, 29).

Bacteriocins are proteinaceous, ribosome synthesized, antibacterial compounds. Among bacteriocins, the lantibiotic family is characterized by the presence of posttranslationally modified residues such as lanthionines, β-methyllanthionines, and didehydrated residues. Their production requires a complex machinery responsible for biosynthesis, posttranslational modification, export, self-immunity, and in some cases regulation. The genes encoding these functions are generally grouped in clusters according to similar organizational schemes (15, 32).

Ruminococcin A (RumA), a lantibiotic produced by a *Ruminococcus gnavus* strain from a human fecal sample, was recently characterized both biochemically (3) and genetically (9). The genes responsible for RumA biosynthesis were shown to be organized into three transcriptional units (synthesis and export, immunity, and regulation). Their regulation was shown to be dependent on the proteolytic activity of trypsin and is probably mediated by a two-component signal transduction system (9). The aims of the present study were to determine (i) whether the structural gene encoding RumA is present among bacteria of the dominant human fecal microbiota and (ii) whether *rumA*-positive strains belong to the *R. gnavus* species. We show here that the structural genes encoding RumA have been distributed among strains of *R. gnavus* and other phylogenetically related bacteria isolated from healthy children and

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TABLE 1. Identification of bacterial strains isolated from human fecal samples

Strain[a]	Origin[b]	G+C content (%)	% DNA-DNA homology with *R. gnavus*	Max 16S rDNA identity (%)/ reference strain	% DNA-DNA homology/reference strain[c]
LEMG25	HC	42	86		
LEMB04*	HC	39	39	99.4/*C. nexile*	81/*C. nexile*
LEMV95*	HC	41	82		
LEMV98*	HC	38	39	97.4/*R. hansenii*	90/*R. hansenii*
LEMV99*	HC	43	86		
LEMB86	HC	42	71		
LEMV61	HA	41	20	94/*E. formicigenerans*[d]	New OTU
E1**	HA	43	80		
LEMV62**	HA	42	87		
LEMV63**	HA	41	25	95/*R. torques*	New OTU
LEMV78	HA	43	20	95/*R. gnavus*	New OTU
LEMB53	CP	42	85		
LEMV50	CP	41	81		
LEMV58	CP	40	86		
LEMV66	CP	41	85		

[a] Strains isolated from the same fecal sample are marked with one asterisk. Strains isolated from the same volunteer but in fecal samples obtained after at least 1-year intervals are marked with two asterisks.
[b] Bacterial strains producing a trypsin-dependent anti-*C. perfringens* substance were isolated from human fecal samples of healthy children (HC), healthy adults (HA), or adults with chronic pouchitis (CP).
[c] Strains exhibiting a 16S rDNA identity <97% with any sequence of a cultivable organism present in the data bank were considered as a new OTU.
[d] *Eubacterium formicigenerans*.

adults, as well as from adults with chronic pouchitis. The dissemination of these genes within bacteria of the dominant fecal microbiota reinforces the hypothesis that RumA could be involved in bacterium-bacterium interactions that take place within the human gut.

MATERIALS AND METHODS

Bacterial strains and media. The following type strains were used in this study: *R. gnavus* ATCC 29149^T (American Type Culture Collection, Manassas, Va.), *Clostridium nexile* DSMZ 1787^T, and *R. hansenii* DSMZ 20583^T (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). *R. gnavus* strain E1 was been isolated from the predominant fecal microbiota of a healthy adult (27). The *C. perfringens* CpA strain (27) was used as the reference target strain. Other strains were isolated during this work (Table 1).

All strains were grown in an anaerobic cabinet in brain heart infusion broth supplemented with yeast extract and hemin (BHI-YH; BHI [Difco Laboratories, Detroit, Mich.] supplemented with 5 g of yeast extract [Difco Laboratories] and 5 mg of hemin [Sigma-Aldricht Chimie, St. Quentin Fallavier, France]/liter). When required, trypsin from bovine pancreas (type XIII; L-1-tosyl-amide phenylalanyl chloride treated; Sigma-Aldricht Chimie) was added to BHI broth or solid medium at a final concentration of 50 μg/ml.

Isolation of strains putatively producing a trypsin-dependent anti-*C. perfringens* substance. Immediately after collection, 1-g aliquots of the fecal samples were introduced into an anaerobic cabinet, 10-fold serially diluted with LCY broth (11), plated onto BHI-YH agar plates, and incubated for 48 h at 37°C. One hundred clones from a 10^{-9} or 10^{-8} dilution were placed by using a toothpick onto BHI-YH plates supplemented with trypsin and allowed to grow for 48 h. Then, 100 μl of culture of the sensitive indicator strain *C. perfringens* CpA (37°C, 16 to 24 h) was added to 10 ml of BHI-YH agar medium and overlaid onto the plates prior to an additional 24 h of incubation at 37°C. Fecal clones were considered positive when they were surrounded by a clearing zone reflecting growth inhibition of the CpA strain.

DNA extraction and manipulation. Bacterial chromosomal DNA was isolated by using a Nucleospin C+T kit (Machery-Nagel GmbH & Co., Düren, Germany). Restriction enzymes and T4 polynucleotide kinase were purchased from Gibco-BRL (Life Technologies SARL, Eragny, France) and MBI Fermentas (Vilnius, Lithuania) and used as recommended by the manufacturers. When necessary, DNA fragments were recovered from agarose gels by using a Nucleospin Extract kit (Machery-Nagel).

RAPD fingerprinting. Randomly amplified polymorphic DNA (RAPD) profiles were obtained according to a previously described protocol (35) with three different primers—OlB06 (5′-TGCTCTGCCC-3′), OlB07 (5′-GGTGACGCA G-3′), or OlB08 (5′-GTCCACACGG-3′)—in independent reactions. Amplification reactions were carried out in a GeneAmp PCR system 2400 (Perkin-Elmer-Cetus, Norwalk, Conn.) by using 20 to 100 ng of the bacterial chromosomal DNA as a template. The reaction mixture included 1.5 mmol of $MgCl_2$/liter, 2 μmol of primer/liter, 7.5 U of DNA polymerase (AmpliTaq; Appligène, Strasbourg, France), 200 μmol of each deoxynucleoside triphosphate/liter, and 10 mmol of Tris-HCl (pH 9.0)/liter in a 100-μl final volume. The PCR conditions used (30 cycles) included annealing at 36°C (2 min), polymerization at 72°C (2 min), and denaturation at 94°C (1 min).

Analysis and comparison of the RAPD profiles. Portions (20 μl) of the PCR amplification products were separated by electrophoresis in 1% Seakem GTG agarose (Tebu, France) gels in Tris-borate-EDTA alongside the 123-bp ladder from Gibco-BRL (Life Technologies SARL, Eragny, France). Each gel contained 10 lanes of PCR products and 3 lanes of ladder located at both sides and in the center. Ethidium bromide-stained gels were photographed under UV light by using Polaroid film type 665, and negative pictures were digitized by using a Hewlett-Packard Scanjet IIcx/T linked to a computer. For each strain, the three RAPD patterns were merged for analysis and comparison by using the GelCompare Programme (Applied Maths, Sint-Martens-Latem, Belgium) (40). This program allows (i) normalization of electrophoresis patterns to compensate for minor differences in migration, (ii) calculation of a Pearson's coefficient of similarity between patterns, and (iii) clustering of the patterns by using the unweighed pair group method for arithmetic averages (UPGMA).

Bacterial identification by DNA-DNA hybridization. The degree of DNA-DNA binding was determined quantitatively by spectrophotometry from renaturation rates according to a modification of the method of De Ley et al. (4). The temperature of renaturation was 25°C. DNA-DNA relatedness values were calculated after incubation for 21 and 24 min, after removal from the calculation of the first 3 min of renaturation. The G+C composition of the strains was determined as described by Marmur and Doty (24).

Amplification and cloning of 16S rDNA. Amplification of 16S ribosomal DNA (rDNA) was performed with the oligonucleotide primers F515 [5′-GTGCCAG C(AC)GCCGCGG-3′], R930 [5′-G(CT)CCCCGTCAATTC(AC)T-3′], F915 [5′-A(GT)GAATTGACGGGG(GA)C-3′], and R1406 [5′-ACGGGCGGTGTG T(GA)C-3′], which correspond to conserved sequences of the bacterial 16S rRNA gene (from positions 515 to 530, 930 to 915, 915 to 930, and 1406 to 1392 on the *Escherichia coli* 16S rRNA, respectively) (23). PCR conditions used included annealing at 42°C (for 30 s) for fragment A (position 515 to position 930) or at 55°C (for 30 s) for fragment B (position 915 to position 1406), extension at 72°C (45 s), and denaturation at 94°C (2 min). Amplification reactions (30 cycles) were carried out in a GeneAmp PCR system 2400 (Perkin-Elmer-Cetus) with 75 ng of the E1 strain chromosomal DNA as a template. Fragments A and B were then cloned by using the LigATor kit (R&D Systems, Abingdon, United Kingdom) according to the manufacturer's recommendations.

***rumA*-like gene detection.** The presence of *rumA*-like genes in the genome of bacterial strains producing a tryspsin-dependent antimicrobial substance was explored by Southern blot hybridization according to standardized methods (30). The oligonucleotide ol30 (5′-AAAAACAATCAGCCACGAATGCAATATGA A-3′) was γ-^{32}P labeled and used as a *rumA*-specific probe (9). Hybridization experiments were carried out under highly stringent conditions at 50°C.

***rumA*-like gene amplification.** The chromosomal region harboring the *rumA*-like genes was amplified by using the oligonucleotide OlK1 (5′-GGAGGAAA GATAAAAATTGATAGTGAACCTAATAG-3′) and OlA4 (5′-AGACCACA AAACTTCTTATGCATACTTACCTCCTG-3′) designated on the basis of the RumA locus sequence (accession number AJ276653 [9]). The PCR conditions included annealing at 50°C (for 30 s), extension at 72°C (for 3 min), and denaturation at 94°C (for 1 min). Amplification reactions (30 cycles) were carried out in a GeneAmp PCR system 2400 (Perkin-Elmer-Cetus) with 500 ng of the bacterial strains chromosomal DNA as a template.

DNA sequencing and analysis. Nucleotide sequences were determined by the dideoxy chain terminator method (31) by using the Prism TM Ready Reaction D-Rhodamine Terminator sequencing kit (Applied Biosystems Division) in a ABI PRISM 310 Genetic Analyzer (Perkin-Elmer-Cetus). DNA or protein homology searches were carried out with the programs included in the Genetics Computer Group sequence analysis software package (University of Wisconsin).

Nucleotide sequence accession numbers. The nucleotide sequences reported in this study were deposited in the NCBI (Bethesda, Md.) GenBank Sequence Database. The accession numbers for partial 16S rDNA sequences are as follows: LEMV61, AF446129; LEMV63, AF446128; and LEMV78, AF446130. The accession numbers of the sequences of the chromosomal region harboring the *rumA*-like genes are as follows: LEMB04, AF439551; LEMB53, AF439554; LEMV58, AF439549; LEMV66, AF439548; LEMV95, AF439550; LEMV98, AF439552; and LEMV99, AF439553.

RESULTS

Isolation and RAPD analysis of strains producing a trypsin-dependent anti-*C. perfringens* substance. Eighteen human fecal samples obtained from various ecological contexts, including five from healthy children between 6 and 10 months of age, nine from healthy adults, and four from adults with chronic pouchitis, were freshly collected. The total bacterial populations were estimated on BHI-YE medium to be between 5×10^{10} and 5×10^{11} CFU/g (wet weight) of feces. Randomly chosen bacterial isolates representing at least 1/100 of the culturable population (bacterial counts of between 5×10^{8} and 5×10^{9} CFU/g of feces) were screened for the production of a trypsin-dependent anti-*C. perfringens* substance. Two healthy-child and six healthy-adult samples were negative, but 1 to 4 positive clones, all of which were strict anaerobic gram-positive cocci, could be isolated from all of the other samples (three from healthy children, three from healthy adults, and four from adults with chronic pouchitis), providing us with 18 clones.

Since several bacterial isolates could be obtained from the same sample, RAPD patterns of positive clones were compared to eliminate redundant isolates. The *R. gnavus* ATCC 29149^{T} reference strain and the previously characterized *R. gnavus* E1 strain, which is capable of producing the trypsin-dependent lantibiotic RumA, were also included in the analysis. RAPD profiles were obtained with three different primers, OlB06, OlB07, and OlB08, in separate reactions. Fourteen strains exhibited clearly differentiated profiles (Table 1 and Fig. 1); redundant clones were eliminated from further experiments.

At a cutoff level of 30 (Pearson's coefficient $\times$ 100), 5 of these 14 strains strains (i.e., LEMG25, LEMV62, LEMV58, LEMB86, and LEMV95) clustered with *R. gnavus* ATCC 29149^{T}, and 4 strains (LEMV99, LEMV66, LEMV50, and LEMB53) clustered with *R. gnavus* E1 (Fig. 1). The RAPD patterns of five other strains (LEMV61, LEMV63,LEMV98, LEMV78, and LEMB04) took up separate positions in the analysis.

Identification of the producing strains. Since similarity in the RAPD patterns suggested that the majority of the positive strains might be related to the *R. gnavus* species, DNA-DNA hybridization experiments were carried out with the *R. gnavus* ATCC 29149^{T} reference strain. As expected, the nine strains (LEMG25, LEMV62, LEMV58, LEMB86, LEMV95, LEMV99, LEMV66, LEMV50, and LEMB53) related to *R. gnavus* according to their RAPD profiles exhibited more than 70% DNA-DNA homology with ATCC 29149^{T} (Table 1), demonstrating that they belonged to *R. gnavus*. For the other five strains (LEMV61, LEMV63, LEMV98, LEMV78, and LEMB04), the level of DNA-DNA homology was <40%, indicating that they did not belong to this species.

The non-*R. gnavus* strains were identified by 16S rDNA sequencing. Two fragments, A and B, of the 16S rDNA were amplified, cloned, and sequenced, providing sequence information on an 861-bp rDNA fragment. The 16S rDNA sequence of LEMB04 strain showed 99.4% identity to *C. nexile.* DNA-DNA hybridization experiments demonstrated that LEMB04 strain had 81% homology with *C. nexile* DSMZ 1787^{T} and thus confirmed that it belonged to this species (Table 1). Similarly, LEMV98 was shown to belong to the *R. hansenii* species since it shared 97.4% 16S rDNA identity and had 90% DNA-DNA homology with the type strain of this species (DSMZ 20583^{T}) (Table 1).

For LEMV78, the16S rDNA sequence percentage of identity to known species was not >95% (*R. gnavus*, Table 1). For the LEMV61 and LEMV63 strains, the highest percentage of identity of their 16S rDNA sequence was observed with unculturable bacteria from human intestinal communities adhufec420 (34) and L37A (accession number AF253389), respectively. The percentage of identity to cultured bacterial strains was <95% (*Eubacterium* species, Table 1). Thus, these three strains should be considered new operational taxonomic units (OTUs) (8).

All of the strains isolated in here belonged to the *C. coccoides* phylogenetic group (RDP group 2.30.4.1) (22) that includes some of the predominant bacterial genera found in the human large bowel (34).

In a second series of experiments, RAPD profiles were obtained from all strains, including *R. hansenii* DSMZ 20583^{T} and *C. nexile* DSMZ 1787^{T} (Fig. 1). Previous results for the strains identified as *R. gnavus* were confirmed: they were grouped in two clusters containing *R. gnavus* ATCC 29149^{T} or *R. gnavus* E1. LEMV98 and LEMB04 were grouped with *R. hansenii* DSMZ 20583^{T} and *C. nexile* DSMZ 1787^{T}, respectively (Fig. 1). RAPD patterns of LEMV61, LEMV63, and LEMV78, putative members of new OTUs, did not exhibit a significant homology with any other strain.

Detection of *rumA*-like genes. In order to determine whether the trypsin-dependent antimicrobial substance produced by the 14 newly identified strains could be related to RumA, the detection of *rumA*-like genes on their genome was undertaken. Chromosomal DNA was extracted, digested with *Eco*RI, and analyzed by Southern blot hybridization by using the specific-*rumA* oligonucleotide ol30 as a probe. *R. gnavus* E1 and ATCC

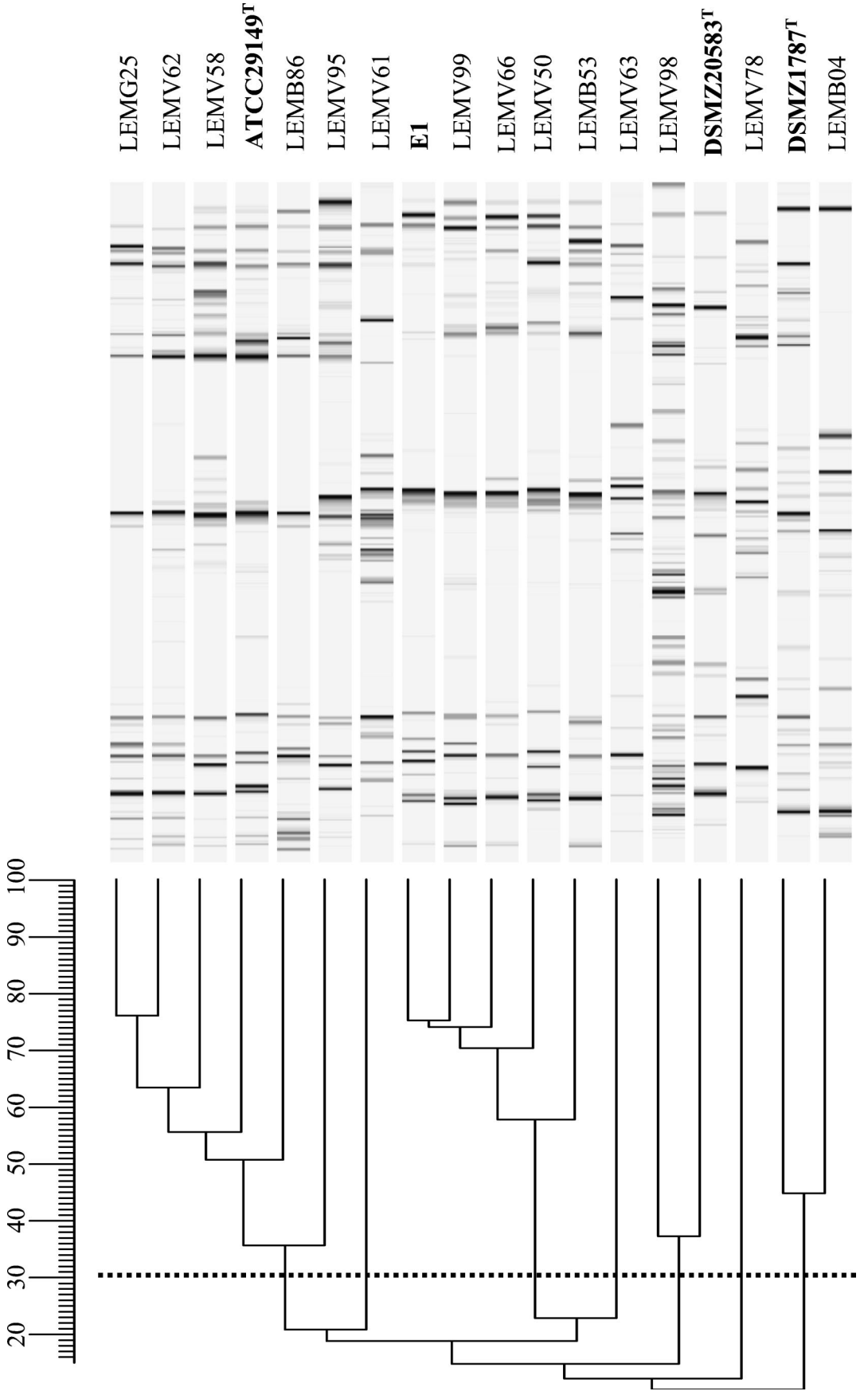

FIG. 1. Comparison of the RAPD patterns. RAPD profiles were obtained in three independent reactions with the oligonucleotide primers OlB6, OlB7, and OlB8 and then merged for analysis. Reference strains *C. nexile* DSMZ 1787^T, *R. hansenii* DSMZ 20583^T, and *R. gnavus* ATCC 29149^T and the *R. gnavus* E1 strain are indicated in boldface letters. The horizontal dotted line indicates the value of 30 for the coefficient of similarity (Pearson's coefficient × 100).

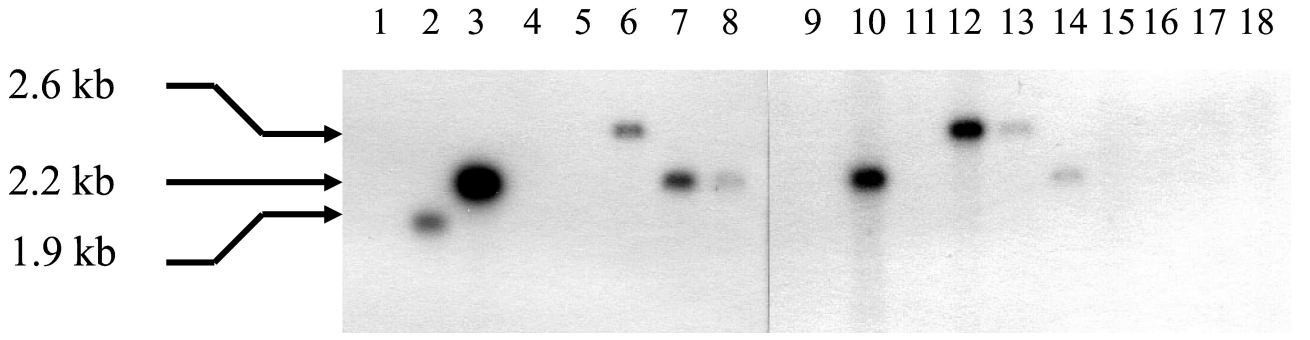

FIG. 2. Detection of *rumA*-like genes by Southern blot hybridization. Lanes: 1 and 9, *R. gnavus* ATCC 29149^T; 2, LEMB53; 3, LEMV58; 4, LEMV50; 5, LEMB86; 6, LEMV98; 7, LEMV95; 8 and 14, *R. gnavus* E1; 10, LEMV66; 11, LEMG25; 12, LEMB04; 13, LEMV99; 15, LEMV78; 16, LEMV63; 17, LEMV61; 18, LEMV62. The sizes of the *Eco*RI restriction fragments hybridizing with the ol30 *rumA*-specific probe are indicated on the left side of the figure.

29149^T strains were included as positive and negative controls, respectively. Apart from strain E1, 7 of the 14 strains gave a single positive signal (Fig. 2). Because of the highly stringent conditions used in this experiment, this result suggested that these strains probably harbored a *rumA*-like gene that is very similar to the one identified in strain E1 (9).

DNA amplification and sequencing of the *rumA*-like genes. In strain E1, the structural gene encoding the RumA precursor is present in three copies located in between the *rumK* and *rumM* genes. These are proposed to encode a sensor histidine kinase and a modification enzyme supposed to catalyze dehydration and thioether bridge formation of lantibiotic precursors, respectively (9).

Two primers, OlK1 and OlA4, located at the 3′ terminus of *rumK* and at the 5′ terminus of *rumM*, respectively (Fig. 3A), were used to amplify total DNA extracted from the seven strains positively responding to *rumA*-specific probe ol30. For each strain, one main amplified fragment was obtained, with a size ranging from ca. 900 bp to 3.7 kbp. The fragments were extracted from agarose gels, purified, and sequenced.

The analysis of the sequence data revealed that, as in strain E1, three copies of the structural *rumA* gene were systematically present on each amplified fragment (Fig. 3). The *rumA1*-, *rumA2*-, and *rumA3*-like genes were located on a 700-bp fragment exhibiting >90% identity to the corresponding strain E1 fragment. The G+C content of this fragment ranged from 32 to 34%, a level clearly different from the average values for the rest of the chromosome (38 to 43%, Table 1). For a given strain, the three copies of the *rumA*-like genes encoded the same putative peptide. Moreover, the peptide sequence deduced from the *rumA*-like genes of four strains (LEMB53, LEMV58, LEMV66, and LEMV95) were identical to the hypothetical RumA precursor previously characterized (9). In the three other strains (LEMB04, LEMV98, and LEMV99), one difference in the same putative residue (at position 15) was observed.

In strains LEMV58, LEMV66, and LEMV95, the amplified fragment was the same size as in strain E1 and showed >92% identity to the fragment of strain E1 (Fig. 3B).

In strains LEMB04, LEMV98, and LEMV99, the amplified fragments were 1,160 bp long and shared >99% identity. The supplementary 400-bp fragment, located between the 3′ terminus of *rumK* and the 5′ terminus of *rumA$_1$* (Fig. 3C), did not show any significant homology with the DNA sequence described above. This fragment harbored a 27-bp imperfect inverted repeat, which was predicted to form a stem-loop structure with a ΔG of −15.1 kcal/mol as shown by Tinoco et al. (38), that might act as a rho-independent transcription terminator. As mentioned above, in these strains, the peptide sequence deduced from the *rumA*-like genes exhibited one difference with the putative RumA precursor deduced from all other strains: one Asn residue located in position 15 of the hypothetical leader peptide replaced a Lys residue. Remarkably, LEMB04, LEMV98, and LEMV99 belonged to three different species (*C. nexile*, *R. hansenii*, and *R. gnavus*, respectively) and had been isolated from the same fecal sample together with the LEMV95 strain (Table 1).

The fragment amplified from LEMB53 strain was the largest. In addition to the *rumA*-like genes, three open reading frame genes—*orf1*, *orf2*, and *orf3*—were found (Fig. 3D) and predicted to code for hypothetical proteins consisting of 137, 114, and 538 amino acids, respectively. Orf1, Orf2, and Orf3 were similar to putative proteins associated with mobile genetic elements previously described in *Yersinia pestis* (26), *Streptococcus pneumoniae* TIGR4 (37), *S. pyogenes* (6), and *E. coli* (25), with some of them belonging to the IS*66* family. The hypothetical genes *orf1*, *orf2*, and *orf3* were located in between two imperfect 29-bp inverted repeats, suggesting that the complete structure could constitute an insertion sequence of the IS*66* family. This putative mobile genetic element was located immediately downstream of the probable rho-independent transcription terminator t2 (Fig. 3D). No direct repeat could be detected close to this structure.

DISCUSSION

Fourteen bacterial strains capable of producing trypsin-dependent antimicrobial compounds active against *C. perfringens* were isolated from the dominant population of fecal samples collected in various ecological contexts from healthy adults and children and from adults with chronic pouchitis. They were identified as *R. gnavus* or closely related species belonging to the phylogenetically defined cluster *C. coccoides*. Three of them were new OTUs. The analysis of RAPD profiles showed that all of the *R. gnavus* strains clustered together with either the reference strain of this species, *R. gnavus* ATCC 29149^T, or

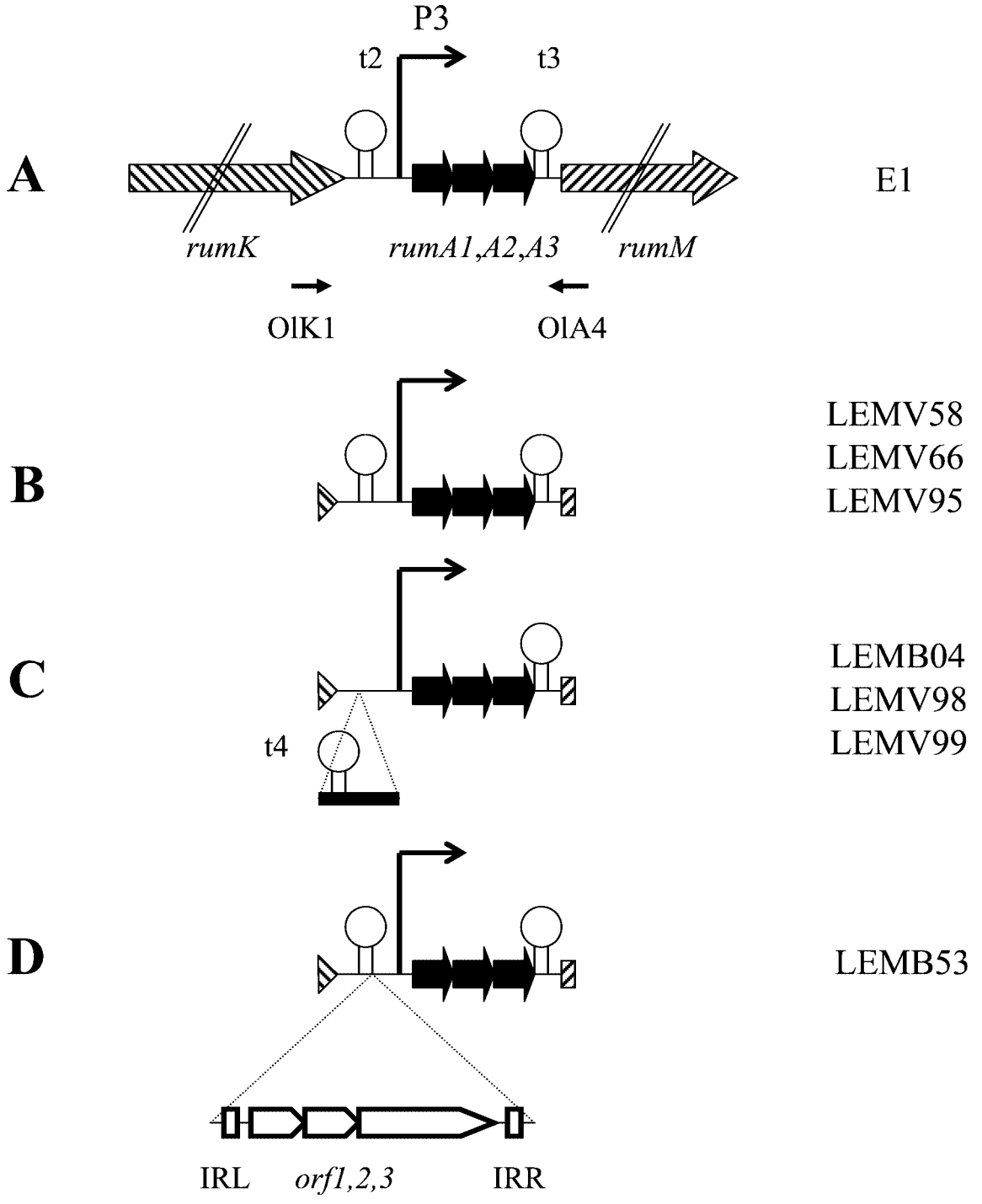

FIG. 3. Schematic representation of the chromosomal region harboring the *rumA*-like genes. The positions of *rumA1*, *rumA2*, and *rumA3* are indicated with respect to *rumK* and *rumM* on the chromosome of strain E1. *rumK* and *rumM* genes are indicated as dashed arrows, *rumA*-like genes are indicated as black arrows, and *orf1*, *orf2*, and *orf3* are iindicated as open arrows. Their orientation indicates the direction of transcription. OlK1 and OlA4 are primers used to amplify and sequence the corresponding DNA region. The strains corresponding to a given organizational scheme are indicated on the right side. t2, t3, and t4 are putative stem-loop structures that might act as rho-independent transcription terminators. P3 was previously suggested to act as the promoter of *rumA* genes in strain E1 (9). White boxes indicate the two imperfect 27-bp inverted repeats IRL and IRR bordering *orf1*, *orf2*, and *orf3*.

the *R. gnavus* E1 strain, which produced the trypsin-dependent lantibotic RumA, recently characterized by our group (3).

When the newly isolated strains were hybridized with a probe specific for the *rumA* structural gene, seven gave a positive response and seven were negative. Since the hybridization experiments were performed under highly stringent conditions, the trypsin-dependent anti-*C. perfringens* activity detected in the seven *rumA*-negative strains during the screening test may

be due to the production of bacteriocins encoded by genes significantly different from those previously identified in the E1 strain. However, it may also be due to non-RumA-related antimicrobial compounds.

The seven positive strains were shown to harbor the structural gene encoding RumA (9). The peptide sequence deduced from these genes was nearly identical to the hypothetical RumA precursor. Only one variation in the amino acid composition of the leader peptide could be noticed in three strains isolated from the same sample. Variations in the amino acid composition of the leader peptide of a lantibiotic were previously demonstrated in M-type 49 group A *Streptococcus* strains, which harbor two tandem copies of the structural gene (14). As already described for strain E1 (9), all of the positive strains harbored three consecutive copies of the *rumA* gene, exhibiting a high percentage of nucleotide identity (>90%) and coding for the same probable peptide. This arrangement of genes was previously reported for strain E1 (9). However, the detection of a similar structure disseminated among several bacterial species of the dominant fecal microbiota was puzzling. The role, if any, of this particular structure on the regulation, the level of expression, or the structural DNA stability of this region remains to be evaluated.

In all of our strains, the *rumA* region exhibited a low G+C content (ca. 32 to 34%), which is clearly different from the rest of the chromosome of *R. gnavus* or phylogenetically related species (38 to 43%). This observation suggests that the *rum* genes were recently acquired by *R. gnavus* via horizontal genetic transfer from a bacterium with a lower G+C content.

Four *rumA*-positive strains were isolated from the same fecal sample collected from a healthy child. Three of these strains belonged to three different species (*R. gnavus*, *R. hansenii*, and *C. nexile*) and harbored an additional 400-bp fragment in between the *rumK* and *rumA1* genes. These strains exhibited >95% identity in a 1.1-kb genome region spanning the *rumA* genes. These three strains were the only ones to harbor the same modification in the leader peptide sequence, suggesting that the genetic transfer of the *rumA* genes occurred within the digestive tract of the volunteer.

Initially, the proteinaceous nature of bacteriocins was evidenced by the loss of the antimicrobial activity after the action of various proteases, including trypsin (16, 18, 19). Therefore, the role of bacteriocins in the digestive tract, if any, has to be local as, for example, when there is a close relationship between the producer and the target strain; otherwise, bacteriocins would be destroyed by host or microbial proteases. In previous studies, it was shown that RumA seems adapted to the digestive ecosystem, since (i) trypsin, one of the main proteases of the gut, is necessary for RumA production; (ii) RumA is resistant to trypsin proteolytic activity; and (iii) RumA is active against two groups of targets: bacterial species belonging to the *C. coccoides* phylogenetic group and pathogenic *Clostridium* spp. (3, 9). Our new results suggest that the *rumA*-like genes may be located on a mobile genetic element transferable to different species of the *C. coccoides* cluster, leading to their dissemination in *R. gnavus* and related species that can reach dominant levels in the bacterial populations of the human gut.

In studies of bacteriocins produced by *E. coli*, Riley and Gordon concluded that "there might be a trade-off between the costs and the benefits of colicin production" (29). This argument is also true for lantibiotics such as RumA, the synthesis of which necessitates a complex genetic system (19). Thus, RumA might be considered as a powerful weapon allowing bacteria that produce it to compete efficiently for the same ecological niches with related species. It is remarkable that the *rumA* genes, initially identified in a bacterium isolated from the fecal microbiota of a healthy adult, were also present in fecal strains from a young child and in adults with chronic pouchitis. Thus, RumA could play a role in various ecological contexts, such as when the digestive microbiota is considered to have reached a steady state (as with healthy adults) and also when it undergoes variations due to increasing complexity (as with children) or profound modification of the ecosystem (as with chronic pouchitis).

Although several *rumA*-positive strains were isolated during the present study, the occurrence of RumA-producing bacteria among the fecal microbiota may still be underestimated. The detection of the *rumA* genes here was undertaken with bacterial strains representing at least 1% of the culturable fecal population. Previous studies have shown that only 21% of the total population detected microscopically through DAPI (4′,6′-diamidino-2-phenylindole) staining and 32 to 37.5% of the total population detected by oligonucleotide probe hybridization were able to grow anaerobically on a nonselective medium (34, 36). During our screening, no bacterial isolate producing a trypsin-dependent anti-*C. perfringens* substance could be obtained from two samples from healthy children and six samples from healthy adults. The assumption that positive strains simply did not exist in these samples cannot be rejected. However, such strains could be present but unculturable or at population levels lower than 5×10^8 CFU/g of feces.

Further studies are now needed to more precisely evaluate the impact of RumA production on the colonization capabilities of the producing strains and on the protection of the host against invasion by pathogenic *Clostridium* spp.

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