Ruminococcin A, a New Lantibiotic Produced by a Ruminococcus gnavus Strain Isolated from Human Feces

J. DABARD,1 C. BRIDONNEAU,1 C. PHILLIPE,1 P. ANGLADE,2 D. MOLLE,3 M. NARDI,2 M. LADIRÉ,1 H. GIRARDIN,4 F. MARCILLE,1 A. GOMEZ,1 AND M. FONS1*

Unité d’Écologie et de Physiologie du Système Digestif and Unité de Biochimie et Structure des Protéines,2 Institut National de la Recherche Agronomique, 78352 Jouy-en-Josas Cedex, Laboratoire de Recherches de Technologie Laitière, Institut National de la Recherche Agronomique, 35042 Rennes Cedex, and Laboratoire de Microbiologie, Institut National de la Recherche Agronomique, 84914 Avignon Cedex 9, France

Received 17 April 2001/Accepted 21 June 2001

When cultivated in the presence of trypsin, the Ruminococcus gnavus E1 strain, isolated from a human fecal sample, was able to produce an antibacterial substance that accumulated in the supernatant. This substance, called ruminococcin A, was purified to homogeneity by reverse-phase chromatography. It was shown to be a 2,675-Da bacteriocin harboring a lanthionine structure. The utilization of Edman degradation and tandem mass spectrometry techniques, followed by DNA sequencing of part of the structural gene, allowed the identification of 21 amino acid residues. Similarity to other bacteriocins present in sequence libraries strongly suggested that ruminococcin A belonged to class IIA of the lantibiotics. The purified ruminococcin A was active against various pathogenic clostridia and bacteria phylogenetically related to R. gnavus. This is the first report on the characterization of a bacteriocin produced by a strictly anaerobic bacterium from human fecal microbiota.

It has been hypothesized previously that the capability to produce antibacterial compounds could play a key role in colonization and protection processes that take place in complex environments (25). Bacteriocin-producing bacteria have been isolated from mammalian digestive ecosystems (2, 13, 14, 17, 29), thus suggesting that they might be involved in colonization resistance, a fundamental function of gut microbiota.

Bacteriocins are proteinaceous compounds ribosomally synthesized. Their production by gram-positive bacteria has been documented extensively in the last few years, and they can be sorted into four distinct classes (15). Class I comprises lantibiotics which are characterized by (i) small size, (ii) activity toward the membrane, and (iii) the presence of dehydrated or unusual residues resulting from posttranslation modification events.

Lanthionine (Lan) and 3-methylanthionine (Me-Lan) are the most common modified residues. They result from the formation of a thioether bond between a cysteine (Cys) residue and either a serine (Ser) or a threonine (Thr) residue, previously dehydrated as dihydroalanine (Dha) or dihydrobutyrylipyline (Dhb), respectively. The dehydration step is catalyzed by a specific enzyme encoded by a gene included in the bacteriocin biosynthetic cluster. However, the number of dehydro residues generated is often larger than the number of potential Cys residues capable of further reacting with them. Therefore, many lantibiotics contain one or more dihydroamino acids (26). It was suggested that lantibiotics be grouped into type A and type B peptides according to their structure and activity. Type A lantibiotics are elongated and flexible peptides forming pores in the cytoplasmic membrane, resulting in, among other things, the loss of ion gradients, whereas type B lantibiotics are more globular and inhibit enzymatic functions (12). The increasing number of novel characterized lantibiotics with intermediate behavior makes this classification more and more difficult.

In the initial definition, bacteriocins were defined by inhibitory activity against closely related bacteria. Results obtained more recently have tended to show that this criterion should not be taken into account anymore (15).

In a previous work, we had demonstrated that strain E1, isolated from the fecal microbiota of a healthy man, was able to produce a trypsin-dependent antibacterial substance active against Clostridium perfringens (24). Initially, the E1 strain was identified as a Peptostreptococcus sp. (24) according to phenotypic characterization. Here we report on the isolation and characterization of ruminococcin A (RumA), a lantibiotic produced by the E1 strain in liquid culture medium and in the presence of trypsin. RumA is of particular interest since it is active against C. perfringens and various other enteric pathogens, suggesting that it could play an essential role in the protection of the host.

MATERIALS AND METHODS

Bacterial strains and media. The E1 strain had been isolated from the predominant fecal microbiota of a healthy adult (24). Other bacterial strains used in this study are listed in Table 1. The C. perfringens Cpa strain (24) was used as the reference target strain. Bacillus cereus strains were isolated from mashed vegetables (Collection INRA, Avignon, France). Clostridium difficile strains 79-685 (32), C253 (4), ATCC 43598 (5), and M-1 and B-1 (51) were kindly provided by T. Karjalainen (Université Paris Sud, Chatenay-Malabry, France), P. Mastrantonio (Istituto Superiore di Sanita, Rome, Italy), M. Delméé (Leuven Universiteit, Brussels, Belgium), and P. Borriello (Central Public Health Laboratory, London, United Kingdom), respectively. Other strains were from culture collections: ATCC (American Type Culture Collection, Manassas, Va.), CIP (Collection Institut Pasteur, Paris, France), NCTC (National Collection of Type Cultures, London, United Kingdom), and VPI (Virginia Polytechnic Institute, Blacksburg).

Strain E1 was 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] per liter and 5 mg of hemin [Sigma-Aldrich Chimiie, St. Quentin Fallavier, France] per liter). When required, trypsin from bovine pancreas (type XIII; L-1-tosyl-aminomethyl phenylalanine chloride treated; Sigma-Aldrich Chimie) was added at a final concentration of 50 μg/ml.

* B. cereus was plated on J agar (5 g of tryptone [Biokar Diagnostics, Beauvais, France] per liter, 15 g of yeast extract [Biokar Diagnostics] per liter, 3 g of K₂HPO₄ per liter, 2 g of glucose per liter, and 15 g of agar [Difco Laboratories] per liter). * Clostridium butyricum* was propagated on TGY medium (30 g of tryptone per liter, 20 g of yeast extract per liter, 5 g of glucose per liter, 0.5 g of cysteine-HCl per liter, 0.084 g of resazurine per liter, 15 g of agar per liter, pH 7.3). Other strains were plated on solid BHI-agar medium obtained by adding 15 g of agar per liter. Bacteriocin purification. * R. gravis* E1 was inoculated at 1% in 200 ml of trypsin-supplemented BHI-YH broth and incubated at 37°C for 48 h under anaerobic conditions. After growth, bacterial cells were removed by centrifugation; the supernatant was lyophilized, resuspended in 35 ml of 0.05% trifluoroacetic acid, and loaded onto C₈ Sep-Pack 35 cm² reverse-phase cartridges (Waters; Millipore). The column was washed twice with 140 ml of 0.05% trifluoroacetic acid and then with 84 ml of 20% and 35% acetonitrile. The bacteriocin was eluted with 84 ml of 40% acetonitrile. Bacteriocin-containing fractions were pooled, evaporated in a Rotavapor R-134 (Buchi, Switzerland), and then resuspended in 9 ml of 36% acetonitrile before further purification by C₁₈ reverse-phase high-pressure liquid chromatography (RP-HPLC). Five-hundred-microliter aliquots were loaded onto a C₁₈ Novapak preparative column (300 by 7.8 mm). Elution was performed at a flow rate of 2 ml/min under 36% acetonitrile isocratic conditions and then at a flow rate of 0.5 ml/min using a linear gradient of acetonitrile from 36 to 60%. Peptide fractions were detected spectrophotometrically by measuring A₂₁₄ and collected manually. The active fractions were pooled and concentrated under vacuum to a final volume of 9 ml. Protein content, estimated with the Folin reagent, and antagonistic activity were determined at each step. The bacteriocin was purified by a second RP-HPLC step performed under the same conditions. Active fractions were lyophilized and then resuspended in distilled water before further analysis.

Bacteriocin activity assays. Antibacterial activity present in the fractions was determined at each step of purification by a modification of the critical dilution assay (11). One hundred fifty microliters of a BHY-YH broth culture (37°C, 16 to 24 h) of the sensitive indicator strain *C. perfringens* CpA was added to 15 ml of BHY-YH agar medium and poured in a sterile plate. Six-millimeter-diameter wells were then prepared with a vacuum pump. Five hundred microliters of each fraction was concentrated under vacuum to a 50-μl final volume. Then, 30 μl of a serial twofold dilution of ruminococcin A was spotted onto the surface of the plates. The plates were then incubated at 37°C for 16 to 24 h in an anaerobic cabinet. Bacteriocin titers were expressed as the reciprocal highest dilution exhibiting an inhibition halo and were reported in arbitrary units (AU) per milliliter, thus allowing the determination of the total AU present in a given fraction.

For determining the activity spectrum, a solution of Sep-pack-purified ruminococcin A with a total protein concentration of 600 μg/ml was used. Ten microliters of a fresh broth culture (containing approximately 10⁷ CFU) of the target strain was spread on appropriate agar plates. After 90 min of incubation at room temperature, 10 μl of a serial twofold dilution of ruminococcin A was spotted onto the surface of the plates. The plates were then incubated until confluent growth of the target strain. The MIC of ruminococcin A was compared to that of metronidazole (Sigma-Aldrich Chimie), an antibiotic frequently used in the case of anaerobic infections of the digestive tract.

Amino acid composition analysis. Purified bacteriocin was hydrolyzed in 6 N HCl under vacuum at 110°C for 24 h. The amino acid composition was determi...
mined on an LC3000 analyzer (Eppendorf Biotronik, Munich, Germany) using a ninhydrin postcolumn derivative system (20).

Mass spectrometry. Mass measurement of the purified bacteriocin was performed by electron spray mass spectrometry, using a VG Bio-Q quadrupole (Bio-Tech, Manchester, United Kingdom) in the positive mode. The protein was dissolved in H2O:CH3CN (50:50 [vol/vol]) ratio with 1% acetic acid at a concentration of about 5 pmol/μl (by volume); 10-μl aliquots were introduced into the ion source at a flow rate of 4 μl/min. Scanning was usually performed from m/z = 500 to m/z = 1,500 in 10 s with the resolution adjusted so that the peak at m/z = 998 from horse heart myoglobin was 1.5 to 2 wide on the base. Calibration was performed by using the multiply charged ions produced by a separate introduction of horse heart myoglobin (16,950.4 Da) (10).

Amino acid sequence analysis. The amino acid sequence of the purified bacteriocin was determined by Edman degradation using an Applied Biosystems model 477A (Foster City, Calif.) pulsed liquid sequencer, connected to a 120A terminator system at a flow rate of 4 μl/min. Scanning was usually performed from m/z = 500 to m/z = 1,500 in 10 s with the resolution adjusted so that the peak at m/z = 998 from horse heart myoglobin was 1.5 to 2 wide on the base. Calibration was performed by using the multiply charged ions produced by a separate introduction of horse heart myoglobin (16,950.4 Da) (10).

DNA sequencing and analysis. Nucleotide sequences were determined by the dyeoxy chain terminator method (26) using the Prism Ready Reaction d-Rhamamine Terminator sequencing kit (Applied Biosystems Division) in an ABI Prism 310 Genetic Analyzer (Perkin-Elmer Cetus). DNA or protein homology searches (GenBank, EMBL, and SWISS-PROT) were carried out with the programs included in the GCG sequence analysis software package (University of Wisconsin). Multiple alignments were edited by the Genedoc program.

RESULTS

Identification of the E1 strain. Two fragments, A and B, of the E1 16S rDNA were amplified, cloned, and sequenced as described in the previous paragraph.

Purification of the bacteriocin produced by the E1 strain. Previous studies had shown that antibacterial activity was present in the supernatant of strain E1 cultivated in the presence of trypsin (24). The purification of the antibacterial substance was achieved by reverse-phase chromatography (Sep-Pack followed by HPLC) (3). The first step of purification removed a significant amount of contaminating proteins (Table 2). The biologically active fraction eluted with 40% acetonitrile and was loaded onto a C18 Novapak preparative column. The antibacterial substance eluted with fractions collected after a 27- to 32-min retention time (Fig. 1A). A second RP-HPLC purification performed under the same conditions yielded two peaks corresponding to fractions F1 and F2 that eluted from 27.4 to 29.3 min and from 29.3 to 31.15 min, respectively (Fig. 1B). Both fractions were active against C. perfringens.

Samples corresponding to fractions F1 and F2 were subjected to mass spectrometric analysis. The molecular mass deduced from fraction F1 was 2,675 Da (Fig. 2). Fraction F2 appeared to contain a product with a molecular mass of 2,675 Da, contaminated with other molecules (data not shown). Further analysis was performed only on fraction F1, which contained the antibacterial substance now called ruminococcin A (RumA).

RumA antagonistic activity was not affected by high temperatures (10 min of incubation at 100°C).

Peptide analysis and sequencing. Analysis of the amino acid composition of RumA highlighted the presence of both Dhb

<table>
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<th>TABLE 2. Purification yield</th>
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<td>Purification stage</td>
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<td>---------------------</td>
</tr>
<tr>
<td>Supernatant</td>
</tr>
<tr>
<td>Sep-pack</td>
</tr>
<tr>
<td>RP-HPLC 1</td>
</tr>
<tr>
<td>RP-HPLC 2</td>
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DNA isolation. R. gnavus E1 chromosomal DNA was isolated using the Nucleospin kit (Machery-Nagel GmbH & Co., Duren, Germany).

Amplification and cloning of E1 16S rRNA. Amplification of 16S ribosomal DNA (rDNA) was performed using oligonucleotide primers F515 [5'-GAGTTCCAGGCACGGCGCCGG-3'] and R930 [5'-GCTTACACGACTCATATATAAGAGACAG-3'], which corresponded to bacterial 16S rRNA gene conserved sequences (from positions 515 to 530, 930 to 915, 915 to 930, and 1406 to 1392 on the Clostridium perfringens 16S rRNA, respectively) (19). PCR conditions used included annealing at 42°C (30 s) for fragment A (position 515 to position 930) or 55°C (30 s) for fragment B (position 915 to position 1406), polymerization at 72°C (1 min), and denaturation at 94°C (2 min). Amplification reactions (30 cycles) were carried out in a Gene Amp PCR system 2400 cycler (Perkin-Elmer Cetus, Norwalk, Conn.) using 75 ng of the E1 strain chromosomal DNA as a template. Fragments A and B were then cloned using the LigAero kit (R&D Systems, Abingdon, United Kingdom) according to the manufacturer's recommendations.

Amplification and cloning of part of the ruminococcin structural gene. Amplification of the DNA fragment encoding the active bacteriocin was performed with degenerate oligonucleotide primers OH1 and OH2. The OH1 sequence (5'-CAGGAAACACGGATGCAAGGCGAATGACCGGNaNNYNNTNAA-3') was based on the pBSK+ reverse primer followed by the degenerate codons (underlined) encoding the six amino acid residues identified at the N-terminal end of the bacteriocin. The OH2 sequence (5'-TGTTAATCGGCGCAGATRANARRRAAYTGCCA-3') was based on the pBSK+ –21 M13 primer sequence and the degenerate codons (underlined) encoding the five amino acid residues identified at the C-terminal end. PCR conditions used included annealing at 50°C (30 s), polymerization at 72°C (1 min), and denaturation at 94°C (1 min). Amplification reactions (30 cycles) were carried out in a Gene Amp PCR system 2400 (Perkin-Elmer Cetus) using 500 ng of the E1 strain chromosomal DNA as a template.

The 100-bp amplified fragment was extracted from an agarose gel, polished with the PCR polishing kit (Stratagene, La Jolla, Calif.), and subsequently cloned at the blunt-end EcoRV restriction site of the pBR322 vector, using standard molecular genetics techniques (27).

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and Lan residues (data not shown). This latter observation suggested that RumA was a lantibiotic.

The peptide sequence obtained by the Edman degradation technique identified the respective positions of 17 residues, 4 remaining unknown (Table 3). The reaction was blocked after the 21st cycle.

RumA was thus subjected to MS/MS. Fragmentation of the doubly charged ion (m/z 1338) of RumA yielded two b series ions. The first series corresponded to the successive cleavage of the sequence from the N-terminal part (Fig. 3). The observed singly charged product ions, i.e., m/z 172, 229, 328, 441, 569, 652, and 765, corresponded to the sequence GNGV(I/L)KDhb(I/L), consistent with the RumA N-terminal sequence obtained by the Edman degradation method (Table 3). It made it possible to identify the seventh residue as a Dhb (Dhb7). The second series (Fig. 3), constituted by doubly charged product ions (m/z 812, 878, 935, 977, 1070, 1134, 1208, and 1264), corresponded to a successive loss of MNDhbWQF(I/L)F residues from the C-terminal region of the precursor ion (m/z 1338). Again, these data were consistent with the RumA C-terminal sequence obtained by the Edman degradation method (Table 3). They made it possible to identify the 16th residue as a Dhb (Dhb16). It was noticeable that the signals corresponding to Dhb7 and Dhb16 were comparable, suggesting that the two residues were at the same stage. From these results, it could also be deduced that this last fragment was an internal sequence of RumA, since the molecular mass difference between m/z 1338 and 1264, i.e., 148 Da, corresponded to the Mr of Phe minus 17 Da (OH group), underlining the fact that this amino acid, i.e., Phe21, was naturally involved in two peptide bonds and hence demonstrating that it was not the last C-terminal residue of RumA.

DNA amplification and sequence. As residues 9 and 12 still remained unidentified, two degenerate oligonucleotide primers were designed on the basis of residues 1 through 6 and 17 through 21 of the active peptide, respectively; all the possible encoding sequences were represented. These two primers, Ol1 and Ol2, were then used to amplify total DNA extracted from the E1 strain. One main amplified fragment could be visualized, sizing the expected value at around 100 bp (data not shown). As direct sequencing with the 221 and reverse primers was not possible, this fragment was cloned onto the pBR322 vector and the nucleotide sequence was obtained.

The peptide sequence deduced from the DNA sequence exhibited high levels of homology with the sequences obtained previously by biochemical techniques (Table 3), demonstrating that the amplified fragment was part of the structural gene coding for the active RumA. Two amino acids not previously identified by biochemical techniques could be deduced from the DNA sequence. They corresponded to one Ser and one Cys residue, Ser9 and Cys12, respectively.

Compared with databases, the RumA peptide sequence exhibited high similarity to various other lantibiotics belonging to
group AII of the lantibiotics (21) (Fig. 4). This result was in agreement with the previous detection of a Lan residue.

**Cyanogen bromide cleavage.** RumA was incubated for 72 h in the presence of CNBr and then analyzed by mass spectrometry. The action of CNBr resulted in a single peptide with a 31-Da loss compared to the 2,675-Da original molecule. This result was typical of the presence of covalent linkages spanning the Met residue and holding peptide fragments together.

**Resistance of RumA to trypsin.** It had been previously demonstrated that the presence of trypsin was necessary just before the inoculation of strain E1, to promote the generation of antibacterial activity (24). However, a Lys residue (known to be a specific cleavage site for trypsin) was evidenced in the N-terminal region of RumA (Lys6). The solution containing the purified RumA was adjusted to neutral pH, and various amounts of trypsin were added before incubation. The activity of RumA against *C. perfringens* was not affected despite 2 h of incubation in the presence of concentrations of trypsin as high as 500 μg/ml. Control experiments demonstrated that trypsin was active despite the presence of acetonitrile in the reaction mixture. Mass spectrometry analysis of RumA incubated with the highest amount of trypsin revealed the presence of only one peak, with a 2,675-Da mass, confirming that RumA had not been cleaved, probably because Lys6 was not accessible to trypsin.

**Activity spectrum.** The activity of RumA was tested against different target strains chosen as follows: (i) anaerobic gram-negative bacteria representative of the human predominant intestinal microbiota (*Bacteroides* spp.), (ii) anaerobic gram-positive bacteria representative of the human predominant microbiota.

![MS/MS analysis of RumA](image)

**FIG. 3.** MS/MS analysis of RumA. The peaks representing cleavage at peptide bonds are identified by the vertical arrows. It is noticeable that the masses of the two Phe residues present at the C-terminal region of the molecule are identical.

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**TABLE 3. RumA peptide sequence**

<table>
<thead>
<tr>
<th>Origin</th>
<th>Residue at position:</th>
</tr>
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<tbody>
<tr>
<td>Ed.</td>
<td>G, N, G, V, L, K, N</td>
</tr>
<tr>
<td>MS/MS</td>
<td>G, N, G, V, I/L, K</td>
</tr>
<tr>
<td>DNA</td>
<td>G, N, G, V, L, K, T</td>
</tr>
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</table>

*a* Comparison of the peptide sequence information obtained by the Edman degradation technique (Ed.) or MS/MS or deduced from the gene sequence (DNA). Residues present at positions 7, 9, 12, and 16 could not be identified by Edman degradation (NI). Residues at positions 5 and 20 appeared as Leu or Ile (I/L) by MS/MS.
intestinal microbiota (Bifidobacterium spp.) or phylogenetically closely related to the E1 strain (Ruminococcus spp., Eubacterium sp.; Clostridium oroticum and Clostridium nexile), and (iii) pathogenic clostridia (C. difficile, C. perfringens, C. sordellii, C. bifermentans, C. sporogenes, C. septicum, C. histolyticum, and C. botulinum) and the food-borne pathogen B. cereus. Complete results are summarized in Table 1. All the Bacteroides sp. strains tested were resistant to RumA. The Bifidobacterium sp. strains displayed intermediate sensitivity, except for Bifidobacterium longum, which was as sensitive as C. perfringens to RumA. Strains phylogenetically related to R. gravisus were mainly sensitive to RumA, except for Ruminococcus torques and C. oroticum. Pathogenic Clostridium species were sensitive to RumA with the exception of C. sporogenes. Most of the B. cereus strains tested were rather insensitive or resistant to RumA activity. Metronidazole, an antibiotic commonly used in intestinal infections due to anaerobic bacteria (7), was also tested in order to compare the RumA antibacterial activities (Table 1).

**DISCUSSION**

Ruminococcin A (RumA) is the first bacteriocin produced by strictly anaerobic bacteria (R. gravisus) of the human gut so far characterized at the molecular level. Purification was achieved by reverse-phase chromatography, leading to a 10,000-fold increase in specific activity. The presence of Dhb and Lan modified residues, evidenced by amino acid composition, allowed us to conclude that RumA was a lantibiotic.

The first attempt at peptide sequencing was realized by the Edman degradation technique. Seventeen out of the 21 N-terminal amino acids were identified, and then the reaction was blocked. Thus, RumA was subjected to MS/MS analysis. Two series of b ions were obtained. The first one corresponded to the N-terminal cleavage of the molecule. It confirmed seven out of the first eight residues previously identified by Edman degradation and evidenced that the residue at position 7 was a Dhb (Dhb7). The second series, corresponding to the cleavage of residues present in the C-terminal part of the molecule, also confirmed previous results obtained by Edman degradation and highlighted the presence of another Dhb residue (Dhb16).

It had been shown previously that MS/MS detection signals corresponding to Dhb may vary, depending on whether the residues are involved in Lan-Me residue or remain unreacted (16). In our experiments, MS/MS signals corresponding to both Dhb7 and Dhb16 were comparable. As the presence of Me-Lan structures generally leads to blockages during recurrent degradation of lantibiotics (26), these observations strongly suggested that both Dhb residues remained unreacted in RumA.

At this step, Edman degradation and MS/MS techniques made it possible to identify 19 amino acids but neither Cys nor Ser residues capable of generating the lanthionine structure evidenced previously. Thus, degenerate oligonucleotides deduced on the basis of the first six N-terminal residues and the last five C-terminal residues identified, respectively, were used to amplify, clone, and sequence part of the structural gene encoding RumA. The deduced peptide sequence was in agreement with the already known RumA sequence and made it possible to identify two new residues present in the central part of the molecule, Ser9 and Cys12, that had not been detected by biochemical techniques.

However, two observations suggested that the sequence that we were able to obtain was still only partial: (i) the RumA molecular mass measured (2,675 Da) was 288 Da higher than the one calculated from experimental data (2,387 Da), and (ii) the last residue identified by MS/MS, appeared to be involved in two peptide bonds, highlighting the presence of other residues at the C-terminal end of the molecule.

The comparison of the RumA sequence with bacteriocins described already highlighted high levels of homology to group AII of the lantibiotics (21). It was noticeable that the TCCS or TCC motif present at their C-terminal end had not been detected in RumA. DNA sequence data (A. Gomez et al., submitted for publication) confirmed that the TCC motif is also present at the C-terminal part of RumA, and so a most probable structure of the molecule can be proposed (Fig. 5).

![FIG. 5. Hypothetical structure of RumA. Considering the presence of the TCC motif at the C terminus of RumA was confirmed, the measured molecular mass of the molecule (2,675 Da) makes it necessary to consider the presence of two unreacted Dhb, one Lan, and one Me-Lan residue. Since Me-Lan residues are well known for being responsible for blockages during Edman degradation, Dhb7, Dhb16, and Cys12 cannot be involved in such a structure. The association between Ser9 and Cys12 in a Lan structure is not possible, since the CNBr reaction highlighted the presence of a covalent bond spanning the Met14 residue. Thus, it is highly probable that (i) Ser9 and the putative Cys12 and (ii) the putative His22 and Thr24 are associated in a Lan and a Me-Lan residue, respectively. Unreacted Dhb7 and Dhb16 residues are noted as T*; circled numerals 1 and 2 indicate the putative Lan and Me-Lan structures, respectively.](http://aem.asm.org/...)

**Fig. 5.** Multiple sequence alignment of RumA with homologous type IIa lantibiotics. RumA was compared to the peptide sequences of butyrivibrioicin OR79A (Bvi79A) (13), lacticin 481 (LctA) (22), variacin (VarA) (23), streptococcin A-M49 and A-FF22 (ScnA) (8, 9), and mutacin II (MutA).
structure, containing two unreacted Dhb residues and both a Lan and a Me-Lan residue (resulting from the Ser\(^{9}\)-Cys\(^{23}\) and Thr\(^{22}\)-Cys\(^{24}\) associations, respectively), the theoretical molecular mass would fit in perfectly with the measured one. One covalent bond due to the Lan structure would span the Met residue, keeping the CNbr-derived peptides together as observed in other lantibiotics (16).

RumA antibacterial activity is targeted against two groups of microorganisms: phylogenetically \(R\). \textit{gnavus}\)-related bacteria, including the reference strain ATCC 29149, and pathogenic \textit{Clostridium} spp. All lantibiotic-producing strains harbor self-protection mechanisms preventing cell death by the action of their own bacteriocin (1). The genes coding for immunity factors are included in the clusters encoding the biosynthetic machinery (26). As \(R\). \textit{gnavus} ATCC 29149 is sensitive to RumA and was not shown to produce an antibacterial substance, one can conclude that this strain probably does not carry the genetic system responsible for the biosynthesis of RumA. Comparison with metronidazole shows that the activity spectrum of RumA is narrower, since \textit{Bacteroides} spp. are mainly resistant. However, metronidazole and RumA activities against \textit{C. perfringens} and \textit{C. difficile} are comparable, suggesting that RumA could be used as a therapeutic agent targeting these pathogens.

This observation gives reason to believe that RumA could play a double role in gut colonization processes and its protection against invasion by pathogens. It is generally hypothesized that, if produced in the digestive tract, bacteriocins might play a local role. Since their proteinaceous nature makes them sensitive to proteases, a close relationship between the producer and the target strain is necessary, or otherwise they would be destroyed. RumA resistance to trypsin, one of the major host proteases, indicates that it could constitute a powerful weapon in bacterium-bacterium antagonisms potentially applicable to human health.

Previous results suggested that RumA was involved in the induction of bacteriocin synthesis rather than in the hypothetical activation of the secreted peptide by proteolysis (24). Additional studies aiming to characterize the genetic determinants of RumA biosynthesis and determine the exact role of trypsin are in progress.

**ACKNOWLEDGMENTS**

We are indebted to M. Laurière and C. Beaumeliot for constructive discussion and to A. M. Wall for correcting the manuscript.

This work was supported by the grants Actions Concertées Coordonnées Sciences du Vivant from the French Ministry for Research and Technology and FAIR CT 95-0433 from the European Community.

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