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Lactobacillus rhamnosus GG Outcompetes Enterococcus faecium via Mucus-Binding Pili: Evidence for a Novel and Heterospecific Probiotic Mechanism

Hanne L. P. Tytgat,[a] François P. Douillard,[b] Justus Reunanen,[b] Pia Rasinkangas,[b] Antoni P. A. Hendrickx,[c] Pia K. Laine,[d] Lars Paulin,[d] Reetta Satokari,[b,e] Willem M. de Vos[a,b,e]

Laboratory of Microbiology, Wageningen University, Wageningen, The Netherlands[a]; Department of Veterinary Biosciences, University of Helsinki, Helsinki, Finland[b]; Department of Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands[c]; Institute of Biotechnology, University of Helsinki, Helsinki, Finland[d]; RPU Immunobiology, Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland[e]

ABSTRACT

Vancomycin-resistant enterococci (VRE) have become a major nosocomial threat. *Enterococcus faecium* is of special concern, as it can easily acquire new antibiotic resistances and is an excellent colonizer of the human intestinal tract. Several clinical studies have explored the potential use of beneficial bacteria to weed out opportunistic pathogens. Specifically, the widely studied *Lactobacillus rhamnosus* strain GG has been applied successfully in the context of VRE infections. Here, we provide new insight into the molecular mechanism underlying the effects of this model probiotic on VRE decolonization. Both clinical VRE isolates and *L. rhamnosus* GG express pili on their cell walls, which are the key modulators of their highly efficient colonization of the intestinal mucosa. We found that one of the VRE pilus clusters shares considerable sequence similarity with the SpaCBA-SrtC1 pilus cluster of *L. rhamnosus* GG. Remarkable immunological and functional similarities were discovered between the mucus-binding pili of *L. rhamnosus* GG and those of the clinical *E. faecium* strain E1165, which was characterized at the genome level. Moreover, *E. faecium* strain E1165 bound efficiently to mucus, which may be prevented by the presence of the mucus-binding SpaC protein or antibodies against *L. rhamnosus* GG or SpaC. These results present experimental support for a novel probiotic mechanism, in which the mucus-binding pili of *L. rhamnosus* GG prevent the binding of a potential pathogen to the host. Hence, we provide a molecular basis for the further exploitation of *L. rhamnosus* GG and its pilins for prophylaxis and treatment of VRE infections.

IMPORTANCE

Concern about vancomycin-resistant *Enterococcus faecium* causing nosocomial infections is rising globally. The arsenal of antibiotic strategies to treat these infections is nearly exhausted, and hence, new treatment strategies are urgently needed. Here, we provide molecular evidence to underpin reports of the successful clinical application of *Lactobacillus rhamnosus* GG in VRE decolonization strategies. Our results provide support for a new molecular mechanism, in which probiotics can perform competitive exclusion and possibly immune interaction. Moreover, we spur further exploration of the potential of intact *L. rhamnosus* GG and purified SpaC pilin as prophylactic and curative agents of the VRE carrier state.

For years, enterococci were viewed as harmless endogenous members of the human microbiota (1), with some strains even being used as probiotics (2). However, over 25 years ago, concerns started to grow as vancomycin-resistant enterococci (VRE) developed into a rapidly spreading nosocomial threat (3–6).

Presently, the prevalence of nosocomial infections caused by VRE is escalating globally (4, 5, 7, 8). These *Enterococcus* species are successful nosocomial agents since they are excellent colonizers, show striking genome plasticity, and persist for long periods of time in the intestinal tract, on medical devices, or on household appliances (4). Especially in immunosuppressed patients, VRE rapidly colonize the small intestine, cecum, and colon, thus outcompeting healthy gut microbiota. The virulence factors of VRE have yet to be fully unraveled, but the high colonization capacity of VRE is considered to be the main factor contributing to its pathogenicity (7, 9, 10). Moreover, VRE are remarkably efficient at acquiring antimicrobial resistances, further enhancing their persistence and colonization potential. The multiple antibiotic resistances of these enterococci are of special concern, as treatment of these infections becomes increasingly challenging. This further stimulates gut colonization by VRE, as the healthy gut microbiota

is detrimentally affected during antibiotic treatment courses. The rapid acquisition of vancomycin resistance genes (*van* genes) is particularly worrisome (7, 8, 11). Vancomycin is the go-to antibiotic used to treat infections of Gram-positive bacteria that are known to be antibiotic resistant, and it is featured on the list of essential medicines of the WHO (12).

While initially the highly pathogenic *Enterococcus faecalis* was viewed as the major threat, it has been surpassed slowly but surely

by its less pathogenic relative *Enterococcus faecium*. This trend is especially alarming, as *E. faecium* is highly efficient in the acquisition and accumulation of antibiotic resistance genes (4, 8, 10, 11).

To win the arms race between this bacterium and the search for new antibiotics, the need to evaluate alternative strategies is high. Several experts point out that the high colonization capability of *E. faecium* is the key feature to tackle (7, 9, 13). A plethora of strategies has been proposed, including "using the good to weed out the bad," i.e., the administration of probiotics as a decolonization strategy (13, 14). In this respect, the capacity of *Lactobacillus rhamnosus* GG to eradicate VRE carriage has been evaluated in multiple clinical trials (13, 15–17). Administration of yogurt containing *L. rhamnosus* GG to renal patients resulted in the clearance of VRE in the treatment group, with most patients remaining VRE negative for at least 4 weeks after trial completion (16). In a Polish trial focusing on VRE-colonized children, *L. rhamnosus* GG treatment resulted in the temporary elimination of the VRE carrier state (17). In another clinical trial, focusing on adults with comorbidities, *L. rhamnosus* GG seemed to have no effect on VRE colonization, which may be due to the comorbidities presented by the patients and the short administration period (15). Based on these clinical trials and the revision of new case studies, Cheng and coworkers have suggested that a combination of antimicrobial therapy (including patient isolation and disinfection) and probiotic treatment with *L. rhamnosus* GG may hold the key for successful sustained decolonization of gastrointestinal carriage of VRE (13).

In this work, we provide new molecular insight into the mechanism underlying the role of *L. rhamnosus* GG in consolidation of VRE decolonization. Various Gram-positive species, including some lactobacilli, harbor pilus gene clusters that may be expressed as proteinaceous cell wall appendages, which contribute to their adhesion and signaling capacities (18–20). *L. rhamnosus* GG produces sortase-dependent pili that are decorated with the mucus-binding protein SpaC, to which its colonization capacities are attributed (21–24). Earlier work reported the enrichment of intact pilus gene clusters (containing LPXTG-type pilin proteins and a cognate class C-type sortase) in hospital-acquired *E. faecium* isolates (25, 26). We determined the complete genome sequence of the clinical *E. faecium* strain E1165. Comparison of pilin gene cluster 3 (PGC-3) of *E. faecium* E1165 and the SpaCBA pili of *L. rhamnosus* GG revealed a high level of sequence similarity between the two pilus gene clusters. Hence, we tested the capacity of the *L. rhamnosus* GG pili, and in particular the adhesive SpaC subunit, as well as antibodies raised against these components to interfere with *E. faecium* E1165 and its mucus interaction. Our findings not only demonstrate the existence of a novel immunogenic probiotic mechanism in the widely exploited intestinal isolate *L. rhamnosus* GG, but they also hold great promise for the development of prophylactic treatments.

MATERIALS AND METHODS

Bacterial strains and culture conditions. *Lactobacillus rhamnosus* GG and its pilus-deficient derivative strain PB12 were grown at 37°C in MRS broth (Difco) in nonshaking conditions. *Enterococcus faecium* was grown stationary in brain heart infusion (BHI) broth (Difco) at 37°C. The three strains were grown in semianaerobic conditions, i.e., stationary growth and minimal oxygen. *Escherichia coli* was cultured aerobically at 37°C in LB medium under shaking conditions.

Genome sequencing. The genomic DNA of *E. faecium* isolate E1165 was extracted using the Wizard genomic DNA purification kit (Promega) per the manufacturer's recommendations. Genomic DNA sequencing of *E. faecium* isolate E1165 was performed using the Pacific Biosciences se-

quencing technology of the DNA Sequencing and Genomics Laboratory of the Institute of Biotechnology, University of Helsinki. In short, the PacBio library kit was used to construct a library with size targeted to 10 kb. Two single-molecule real-time (SMRT) cells were run on a PacBio RS II system using P6/C4 chemistry and 240 min of video time. A total of 78,536 reads (N_{50} length, 13,229 bp) were assembled using the HGAP.3 implemented in the SMRT analysis system (smrtanalysis_2.0.3; Pacific Biosciences). Apart from the complete chromosome (2,766,743 bp), a megaplasmid of 192,533 bp and one smaller plasmid of 32,402 bp were obtained with mean coverages of 190×, 210×, and 260×, respectively. Additionally, a circular phage genome (38,742 bp, 50×) was found. Using the RAST server (27), 3,024 open reading frames (ORFs) were predicted in the *E. faecium* E1165 genome and phage.

Production of antiserum. Serum samples were obtained from four patients that had *E. faecium* bacteremia. Samples were obtained during normal blood withdrawal and were approved by the medical ethical committee of the University Medical Center of Utrecht. PilB-specific rabbit immune serum and SpaC antiserum were produced as described earlier (25, 28). Antiserum against *L. rhamnosus* GG was produced as described recently (29). Controls with preimmune sera of PilB, SpaC, and *L. rhamnosus* GG were performed earlier and rendered negative results (25) (results not shown).

Production of recombinant SpaC protein in *E. coli*. The coding sequence of *spaC* (*LGG_00444*), excluding the C-terminal cell wall sorting signal and the N-terminal signal peptide, was PCR amplified from a codon-optimized *spaCBA* gene cluster (primers BG3764 ttcTCATGActg ataacattcgcccaacc and BG3765 ttcGCGGCCGCcggcaaaattgcaagtgg). The purified PCR product was digested with BspHI and NotI (marked in capital letters in the primer sequences) and cloned into the pWUR533 vector, resulting in pWUR533_SpaC. This vector is a derivative of the pET52-1b vector in which the C-terminal thrombin protease site was replaced by an HRV 3C protease site and a Strep-tag II instead of a His tag. One percent of an overnight culture of *E. coli* BL21(DE3)/pSJS1244, in which the pWUR533_SpaC vector was introduced, was grown in LB medium with ampicillin (100 µg/ml) and spectinomycin (50 µg/ml). After 3 h of incubation at 37°C, protein production was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM). Three hours later, cells were harvested by centrifugation. Purification of the recombinant SpaC protein was performed in accordance with the Strep-Tactin resin manufacturer's recommendations (IBA). The Strep-tag was removed using the HRV 3C protease (EMD Millipore) following the protocol of the manufacturer. The tag and protease were subsequently removed using a Vivaspin 20 centrifugal concentrator (Sartorius).

Mucus-binding assays. Assessment of the binding of strains to mucus was performed as described earlier (30, 31). Bacterial cells were grown overnight in an appropriate medium supplemented with 1 mCi/ml [³H]thymidine (Perkin-Elmer Life Sciences). Bacteria (optical density at 600 nm [OD_{600}], 0.25) were applied to a Nunc MaxiSorp plate coated with 0.5 mg/ml type II porcine mucus (Sigma-Aldrich, MO, USA). Optionally, 1/100 dilutions of antisera against total *L. rhamnosus* GG cells (raised as described in reference 29) and the SpaC subunit of the pili of *L. rhamnosus* GG (28) were added immediately to allow competition. Recombinant SpaC was added in a final concentration of 4.6 ng/µl. Bacteria were allowed to bind to mucus for 1 h at 37°C. After this, the plate was washed a few times with phosphate-buffered saline (PBS) prior to cell lysis (1 h at 60°C). The ³H measurement protocol has been extensively described previously (31).

Immunogold electron microscopy. Immunogold electron microscopy was performed as described earlier with minor modifications (21, 23, 25). In the first labeling step, the grids were incubated with rabbit SpaC antiserum diluted 1:120 in blocking solution or PilB antiserum diluted 1:5, followed by washing with 0.1% bovine serum albumin (BSA) in PBS. In the case of double-labeling experiments, a second labeling was performed using antisera in similar concentrations as described earlier. The grids were analyzed using a JEM-1400 transmission electron microscope (JEOL).
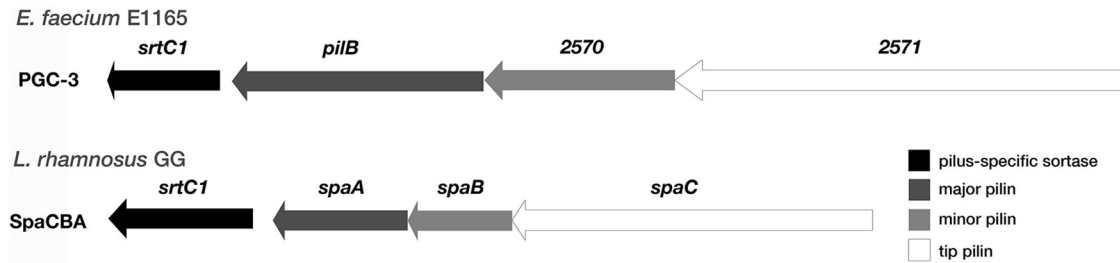
FIG 1 Sequence homology of pilus gene clusters of *Enterococcus faecium* E1165 and *Lactobacillus rhamnosus* GG. Pilus gene clusters of both *E. faecium* E1165 (~99% similar to the TX16 type strain) (25) and *L. rhamnosus* GG (23) are shown. Overall, the clusters have a similar buildup and share a high sequence homology (>50% similarity), and both harbor a sortase (black), a tip adhesin (white), and two pilins (gray). The tip pilins of both strains ORF *2571* (TX16 and E1165) and *spaC* share 33% identity (49% positive matches). Arrow sizes reflect gene sizes.

Data analysis. Data analysis was performed using GraphPad Prism 6. Significant differences between two values were calculated using paired *t* tests, and the significance level was set at a *P* value of <0.05.

Accession number(s). The complete circular genome, the two plasmid sequences, and phage sequences were deposited in the European Nucleotide Archive (ENA) under project ID PRJEB12401 and genome accession numbers LN999987 to LN999990.

RESULTS

Homology of pili of hospital-associated VRE and *Lactobacillus rhamnosus* GG. To grasp the molecular basis of the decolonization effect of *L. rhamnosus* GG on VRE shown in clinical trials (13), we focused on their surface adhesion molecules. Specifically, SpaCBA pili of *L. rhamnosus* GG are described as primary adhesive molecules, which explains the mucus adhesion capacity of this probiotic (21, 22, 24, 31, 32). In addition, enterococci are known to produce pili that are important in colonization and adhesion to (a)biotic surfaces (19, 33, 34). The *E. faecium* type strain TX16 harbors four distinct pilin gene clusters (PGCs) (35), and hospital isolates of *E. faecium* are enriched in PGCs (25, 26).

A homology search exploring potential similarity between the PGCs of *L. rhamnosus* GG and *E. faecium* resulted in a striking similarity (>50%) between the SpaCBA-SrtC1 pilus cluster of *L. rhamnosus* GG and the PGC-3 cluster of *E. faecium*. Scanning the organization of the PGC-3 gene cluster, harboring the PilB-type pili (25), revealed a similar sequence and organization as the well-studied SpaCBA pili of *L. rhamnosus* GG (21, 22, 24, 36) (Fig. 1). Closer investigation revealed that the SpaC pilin of *L. rhamnosus* GG was 33% identical to the tip pilin protein sequence of the *E. faecium* TX16 strain (ORF *2571*). This similarity rose up to 49% when taking the amount of positive matches into account. Overall, mapping of the SpaC protein sequence to the *E. faecium* taxonomy identification number (taxid) 1352 revealed that in some strains, the sequence similarity even surpasses 50% (54% and up to 65% when considering positive matches) (data not shown).

As a case study for hospital acquired VRE, we focused on the clinically relevant *E. faecium* strain E1165, originally isolated from the wound/soft tissue of a hospitalized patient in 1997 (Genoa, Italy). Expression of the PGC-3 cluster-encoded PilB-type pili in *E. faecium* E1165 was already confirmed previously (25). We used PacBio RS II long-read sequencing technology to determine the complete genome sequence of strain E1165, which was deposited in the European Nucleotide Archive (ENA) under project ID PRJEB12401 and genome accession numbers LN999987 to LN999990. Detailed analysis of the E1165 genome revealed that the 2.766-Mb large genome contains three pilin gene clusters, as

well as an additional pilin gene cluster (PilA gene cluster or PGC-1) located on a megaplasmid of 192.533 kb (37). The four gene clusters of E1165 are highly (~99%) homologous to the PGCs of the TX16 type strain (data not shown).

Cross-reactivity of antibodies reveals structural and immunological similarities between pili of *E. faecium* E1165 and *L. rhamnosus* GG. To explore the functional relationship between the *L. rhamnosus* GG and *E. faecium* E1165 pili, we performed immunogold electron microscopy experiments using antibodies directed against the pilin subunits of each of these strains. Interestingly, antibodies raised against the SpaC subunit of the SpaCBA pili of *L. rhamnosus* GG specifically bound to the pili on the surface of E1165 (in immunogold labeling experiments) (Fig. 2). These results suggest that the sequence similarity between the SpaC pilin of *L. rhamnosus* GG and the tip pilin of E1165 may be indicative of a stronger, antigenic relation between the two pilus clusters. Next, we sought to investigate the nature of the targeted pili by setting up a double-labeling experiment that included both antibodies raised against the SpaC pilin of *L. rhamnosus* GG and PilB of E1165. Immunogold electron microscopy revealed that PilB is located in the same *E. faecium* pilus fiber that also reacts with gold-labeled antibodies against SpaC. This colocalization of both antibodies on
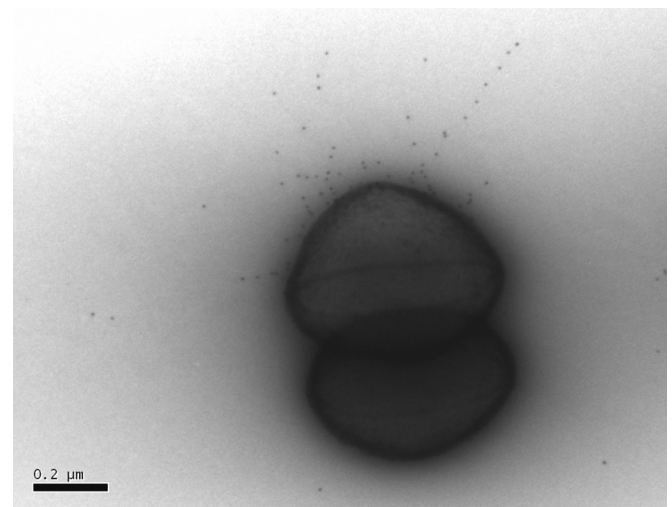

FIG 2 *L. rhamnosus* GG SpaC pilin-specific antibodies bind to E1165 pili. Immunogold labeling microscopy using SpaC antibodies shows binding of these *L. rhamnosus* GG major pilin-specific antibodies to the pili of *E. faecium* E1165 cells. Representative experimental results are shown (scale bar, 200 nm).
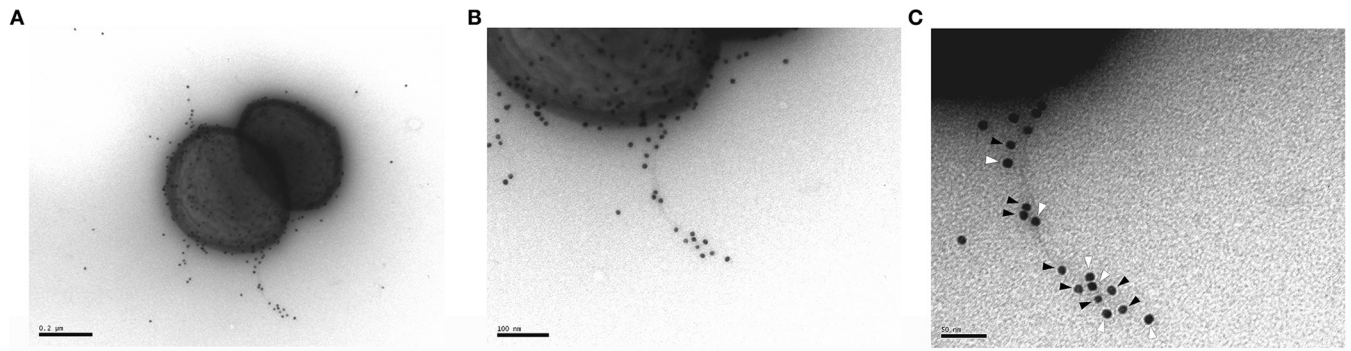
FIG 3 *L. rhamnosus* GG SpaC pilin antibodies colocalize with *E. faecium* PilB pilin antibodies on E1165 pili. *E. faecium* E1165 cells were incubated with gold particles either labeled with PilB antibodies (10-nm particles, black arrows in C) or SpaC antibodies (15-nm particles, white arrows in C). Both labels colocalize on the pili of E1165, indicating their specificity for the PilB-type pili of *E. faecium* E1165 expressed by PGC-3. (A) Complete E1165 cells (scale bar, 200 nm). (B and C) Zoomed-in image of the labeled pili (scale bars, respectively, 100 nm and 50 nm). Results of a representative experiment are shown.

the pili of E1165 confirms them being PilB-type pili expressed by PGC-3 (Fig. 3). This cross-reactivity of *L. rhamnosus* GG and E1165 pilin-specific antibodies illustrates that these pili are not only related on a genetic level but also on an antigenic level. The sequence homology seems to result in structurally related pilins, thus explaining the cross-reactivity of the antibodies.

Next, we set out to relate these results to the clinical effect of *L. rhamnosus* GG administration on VRE colonization (13, 16, 17). Serum samples of patients with VRE bacteremia and healthy persons were isolated. Immunogold electron microscopy revealed that the serum samples of all VRE-colonized patients bound to *L. rhamnosus* GG, with the highest binding density on the SpaCBA pili (Fig. 4A and B). As expected, this interaction was absent when testing serum samples from healthy patients (Fig. 4C). These results suggest that the pili of both species are indeed related and that their sequence and antigenic homology might underpin the clinical results.

L. rhamnosus GG-derived molecules effectively displace *E. faecium* from mucus. Ultimately, we investigated whether the sequence and antigenic homology between the pili of *L. rhamnosus* GG and E1165 could be exploited in the decolonization of *E. faecium* as suggested by clinical trials (13). As pili are known to be key in the colonization and adhesion of microbes in the human intestinal tract by binding to human mucus (38, 39), we investigated the mucus-binding capacities of *L. rhamnosus* GG and *E. faecium* E1165 in the presence and absence of specific antibodies against the former.

In accordance with earlier results, *L. rhamnosus* GG strongly

bound to mucus (21, 24, 31). This interaction may be attributed largely to the presence of the SpaCBA pili, as a pilus-deficient derivative of *L. rhamnosus* GG, strain PB12, can only minimally adhere to mucus (31) ($P < 0.0001$) (Fig. 5). *E. faecium* E1165 was also found to interact strongly with the mucus (Fig. 5). Interaction levels were significantly higher ($P < 0.05$) for strain E1165 than those for *L. rhamnosus* GG, which may be indicative of a stronger binding of E1165 to mucus or the presence of more pili per cell. In view of the similarity between the pili of *E. faecium* and those of *L. rhamnosus* GG, we hypothesized that the *E. faecium* E1165 pili are the principal actors of its strong mucus adhesion. Hence, we sought to study the capacity of antibodies raised against *L. rhamnosus* GG and its SpaC pilin to affect *E. faecium* E1165 mucosal adhesion. Antibodies that were raised against the SpaC adhesive pilin effectively interacted with the binding of E1165 to mucus (Fig. 5). In addition, antibodies raised against intact *L. rhamnosus* GG cells could significantly interfere with *E. faecium* E1165 mucus binding ($P < 0.0001$) (Fig. 5). The effectiveness of the anti-*L. rhamnosus* GG antibodies can most probably be explained by the presence of reactive anti-SpaC antibodies in the polyclonal serum. Finally, the SpaC pilin produced and purified from *E. coli* was found to significantly reduce ($P < 0.05$) the mucus binding of *E. faecium* E1165 (and *L. rhamnosus* GG) (data not shown) (Fig. 5). In conclusion, these data indicate that *L. rhamnosus* GG and *E. faecium* E1165 pili compete for the same binding sites on mucus.
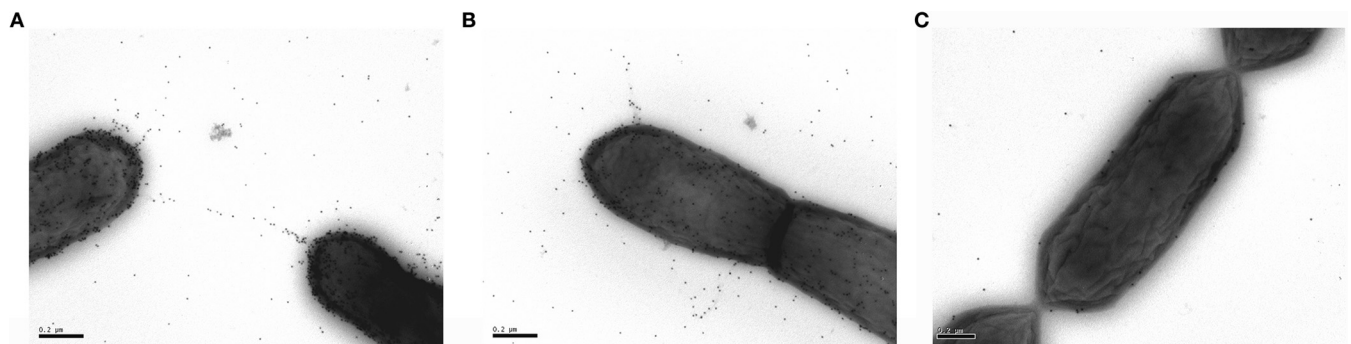

FIG 4 Serum from VRE-colonized patients interacts with *L. rhamnosus* GG pili, as shown by immunogold labeling microscopy of *L. rhamnosus* GG in patient serum samples. (A and B) Serum samples from two different patients infected with VRE. (C) Serum from a healthy patient (scale bar, 200 nm). Pictures of representative experiments are shown.
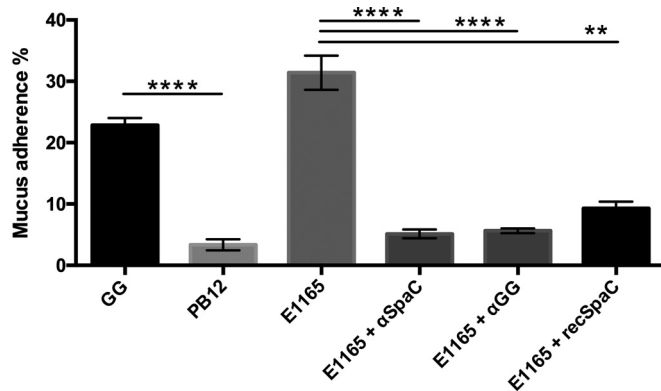
FIG 5 *L. rhamnosus* GG-specific antibodies and purified SpaC pilin interfere with the mucus-binding capacity of *E. faecium* E1165. The high adhesion capacity of *L. rhamnosus* GG to mucus can be explained by the presence of its SpaCBA pili, as adhesion of PB12, the mutant lacking pili, significantly drops ($P < 0.0001$). *E. faecium* E1165 can adhere well to mucus. Antibodies raised against the SpaC pilin of *L. rhamnosus* GG significantly reduce binding of *E. faecium* E1165 to mucus ($P < 0.0001$), as do antibodies raised against complete *L. rhamnosus* GG cells ($P = 0.0004$). The mucus-binding SpaC pilin purified from *E. coli* may also significantly interfere with binding of E1165 to mucus ($P = 0.0011$). Error bars depict the standard error of the mean (SEM) and data represent findings from at least triplicate experiments.

DISCUSSION

Infections with vancomycin-resistant *E. faecium* or VRE have become a major nosocomial threat (4). Effective treatment can no longer rely on the application of antibiotics, as these bacteria are extremely efficient at accumulating antibiotic resistance genes in their genomes (11). This is related to the notion that multidrug-resistant enterococci lack the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas system, a natural defense system (40). The full genome sequence of *E. faecium* strain E1165 did not reveal the presence of any CRISPR-Cas-related genes, reflecting its capacity to rapidly acquire antibiotic resistance genes (CRISPRdb screening [41]; results not shown).

New strategies to tackle VRE are currently being explored, including the implementation of probiotics such as *L. rhamnosus* GG (13). Several clinical trials have explored the efficacy of *L. rhamnosus* GG to interfere with VRE colonization in different setups (13, 15–17). Various studies, focusing on renal patients (16), children (17), and patients suffering from endocarditis and liver problems (13), illustrated the efficacy of *L. rhamnosus* GG administration in sustained colonization resistance against VRE. The effect of *L. rhamnosus* GG on VRE decolonization has been consistently demonstrated by all successful clinical trials. As the SpaCBA pili of *L. rhamnosus* GG are the main actors of its high adhesive phenotype, we sought to investigate the potential role of these molecules in its beneficial clinical effect against VRE colonization. Intriguingly, the SpaCBA pili of *L. rhamnosus* GG show a striking sequence homology to the PilB-type pili of enterococci encoded by the PGC-3 cluster. This is of special importance, as pilus gene clusters are enriched in pathogenic VRE strains (25, 26). A clinical isolate, *E. faecium* E1165, was earlier shown to express these PilB-type pili (25). The complete genome analysis of E1165 confirmed the presence of an intact PGC-3 cluster in its genome, and this strain was further used throughout the study.

Immunogold electron microscopy experiments revealed that the pili of *L. rhamnosus* GG and *E. faecium* E1165 not only share a

high sequence homology but are also immunologically related. In particular, antibodies raised against the SpaC pilin of *L. rhamnosus* GG specifically interacted with the pili of *E. faecium* E1165. Further double-labeling experiments using specific antibodies against SpaC and PilB resulted in their colocalization on the PilB pili of *E. faecium* E1165. These results underline the antigenic relation between the two pilus clusters. Furthermore, we can relate these results to the clinical trials (13), as patient serum samples from VRE carriers interacted with *L. rhamnosus* GG cells and, in particular, with the surface-exposed and mucus-binding pili.

As colonization of the human intestine is most likely to rely predominantly on the interaction between bacteria, and more particularly on their adhesive pili, and the intestinal mucus (38, 39), the effect of *L. rhamnosus* GG on VRE mucus-binding was explored. These experiments resulted in the conclusion that antibodies raised against *L. rhamnosus* GG and its SpaC pilin interfered with the mucus-binding capacity of *E. faecium* E1165. The importance of the pili in this effect was further underpinned by the capacity of the purified SpaC pilin to drastically reduce the binding of *E. faecium* E1165 to mucus.

Based on our results, we suggest a novel probiotic mechanism in which probiotics are not only applied to perform interspecies competitive exclusion but are also inducers of cross-immunity against potential pathogens. The effect of *L. rhamnosus* GG on the decolonization of VRE implies a specific mechanistic effect of this probiotic on the gut community, which has not been reported to date.

In this work, we have successfully elucidated a molecular mechanism underlying the successful application of *L. rhamnosus* GG to eradicate *E. faecium* in several clinical trials and spur the further implementation of *L. rhamnosus* GG in VRE treatment (13). *L. rhamnosus* GG and its purified SpaC pilin can play important roles in the improvement of VRE treatment, the consolidation of its decolonization, and the development of effective strategies to tackle the (asymptomatic) carrier state of VRE. Our results shed light on a unique mechanism to specifically target VRE without harming commensal microbiota.

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