

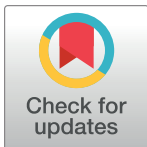
PEARLS

Sugarcoating it: Enterococcal polysaccharides as key modulators of host–pathogen interactions

Yusibeska Ramos¹, Stephanie Sansone^{1,2}, Diana K. Morales^{1*}

1 Department of Obstetrics and Gynecology, Weill Cornell Medicine, New York, New York, United States of America, **2** Department of Urology, Weill Cornell Medicine, New York, New York, United States of America

* dim2018@med.cornell.edu



OPEN ACCESS

Citation: Ramos Y, Sansone S, Morales DK (2021) Sugarcoating it: Enterococcal polysaccharides as key modulators of host–pathogen interactions. *PLoS Pathog* 17(9): e1009822. <https://doi.org/10.1371/journal.ppat.1009822>

Editor: Kimberly A. Kline, Nanyang Technological University, SINGAPORE

Published: September 9, 2021

Copyright: © 2021 Ramos et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: DKM has been funded by the Frueauff Foundation Grant, the Anna-Maria and Stephen Kellen Junior Faculty Fellowship and the U.S. Department of Defense Grants (OC190443 and OC200224). DKM and SS have been supported by the June Allyson Memorial Fund Research Award Grant. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

The human gastrointestinal tract (GIT) harbors a diverse microbial ecosystem that plays a critical role in defining health and disease. Members of the bacterial genus *Enterococcus* sp. are common gut-resident microbes that have the potential to cause life-threatening infections when GIT homeostasis is disrupted due to immunosuppression or prolonged antibiotic treatment [1]. *Enterococcus faecalis* and *Enterococcus faecium*, for instance, are predominant gut inhabitants accounting for the majority of hospital-acquired enterococcal infections [2–5]. The pathogenic success of enterococci is partly attributed to their intrinsic tolerance and acquired resistance to diverse antimicrobials, as well as their ability to endure and thrive in harsh environments [1]. Emerging studies suggest that this environmental persistence is in part mediated by polysaccharides [6], which generally consist of repeating units of diverse oligosaccharides covalently linked to the cell surface [7]. Of note, *Enterococcus* sp. possess highly diverse glycobiological arrangements (Fig 1) given their ability to build not only essential structural polysaccharides, such as peptidoglycan (PG), enterococcal polysaccharide antigen (EPA), and wall teichoic acids (WTAs), but also the capacity to synthesize capsule polysaccharides (CPs), lipoteichoic acids (LTAs), and other extracellular polysaccharides [7–11]. These glycans play a prominent role in maintaining bacterial cell integrity and morphology in conjunction with building a dynamic interface with the environment [11,12]. Enterococcal polysaccharides also coordinate major host–pathogen interactions since they modulate cell adhesion and the formation of microbial multicellular communities. Further, these complex sugars have been proposed to promote GIT colonization and penetration of intestinal barriers [13–16] while enhancing resistance to phages, antibiotics, and even the host’s immune system [17–24]. In this review, we discuss multiple mechanisms through which polysaccharides can shape the physiology and pathogenicity of enterococci, with an especial emphasis on *E. faecalis* and *E. faecium*.

Polysaccharides in the development of complex multicellular communities

Enterococci form structurally complex communities (biofilms) in a number of infections, where they become not only difficult to eradicate, but also a source of bacterial dissemination and a reservoir for antibiotic resistance [25]. Biofilms mainly consist of a population of cells bound together and embedded in a self-produced extracellular matrix [26]. The process of biofilm formation generally involves initial surface attachment, aggregate (microcolony) development, structural maturation (or biofilm growth), and cell dispersal [25]. While the role of polysaccharides in biofilm formation has been extensively delineated in other bacterial species

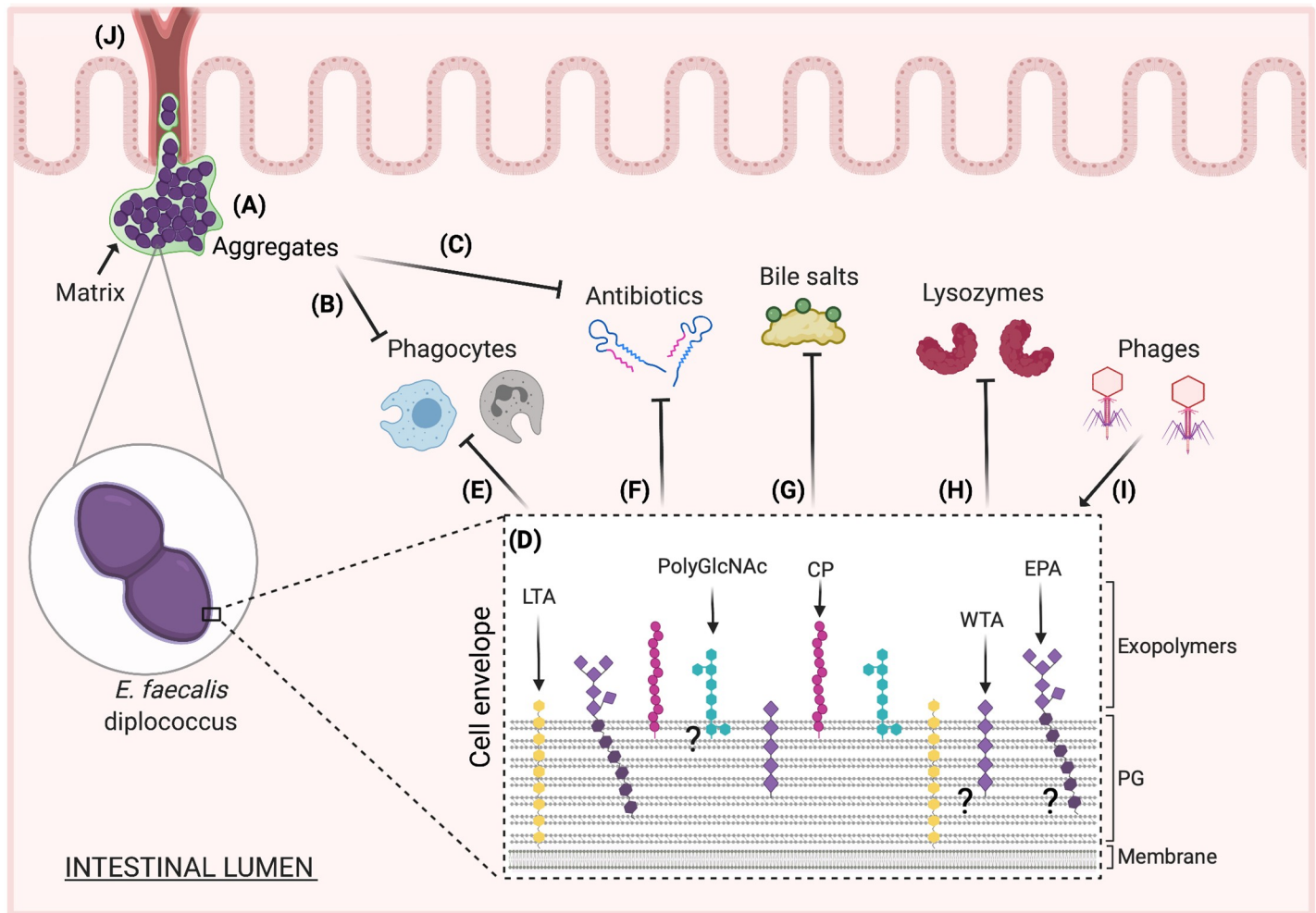


Fig 1. Proposed physiological roles of *Enterococcus faecalis* polysaccharides in the gut. (A) *E. faecalis* intestinal colonization has been proposed to be mediated by the formation of multicellular aggregates covered with and connected by a matrix (green) partly composed of exopolysaccharides [34,69]. (B) Formation of these complex communities may allow enterococci to evade the immune system by promoting survival within phagocytes (macrophages or neutrophils) and (C) to resist the effect of antibiotics produced by other gut bacteria. (D) Cells within these complex communities possess a variety of glycopolymers that promote aggregate formation and structural development [25]. Among these polymers, PG creates a layer that protects cells from osmotic pressure, reinforces cell shape and size, and shelters cell envelope components, including other structural/nonstructural polysaccharides [28]. The proposed scheme was based on previous publications [7,10,11] and shows an enterococcal cell envelope with relative glycobiochemical arrangements and positions. These glycopolymers, from left to right, include the membrane-anchored LTA (yellow), the exopolysaccharides containing polyGlcNAc (cyan), the covalently PG-bound CP (fuchsia), the WTA (light purple) that may also be bound to PG, and the EPA, which possesses a rhamnan backbone (dark purple) possibly anchored to PG and extracellular exposed WTA decorations (light purple). (E) WTAs, LTAs, EPA, and/or CPs allow *E. faecalis* to evade phagocytosis by macrophages or neutrophils [24,76,80,81,84–86]. These cell envelope components likely act as a shield that prevent complement detection and mediate resistance to phagocytosis. Changes to these polysaccharides may disrupt the cell envelope topography, leaving the bacteria naked or likely exposing “neoepitopes” for recognition by the complement system. (F) Changes in PG and EPA structure or composition may also be contributing factors in the resistance to antibiotics, such as those targeting the enterococcal cell envelope [13,17,20,24,53,55,57]. (G) *E. faecalis* may resist the intestinal bile salt toxicity through rearrangements in the composition of EPA and/or PG that counteract the detergent activity of these amphipathic molecules and the induced external osmotic pressure. (H) This bacterium can evade the immune response and persist in the gut by resisting the antimicrobial effect of lysozymes through modifications of their PG structure [81,95–97]. (I) EPA also acts as a “receptor” that is recognized by phages during viral infection, and changes in EPA decoration can confer resistance to these phages by *E. faecalis* [18,47]. (J) Upon intestinal overgrowth, this bacterium can exit the intestinal lumen to reach the bloodstream and colonize distal anatomical sites. The formation of matrix-covered enterococcal aggregates may represent a new mechanism that promotes *E. faecalis* translocation across the gut epithelial barriers in susceptible hosts, where EPA or/and polyGlcNAc-containing exopolymers may facilitate this migration. (?) denotes that the exact localization of EPA, polyGlcNAc-containing polysaccharides, and WTA in the enterococcal cell envelope is uncertain. CP, capsule polysaccharide; EPA, enterococcal polysaccharide antigen; LTA, lipoteichoic acid; PG, peptidoglycan; polyGlcNAc, β -(1,6)-GlcNAc polymers; WTA, wall teichoic acid.

<https://doi.org/10.1371/journal.ppat.1009822.g001>

[26,27], little is known about how these glycans affect the development of enterococcal communities. Below, we summarize recent findings highlighting the critical role of polysaccharides in enterococcal biofilms:

Cell and surface attachment

Enterococcal glycopolymers, such as PG, LTAs, EPA, and other exopolysaccharides, contribute to the initiation of biofilm formation. PG, which consists of a polymeric mesh (PG sacculus) with strands of repeating $\beta(1,4)$ -linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) disaccharides cross-linked through short peptide stems, can undergo constant remodeling in response to changing environmental conditions [28]. Hence, during biofilm growth, it has been shown that *E. faecalis* alters its PG through modifying its peptide stems, increasing cross-linking, *N*-deacetylation, and reducing *O*-acetylation, in comparison to PG extracted from free-living (planktonic) enterococcal cells in vitro [8]. These changes may impact PG's interactions with other ions and the overall cell charge, which modifies *E. faecalis*' ability to attach to surfaces and enhances cell-to-cell aggregation for biofilm formation. PG also contributes to maintaining cell shape and serves as a scaffold for other components involved in attachment and biofilm formation, including cell wall-anchored LPxTG surface proteins, such as enterococcal surface protein (Esp), and the endocarditis and biofilm-associated pili (Ebp) [29–31]. Along with surface proteins, WTAs, LTAs, CPs, and the EPA polymer are sheltered by the PG layer (Fig 1).

LTAs have also been shown to promote the initial steps of biofilm formation, with the interaction of these long anionic polymers with cell surface adhesins facilitating the attachment of *E. faecalis* to itself and/or other surfaces such as epithelial tissues [9]. Furthermore, different modifications in the carbohydrate composition of EPA, a rhamnan polymer backbone (EPA core) that binds to cell wall-exposed WTAs (ribitol teichoic acids; EPA decorations) [10], alter surface hydrophobicity and charge, thus affecting cell-to-cell attachment [22,24]. Several biofilm-related phenotypes have been attributed to this polymer. For instance, while Teng and colleagues reported that mutations in genes encoding the EPA core (*epaA*, *epaB*, *epaE*, *epaN*, and *epaM*; Fig 2) reduced *E. faecalis* OG1RF biofilm formation [32,33], recent studies using the same parental strain demonstrated that mutants involved in either EPA backbone synthesis ($\Delta epaI$ and $\Delta epaQ$) or EPA decoration ($\Delta epaOX$) decreased biofilm biomass only when grown in different settings with subinhibitory concentrations of antibiotics [20,22]. Moreover, strains carrying insertion mutations in *epaJ* or *epaK* showed no phenotypic differences compared with wild-type (WT) biofilms in the described assays [22]. Consistent with these results, it has been demonstrated that biofilm formation is influenced by the growth medium and nutrient availability [34–36] and that the media composition can also affect the polysaccharide profile of a mutant altered in decorating its EPA backbone [14,15]. Hence, these observations suggest that EPA may be modified in response to external growth conditions, impacting enterococcal biofilm development in multiple ways.

Microcolony formation and biofilm maturation

After initial attachment, the continuous growth of a surrounding matrix composed of exopolysaccharides and other macromolecules promotes cell aggregate/microcolony formation and biofilm growth [37]. The EPA polysaccharide, for instance, has been shown to define the *E. faecalis* biofilm architectural arrangement by stabilizing the maturation of the transient aggregates [22,38]. Indeed, deletion of genes in the core or variable region of the *epa* loci (Fig 2) affects *E. faecalis* cell shape, polysaccharide composition, and membrane potential [14,15,22,38]. This, in turn, promotes the formation of microcolonies that exhibit reduced structural integrity, compared with parental biofilms [22,38], likely due to induced changes in enterococcal adhesion traits. *E. faecalis* biofilm architecture has been found to be altered by specific *epa* mutations [20,22]. In contrast to WT (strain OG1RF) or $\Delta epaOX$, which formed biofilms consisting of monolayers or clumped cells and long chain areas, respectively [20,22],

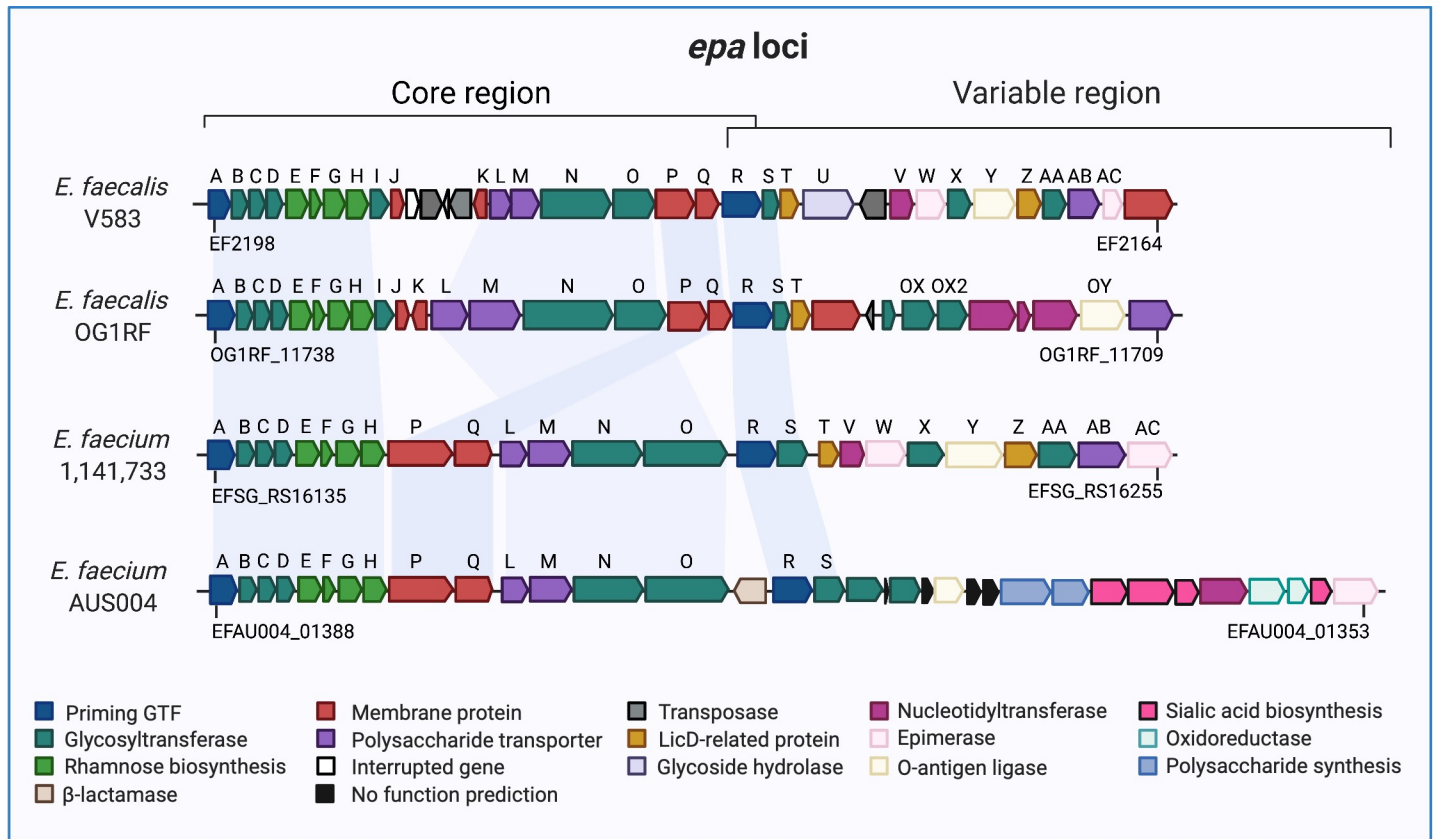


Fig 2. Comparison of predicted *epa*-like loci among enterococcal strains. In *Enterococcus faecalis* V583 and OG1RF, EPA biosynthesis is encoded by a cluster of genes organized in 2 genetic loci: first, a conserved region, consisting of 18 genes, from *epaA* to *epaR*, which participate in the rhamnan backbone biosynthesis (core region), and, second, a downstream cluster of approximately 10–20 genes exhibiting genetic variability among strains, which has been proposed to account for the major differences on EPA decoration among *E. faecalis* isolates (variable region) [10,14,32,105]. The core region of *Enterococcus faecium* is differentially organized in comparison with the EPA core from *E. faecalis*. It does not have homologs of *epaI*, *epaJ*, and *epaK*. Instead, it has the 2 genes, *epaP* and *epaQ*, located at that site [104,105,107]. *E. faecium*'s variable locus is proposed to be divided into 4 main variants based on sequence similarities [104,107]. The scheme depicts variants 2 and 4 for strains 1,141,733 and Aus0004, respectively. Arrow colors correspond to colored boxes (bottom) and indicate predicted open reading frame function according to genome annotations and BLASTP analysis. Blue shades connect homologs of different strains. The absence/variability of *epa* genes suggests that the EPA polysaccharides of the 2 species have different sugar compositions, and, in consequence, may confer diverse physiological functions. The *epa* loci were adapted from [10,20,32,54,104,105,107]. All genes are drawn to scale. EPA, enterococcal polysaccharide antigen; GTF, glycosyltransferase.

<https://doi.org/10.1371/journal.ppat.1009822.g002>

ΔepaQ developed thicker structures of combined architectures including cells arranging in monolayers and large aggregates [22]. While the chemical nature of the exopolysaccharides composing the enterococcal matrix is still unclear, our group identified by immunostaining that *E. faecalis*' aggregates are covered by exopolysaccharides formed partly by polymers of β-(1,6)-GlcNAc (polyGlcNAc) [15]. Although the precise structure of these polysaccharides has not been fully elucidated, we demonstrated that the deletion of *epaX* in *E. faecalis* V583, a gene involved in EPA decoration (Fig 2) [10], caused a marked reduction in the matrix surrounding the aggregates [15]. Notably, this defect correlated with the lack of detection of polyGlcNAc-containing polymers by antibody-based methods, in comparison with the parental counterpart [15]. Studies by Guerardel and colleagues did not find detectable amounts of polyGlcNAc-containing polymers in planktonic cultures of *E. faecalis* [10], indicating that the culture conditions may account for such observations by impacting *E. faecalis* exopolysaccharide production. In fact, it has been reported that the exopolymers' chemical composition can be altered in response to the specific nutrients supplemented [39]. Future work using different

growth conditions may help explain the link between *epaX* and the production of the poly-GlcNAc-containing exopolysaccharides, which might be involved in polyGlcNAc synthesis and/or contribute to retention or binding of the exopolymer through negatively charged EPA decorations. Since the absence of polyGlcNAc-like exopolysaccharides decreases the ability to develop structured biofilms in other gram-positive bacteria [40,41], additional biophysical and chemical analyses are warranted to comprehensively understand the role of enterococcal poly-GlcNAc-containing exopolymers in aggregate/biofilm formation and its matrix physiology.

Polysaccharides in enterococcal adaptation to harsh conditions

Bacteria have evolved sophisticated mechanisms to survive in hostile environments, including the human body. Polysaccharides provide an adaptive advantage to cope with such external stressors. In biofilms, for instance, modifications of exopolysaccharides during glucose starvation increased *E. faecalis*' hydrophobicity, enabling survival in energy-depleted environments such as root canals [42]. Moreover, *E. faecium* probiotic strains have been shown to produce exopolysaccharides that can scavenge reactive oxygen species in vitro, suggesting a potent antioxidant defense mechanism mediated by these glycopolymers [43]. Below, we summarize how polysaccharides promote enterococcal resistance to some environmental stressors.

Resistance to osmotic stress

EPA serves a protective role for enterococci in the GIT where bile salts disrupt bacterial membranes and cause osmotic stress [44]. Intestinal bacteria resist bile by activating efflux systems and remodeling their cell envelope [45]. Under high osmolarity, *E. faecalis* is capable of up-regulating expression of *epa* genes, especially those that code for rhamnose biosynthesis and glycosyltransferases. For example, mutants in the *epa* core region (Fig 2), $\Delta epaB$ and $\Delta epaR$, exhibit increased susceptibility to salt-induced osmotic stress [46,47]. Moreover, the absence of *EpaOX* considerably affects cell envelope integrity [38] by compromising decorations of rhamnan backbone required for optimal polymerization of PG by penicillin-binding proteins [24]. When intact, the PG sacculus protects cells from osmotic rupture and reinforces cell shape and size [28]. Indeed, strains $\Delta epaOX$ in OG1RF and $\Delta epaX$ in V583 showed an altered cell morphology and an increased sensitivity to bile salt components [14,20], suggesting that EPA and PG may play an important role during the remodeling of the cell envelope to counteract changes in the external osmolarity in enterococci.

Resistance to antibiotics

Polysaccharides are also key players in mediating resistance to antibiotics. PG is a major target for many antimicrobials in bacteria including β -lactams and glycopeptides (vancomycin). Enterococci have evolved multiple mechanisms to counteract these antibiotics (for comprehensive reviews, see [48,49]). *E. faecium*, for instance, modifies its PG intermediaries by replacing the D-alanine–D-alanine (vancomycin's target) with D-alanine–D-lactate as the terminal amino acids in lipid II [50]. This modification decreases the interaction between vancomycin and PG due to loss of a single hydrogen bond, conferring high levels of resistance to this bacterium [50]. *E. faecalis*, on the other hand, synthesizes vancomycin-resistant PG precursors terminating in D-alanine–D-serine [51]. The additional hydroxyl group of D-serine also introduces a steric hindrance for interaction with vancomycin, lowering this antibiotic affinity for PG [52,53]. Many of these glycopeptide resistance mechanisms in these strains are encoded in transposons or plasmids and involve the concerted action of multiple enzymes altering the basic structure of the cell envelope to overcome the damaging action of vancomycin [48,49].

Changes in the composition of EPA caused by mutations in the *epa* loci have also been shown to decrease sensitivity to antibiotics that target cell envelope synthesis, such as ceftriaxone or carbapenems in agar plates with Etest strips [54,55]. This adaptation is thought to result in part from reduced rhamnose and/or increased mannose abundance in some of these strains' cell wall polysaccharides [55]. Moreover, a prior study reported that changes in the minimal inhibitory concentration between parental and *epa* core mutants were modest in agar plates with daptomycin using Etest strips [55], whereas Dale and colleagues showed that the deletion of *epaI* (EPA core) and *epaOX* resulted in enhanced susceptibility of *E. faecalis* OG1RF planktonic cells to the same antibiotic [20]. These contrasting findings suggest that the growth conditions used in both studies may differentially affect EPA synthesis/composition, and, thus, the cell envelope integrity necessary to prevent daptomycin from reaching the division septum. Reinforcing the role of EPA in modulating enterococcal resistance to antimicrobials, a recent study demonstrated that mutations in *galU*, which encodes UTP-glucose-1-phosphate uridylyltransferase (an enzyme involved in generating the UDP glucose required for *E. faecalis* galactose fermentation), affects EPA synthesis and changes the enterococcal susceptibility to β -lactams in agar dilution assays [56]. Additional studies have demonstrated that the EPA confers protection to multiple antibiotics, including gentamicin, vancomycin, and fusidic acid exposure under distinct growth conditions [13,17,20,55,57]. While informative, further research considering environmental factors and/or culture settings that may impact polysaccharide composition is needed to understand the precise mechanisms that mediate EPA-driven antibiotic resistance in enterococci.

Enterococci can also acquire antibiotic resistance by forming biofilms. Several characteristics of these structures contribute to enhance antibiotic resistance relative to planktonic cultures, including poor antibiotic penetration into the biofilm, antibiotic sequestration, and the presence of persister cells within the biofilm (for an excellent review, see [25]). Although *E. faecium* is intrinsically more resistant to antibiotics, *E. faecalis* forms thicker biofilms that promote tolerance to antimicrobials [58,59]. It has been previously reported that certain genes of the *epa* loci contribute to biofilm-associated antibiotic resistance [20,22,38]. Mutations in *epaI*, *epaQ*, and *epaOX* have demonstrated reduced biofilm formation in the presence of daptomycin, and only the latter showed to be more sensitive to gentamicin exposure. In sharp contrast, all 3 deletion mutants showed increased resistance to ceftriaxone [22]. Resistance to this antibiotic involves 2 interacting signaling pathways: the eukaryotic-like Ser/Thr kinase *ireK* and the CroS/R 2-component regulatory system [60–62]. *IreK* is considered to monitor the cell wall integrity and respond to cell wall stress [63]. Hence, EPA alterations resulting in cell wall and shape changes [14,20,22], especially in biofilms, may lead to *IreK* activation of this pathway, leading to a higher intrinsic resistance to ceftriaxone. In fact, dysregulated *IreK* or CroS/R pathways (like strains lacking negative regulators for these systems) display increased cephalosporin resistance [64,65]. Further analysis to establish the connection between the cephalosporin resistance phenotype of *epa* mutants and the *IreK* and CroS/R pathways could provide insights into the cell envelope stress signals detected by these systems.

Resistance to bacteriophages

To infect bacteria, phages must bind to cell wall-associated molecules, such as polysaccharides, which serve as receptors during viral infection [66]. EPA can operate as one of these “receptors,” coordinating phage absorption and subsequent infection by lytic *E. faecalis* phages [17]. The ability for phages to infect *E. faecalis* depends specifically on certain genes of both the core and the variable region of the *epa* loci [18,47]. Mutations in these genes have been found to promote phage resistance, but they also increase *E. faecalis*' sensitivity to osmotic stress and

antibiotics that target the cell wall [17,18,47], suggesting that phage resistance in enterococci might be a fitness trade-off. Furthermore, in *E. faecium*, it has been reported that changes in the composition of cell wall polysaccharides like CPs and EPA promote phage resistance adaptation. Notably, mutations in *epaR* and *epaX* limit phage adsorption to *E. faecium*, although to a lesser extent than similar mutations in *E. faecalis* [54]. These adaptive traits act as gatekeepers to phage absorption, conferring additional means through which *Enterococcus* thrives.

Glycopolymers in GIT colonization, exit, and distal spread

Enterococci have been found in the intestinal lumen as well as in the complex mucus layer and crypts of the small intestine [67,68]. GIT colonization by *E. faecalis* seems to be mediated by the formation of microcolonies rather than structured biofilms, as evidenced by the formation of enterococcal aggregates covered by a fibrous, sweater-like, matrix predominantly localized throughout the GIT of germ-free mice [34]. Similarly, McKenney and colleagues demonstrated that vancomycin-resistant *E. faecium* substantially increased the formation of aggregates in the cecum of antibiotic-treated mice supplemented with lithocholic acid. This secondary bile acid, prevalent in the intestine of mice and humans, impaired the separation of growing enterococcal diplococci, causing the formation of long chains in vitro. Thus, mutants locked in the diplococci state were deficient in mouse tissue colonization and persistence, likely due to their inability to form aggregates and compete with the intestinal microbiota [69].

In addition, it has been demonstrated that EPA plays a role in GIT colonization. Studies involving the deletion of *epaX* and *epaS*, or the entire loci variable region, showed decreased intestinal colonization by *E. faecalis*, as evidenced by lower colony-forming units obtained from stool samples of mice treated with the mutants relative to WT V583 [13,14,17]. Notably, $\Delta epaS$ could not overgrow upon antibiotic-driven dysbiosis and failed to be efficiently transmitted to young mice following birth [17], suggesting that EPA promotes *E. faecalis* aggregate stability during GIT colonization. Indeed, loss of a glycosyltransferase from the *epa* variable loci reduces biofilm formation in the presence of sodium cholate (a bile acid component), and aggregates formed by a mutant in this locus exhibit reduced structural cohesion in vitro [20,38]. Although it has been observed that the aggregates formed by *E. faecalis* in vivo are morphologically similar to those formed in continuous flow bioreactors [34,38], the determinants of microcolony structural development in vivo are poorly characterized. Therefore, analyses to determine the role of EPA in aggregate formation/architecture using microscopic imaging of the GIT in murine models deserve further attention.

Upon damage of the gut barrier, enterococcal overgrowth in the lumen can promote breaching of the intestinal epithelium, a process known as translocation [70–72]. Some intrinsic factors have been proposed to contribute directly or indirectly to enterococcal egress from the GIT [15,16,73,74]. Among them, genes in the core and variable region (*epaX*) of the *epa* loci have shown to be necessary for efficient migration through intestinal epithelial barriers in vitro [15,16]. Moreover, using immunofluorescence microscopy, we found that *E. faecalis* WT formed aggregates covered by polyGlcNAc-containing exopolysaccharides that localized with the epithelial actin cytoskeleton during translocation. These polymers, however, were not detected when $\Delta epaX$ strains were used in the same experimental setting [15]. Hence, formation of matrix-covered enterococcal aggregates might represent a new mechanism that promotes colonization and/or migration across the intestinal barriers in susceptible hosts. Further studies using in vivo models are needed to gain a deeper knowledge of the role of EPA and other uncharacterized enterococcal polysaccharides in bacterial translocation through the intestinal epithelium, a key step for switching from gut commensal to pathogen.

Enterococcal polymers facilitate immune resistance and evasion

Once outside the intestine, enterococci can spread and colonize other body sites, where they can become drivers of disease. Both as successful inhabitants of the GIT and potential pathogens, enterococci have developed mechanisms partly mediated by their cell surface polysaccharides to modulate, resist, or evade the host innate immune system (see review for further mechanisms [23]). The host immune response involves physical and chemical barriers, including soluble proteins, pattern recognition receptors, and phagocytes. The formation of biofilms has been proposed as a key strategy used by enterococci to evade the immune system. For instance, when the host epithelial barrier is disrupted, *E. faecalis* has been found to form matrix-encased microcolonies that can be internalized and encapsulated by the wounded tissue. In these settings, reduced cytokine and chemokine induction has been evidenced, suggesting that this bacterium can modulate the immune response to promote its persistence in wounded tissues [75]. Consistent with this finding, previous studies demonstrated that *E. faecalis* biofilms induce lower pro-inflammatory responses and promote survival within phagocytes in comparison with planktonic cells in vitro [76–79]. Thus, biofilms may be better adapted to overcome host defenses in vivo. Further analyses to determine the role of enterococcal polysaccharides in biofilm-mediated immune resistance using animal models deserve attention.

Several intrinsic components of the enterococcal cell envelope also play important roles during these host–pathogen interactions (Fig 1) [24,80]. For example, an *E. faecalis* deletion mutant defective in LTA D-alanylation demonstrated lower capacity to form biofilms and greater antibody recognition, which is an integral part of the humoral immune response capable of blocking adhesion and entry of a pathogen into tissues while promoting opsonophagocytosis [76,81]. Enterococcal LTA has also been proposed to alter macrophage function by promoting autophagy, a lysosome-mediated degradation process of internal cell components [82]. The LTA of *E. faecalis* can also inhibit Toll-like receptor (TLR)-mediated responses in periodontal tissues, which may represent an additional mechanism of macrophage suppression [83]. Disruption of *epa* core genes result in “naked” enterococcal cells lacking the EPA polymer that are more sensitive to killing by phagocytes [84]. While the mechanisms behind this process remain elusive, it is likely that changes in the carbohydrate composition of these mutants may expose “neopeptides” for recognition by the complement system. However, it is unclear whether EPA is directly recognized by immune cells or it encases other cell factors to prevent *E. faecalis* phagocytic clearance. Additional research then demonstrated that EPA decorations also aid in protection against complement-mediated phagocytic killing [24], suggesting that modifications in EPA, like the covalent link of 2 ribitol-containing WTAs [10], rather than the backbone itself, support phagocyte evasion. Consistent with these findings, mutations in *tagB* resulting in the loss of these 2 ribitol-containing WTAs and secondary changes in EPA also led to increased complement recognition and augmented phagocytic killing of *E. faecalis* in vitro [85]. It is conceivable that the absence of WTAs in these mutants could disrupt the cell envelope topography, rearranging the surface localization of EPA, LTAs, and/or CPs. Hence, WTAs may mediate resistance to phagocytosis indirectly by acting as a shield for other surface glycopolymers and preventing complement detection [80,81,85,86]. Whether *E. faecalis*’ ribitol teichoic acids are only linked to EPA backbone or also covalently attached to MurNAc on PG as described in other gram-positive bacteria [87,88] deserves further investigation.

Enterococci are also capable of evading the immune system by resisting the antimicrobial effect of lysozymes, which are critical components of the host innate immune response. In mammals, these enzymes are found abundantly in the blood, liver, secretions, phagocytes, and at mucosal surfaces [89–92]. Lysozymes can hydrolyze the β -(1,4) glycosidic bonds linking PG

monosaccharides (muramidase activity) and/or bind and create pores in negatively charged bacterial membranes, acting as cationic antimicrobial peptides (CAMPs) [93,94]. These lysozyme-dependent effects kill bacteria and release immunomodulatory bacterial components, including PG fragments [89]. *E. faecalis* has developed mechanisms to counteract lysozymes, including modifying its PG structure through GlcNAc deacetylation, MurNAc O-acetylation, or/and reduction of the cell net negative charge with the addition of positively charged D-alanine residues to WTA and LTA [81,95–97]. Recent studies have specifically shown that exposing *E. faecalis* to lysozyme triggers the production of a PG deacetylase that contributes to this bacterium's virulence in the *Galleria mellonella* insect model [97]. Enterococci are also exposed to other CAMPs, which are positively charged products secreted by host cells or intestinal microflora. Mutations that increase the cell envelope's negative charge (deletion of *tagB*, required for WTA biosynthesis) affect LTA D-alanylation (deletion of *dltA*) or/and alter EPA decoration (*epaX*-like mutations) result in increased sensitivity to CAMPs by *E. faecalis* [24,81,85].

CP expression is another strategy that allows enterococci to evade the immune system. These glycopolymers are localized at the cell surface and function as shields to mask underlying cell surface structures to reduce opsonization [98]. In *E. faecalis* and *E. faecium*, insertional inactivation of genes in the CP biosynthetic pathway yielded mutants with enhanced susceptibility to phagocytic killing by neutrophils and compromised *Enterococcus*' ability to persist in regional lymph nodes [99,100]. *E. faecalis* specifically produces 2 CP serotypes (C and D) with higher resistance to complement-mediated opsonophagocytosis than unencapsulated strains [101], contributing to innate immune evasion and increased pathogenesis [102]. These differences observed in opsonophagocytosis have been attributed to the ability of CPs to mask the detection of LTAs and surface bound C3 (a complement component required for activation of this pathway), thus preventing macrophage activation and reducing pro-inflammatory cytokine production such as tumor necrosis factor α [101,103]. However, to date, the chemical structures of these CPs have not been published. On the other hand, genome sequencing analyses revealed that, although several *E. faecium* strains do not possess *cpsC-cpsK* homologs required for CP synthesis in *E. faecalis*, a putative CP biosynthetic region is conserved among all enterococcal species except *E. faecalis* [101,104,105]. Notably, in vancomycin-resistant *E. faecium*, mutations in this novel locus correlate with enhanced biofilm formation, antibiotic tolerance, and lysozyme resistance in vitro [106], suggesting that the absence/modification of CP may confer a fitness advantage to *E. faecium* in the host.

Enterococci have therefore adopted multiple strategies to evade immune surveillance, contributing to its successful transition to a resilient pathogen. It is evident that polysaccharides influence their physiology by enhancing enterococci's ability to form multicellular aggregates, colonize the GIT and extraintestinal sites, endure environmental stressors, evade the host immune system, and resist antibiotics. Upon loss of functional WTAs, LTAs, CPs, or EPA genes, *Enterococcus* sp. lose these remarkable capabilities. Clearly, the diversity of polysaccharides able to be produced in different growth conditions or by strains/species is likely to influence enterococcal fitness and survival. Examples of this diversity are reflected in the differences found in the variable region of the *epa* loci that may change the EPA decoration or structure (Fig 2). Thus, enterococcal polymers represent a high-profile target for future therapeutics, potentially leading to new strategies to effectively control systemic and persistent enterococcal infections. Understanding the vastly unexplored connections between the host, gut microbiota, and enterococcal polysaccharides will provide “sweet” insights into the mechanisms of enterococcal colonization and pathogenesis.

Acknowledgments

We thank Juan R. Cubillos-Ruiz for his comments and suggestions. We apologize to colleagues whose work was not cited in this review due to space limitations. Figures were created with [BioRender.com](https://www.biorender.com).

References

1. Fiore E, Van Tyne D, Gilmore MS. Pathogenicity of Enterococci. *Microbiol Spectr*. 2019; 7(4). Epub 2019/07/13. <https://doi.org/10.1128/microbiolspec.GPP3-0053-2018> PMID: 31298205; PubMed Central PMCID: PMC6629438.
2. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol*. 2008; 29(11):996–1011. Epub 2008/10/25. <https://doi.org/10.1086/591861> PMID: 18947320.
3. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, et al. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *Infect Control Hosp Epidemiol*. 2016; 37(11):1288–301. Epub 2016/10/22. <https://doi.org/10.1017/ice.2016.174> PMID: 27573805.
4. Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, et al. Genomic variation landscape of the human gut microbiome. *Nature*. 2013; 493(7430):45–50. Epub 2012/12/12. <https://doi.org/10.1038/nature11711> PMID: 23222524; PubMed Central PMCID: PMC3536929.
5. Landete JM, Peiroten A, Medina M, Arques JL, Rodriguez-Minguez E. Virulence and Antibiotic Resistance of Enterococci Isolated from Healthy Breastfed Infants. *Microb Drug Resist*. 2018; 24(1):63–9. Epub 2017/07/15. <https://doi.org/10.1089/mdr.2016.0320> PMID: 28708453.
6. Sava IG, Heikens E, Huebner J. Pathogenesis and immunity in enterococcal infections. *Clin Microbiol Infect*. 2010; 16(6):533–40. Epub 2010/06/24. <https://doi.org/10.1111/j.1469-0691.2010.03213.x> PMID: 20569264.
7. Hancock LE, Murray BE, Sillanpää J. Enterococcal Cell Wall Components and Structures. In: Gilmore MS, Clewell DB, Ike Y, Shankar N, editors. *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection*. Boston, Massachusetts; 2014.
8. Chang JD, Wallace AG, Foster EE, Kim SJ. Peptidoglycan Compositional Analysis of *Enterococcus faecalis* Biofilm by Stable Isotope Labeling by Amino Acids in a Bacterial Culture. *Biochemistry*. 2018; 57(7):1274–83. Epub 2018/01/26. <https://doi.org/10.1021/acs.biochem.7b01207> PMID: 29368511; PubMed Central PMCID: PMC5972391.
9. Wobser D, Ali L, Grohmann E, Huebner J, Sakinc T. A novel role for D-alanylation of lipoteichoic acid of *Enterococcus faecalis* in urinary tract infection. *PLoS ONE*. 2014; 9(10):e107827. Epub 2014/10/09. <https://doi.org/10.1371/journal.pone.0107827> PMID: 25296179; PubMed Central PMCID: PMC4189791.
10. Guerardel Y, Sadovskaya I, Maes E, Furlan S, Chapot-Chartier MP, Mesnage S, et al. Complete Structure of the Enterococcal Polysaccharide Antigen (EPA) of Vancomycin-Resistant *Enterococcus faecalis* V583 Reveals that EPA Decorations Are Teichoic Acids Covalently Linked to a Rhamnopolysaccharide Backbone. *mBio*. 2020; 11(2). Epub 2020/04/30. <https://doi.org/10.1128/mBio.00277-20> PMID: 32345640; PubMed Central PMCID: PMC7188991.
11. Hendrickx AP, van Schaik W, Willems RJ. The cell wall architecture of *Enterococcus faecium*: from resistance to pathogenesis. *Future Microbiol*. 2013; 8(8):993–1010. Epub 2013/08/02. <https://doi.org/10.2217/fmb.13.66> PMID: 23902146.
12. Chapot-Chartier MP, Kulakauskas S. Cell wall structure and function in lactic acid bacteria. *Microb Cell Fact*. 2014; 13(Suppl 1):S9. Epub 2014/09/05. <https://doi.org/10.1186/1475-2859-13-S1-S9> PMID: 25186919; PubMed Central PMCID: PMC4155827.
13. Furlan S, Matos RC, Kennedy SP, Doublet B, Serron P, Rigottier-Gois L. Fitness Restoration of a Genetically Tractable *Enterococcus faecalis* V583 Derivative To Study Decoration-Related Phenotypes of the Enterococcal Polysaccharide Antigen. *mSphere*. 2019; 4(4). Epub 2019/07/12. <https://doi.org/10.1128/mSphere.00310-19> PMID: 31292230; PubMed Central PMCID: PMC6620374.
14. Rigottier-Gois L, Madec C, Navickas A, Matos RC, Akary-Lepage E, Mistou MY, et al. The surface rhamnopolysaccharide epa of *Enterococcus faecalis* is a key determinant of intestinal colonization. *J Infect Dis*. 2015; 211(1):62–71. <https://doi.org/10.1093/infdis/jiu402> PMID: 25035517.

15. Ramos Y, Rocha J, Hael AL, van Gestel J, Vlamakis H, Cywes-Bentley C, et al. PolyGlcNAc-containing exopolymers enable surface penetration by non-motile *Enterococcus faecalis*. PLoS Pathog. 2019; 15(2):e1007571. <https://doi.org/10.1371/journal.ppat.1007571> PMID: 30742693.
16. Zeng J, Teng F, Weinstock GM, Murray BE. Translocation of *Enterococcus faecalis* strains across a monolayer of polarized human enterocyte-like T84 cells. J Clin Microbiol. 2004; 42(3):1149–54. <https://doi.org/10.1128/JCM.42.3.1149-1154.2004> PMID: 15004067; PubMed Central PMCID: PMC356884.
17. Chatterjee A, Johnson CN, Luong P, Hullahalli K, McBride SW, Schubert AM, et al. Bacteriophage Resistance Alters Antibiotic-Mediated Intestinal Expansion of Enterococci. Infect Immun. 2019;87(6). Epub 2019/04/03. <https://doi.org/10.1128/IAI.00085-19> PMID: 30936157; PubMed Central PMCID: PMC6529655.
18. Chatterjee A, Willett JLE, Nguyen UT, Monogue B, Palmer KL, Dunny GM, et al. Parallel Genomics Uncover Novel Enterococcal-Bacteriophage Interactions. mBio. 2020; 11(2). Epub 2020/03/05. <https://doi.org/10.1128/mBio.03120-19> PMID: 32127456; PubMed Central PMCID: PMC7064774.
19. Al-Zubidi M, Widziolak M, Court EK, Gains AF, Smith RE, Ansbro K, et al. Identification of Novel Bacteriophages with Therapeutic Potential That Target *Enterococcus faecalis*. Infect Immun. 2019;87(11). Epub 2019/08/28. <https://doi.org/10.1128/IAI.00512-19> PMID: 31451618; PubMed Central PMCID: PMC6803325.
20. Dale JL, Cagnazzo J, Phan CQ, Barnes AM, Dunny GM. Multiple roles for *Enterococcus faecalis* glycosyltransferases in biofilm-associated antibiotic resistance, cell envelope integrity, and conjugative transfer. Antimicrob Agents Chemother. 2015; 59(7):4094–105. Epub 2015/04/29. <https://doi.org/10.1128/AAC.00344-15> PMID: 25918141; PubMed Central PMCID: PMC4468649.
21. Patterson JE, Sweeney AH, Simms M, Carley N, Mangi R, Sabetta J, et al. An analysis of 110 serious enterococcal infections. Epidemiology, antibiotic susceptibility, and outcome. Medicine (Baltimore). 1995; 74(4):191–200. Epub 1995/07/01. <https://doi.org/10.1097/00005792-199507000-00003> PMID: 7623654.
22. Korir ML, Dale JL, Dunny GM. Role of epaQ, a Previously Uncharacterized *Enterococcus faecalis* Gene, in Biofilm Development and Antimicrobial Resistance. J Bacteriol. 2019; 201(18). Epub 2019/03/27. <https://doi.org/10.1128/JB.00078-19> PMID: 30910809; PubMed Central PMCID: PMC6707930.
23. Kao PHN, Kline KA. Dr. Jekyll and Mr. Hide: How *Enterococcus faecalis* Subverts the Host Immune Response to Cause Infection. J Mol Biol. 2019; 431(16):2932–45. Epub 2019/05/28. <https://doi.org/10.1016/j.jmb.2019.05.030> PMID: 31132360.
24. Smith RE, Salamaga B, Szkuta P, Hajdamowicz N, Prajsnar TK, Bulmer GS, et al. Decoration of the enterococcal polysaccharide antigen EPA is essential for virulence, cell surface charge and interaction with effectors of the innate immune system. PLoS Pathog. 2019; 15(5):e1007730. Epub 2019/05/03. <https://doi.org/10.1371/journal.ppat.1007730> PMID: 31048927; PubMed Central PMCID: PMC6497286.
25. Ch'ng JH, Chong KKL, Lam LN, Wong JJ, Kline KA. Biofilm-associated infection by enterococci. Nat Rev Microbiol. 2019; 17(2):82–94. Epub 2018/10/20. <https://doi.org/10.1038/s41579-018-0107-z> PMID: 30337708.
26. Limoli DH, Jones CJ, Wozniak DJ. Bacterial Extracellular Polysaccharides in Biofilm Formation and Function. Microbiol Spectr. 2015; 3(3). Epub 2015/07/18. <https://doi.org/10.1128/microbiolspec.MB-0011-2014> PMID: 26185074; PubMed Central PMCID: PMC4657554.
27. Vu B, Chen M, Crawford RJ, Ivanova EP. Bacterial extracellular polysaccharides involved in biofilm formation. Molecules. 2009; 14(7):2535–54. Epub 2009/07/28. <https://doi.org/10.3390/molecules14072535> PMID: 19633622; PubMed Central PMCID: PMC6254922.
28. Typas A, Banzhaf M, Gross CA, Vollmer W. From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nat Rev Microbiol. 2011; 10(2):123–36. Epub 2011/12/29. <https://doi.org/10.1038/nrmicro2677> PMID: 22203377; PubMed Central PMCID: PMC5433867.
29. Tendolkar PM, Baghdayan AS, Gilmore MS, Shankar N. Enterococcal surface protein, Esp, enhances biofilm formation by *Enterococcus faecalis*. Infect Immun. 2004; 72(10):6032–9. Epub 2004/09/24. <https://doi.org/10.1128/IAI.72.10.6032-6039.2004> PMID: 15385507; PubMed Central PMCID: PMC517584.
30. Heikens E, Bonten MJ, Willems RJ. Enterococcal surface protein Esp is important for biofilm formation of *Enterococcus faecium* E1162. J Bacteriol. 2007; 189(22):8233–40. Epub 2007/09/11. <https://doi.org/10.1128/JB.01205-07> PMID: 17827282; PubMed Central PMCID: PMC2168697.
31. Nallapareddy SR, Singh KV, Sillanpaa J, Garsin DA, Hook M, Erlandsen SL, et al. Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. J Clin Invest. 2006; 116(10):2799–807. Epub 2006/10/04. <https://doi.org/10.1172/JCI29021> PMID: 17016560; PubMed Central PMCID: PMC1578622.

32. Teng F, Singh KV, Bourgogne A, Zeng J, Murray BE. Further characterization of the *epa* gene cluster and *Epa* polysaccharides of *Enterococcus faecalis*. *Infect Immun*. 2009; 77(9):3759–67. <https://doi.org/10.1128/IAI.00149-09> PMID: 19581393; PubMed Central PMCID: PMC2737988.
33. Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by *Enterococcus faecalis*. *Infect Immun*. 2004; 72(6):3658–63. Epub 2004/05/25. <https://doi.org/10.1128/IAI.72.6.3658-3663.2004> PMID: 15155680; PubMed Central PMCID: PMC415661.
34. Barnes AMT, Dale JL, Chen Y, Manias DA, Greenwood Quaintance KE, Karau MK, et al. *Enterococcus faecalis* readily colonizes the entire gastrointestinal tract and forms biofilms in a germ-free mouse model. *Virulence*. 2017; 8(3):282–96. Epub 2016/08/27. <https://doi.org/10.1080/21505594.2016.1208890> PMID: 27562711; PubMed Central PMCID: PMC5411234.
35. Kristich CJ, Li YH, Cvitkovitch DG, Dunny GM. Esp-independent biofilm formation by *Enterococcus faecalis*. *J Bacteriol*. 2004; 186(1):154–63. Epub 2003/12/18. <https://doi.org/10.1128/JB.186.1.154-163.2004> PMID: 14679235; PubMed Central PMCID: PMC365672.
36. Leuck AM, Johnson JR, Dunny GM. A widely used in vitro biofilm assay has questionable clinical significance for enterococcal endocarditis. *PLoS ONE*. 2014; 9(9):e107282. Epub 2014/09/26. <https://doi.org/10.1371/journal.pone.0107282> PMID: 25255085; PubMed Central PMCID: PMC4177788.
37. Islam ST, Vergara Alvarez I, Saidi F, Guiseppi A, Vinogradov E, Sharma G, et al. Modulation of bacterial multicellularity via spatio-specific polysaccharide secretion. *PLoS Biol*. 2020; 18(6):e3000728. Epub 2020/06/10. <https://doi.org/10.1371/journal.pbio.3000728> PMID: 32516311; PubMed Central PMCID: PMC7310880.
38. Dale JL, Nilson JL, Barnes AMT, Dunny GM. Restructuring of *Enterococcus faecalis* biofilm architecture in response to antibiotic-induced stress. *NPJ Biofilms Microbiomes*. 2017; 3:15. Epub 2017/07/08. <https://doi.org/10.1038/s41522-017-0023-4> PMID: 28685097; PubMed Central PMCID: PMC5493694.
39. Staudt AK, Wolfe LG, Shrout JD. Variations in exopolysaccharide production by *Rhizobium tropici*. *Arch Microbiol*. 2012; 194(3):197–206. Epub 2011/08/23. <https://doi.org/10.1007/s00203-011-0742-5> PMID: 21858649.
40. Rohde H, Frankenberger S, Zahringer U, Mack D. Structure, function and contribution of polysaccharide intercellular adhesin (PIA) to *Staphylococcus epidermidis* biofilm formation and pathogenesis of biomaterial-associated infections. *Eur J Cell Biol*. 2010; 89(1):103–11. Epub 2009/11/17. <https://doi.org/10.1016/j.ejcb.2009.10.005> PMID: 19913940.
41. Roux D, Cywes-Bentley C, Zhang YF, Pons S, Konkol M, Kearns DB, et al. Identification of Poly-N-acetylglucosamine as a Major Polysaccharide Component of the *Bacillus subtilis* Biofilm Matrix. *J Biol Chem*. 2015; 290(31):19261–72. Epub 2015/06/17. <https://doi.org/10.1074/jbc.M115.648709> PMID: 26078454; PubMed Central PMCID: PMC4521046.
42. Ran SJ, Jiang W, Zhu CL, Liang JP. Exploration of the mechanisms of biofilm formation by *Enterococcus faecalis* in glucose starvation environments. *Aust Dent J*. 2015; 60(2):143–53. Epub 2015/05/21. <https://doi.org/10.1111/adj.12324> PMID: 25990488.
43. Abdhul K, Ganesh M, Shanmughapriya S, Kanagavel M, Anbarasu K, Natarajaseenivasan K. Antioxidant activity of exopolysaccharide from probiotic strain *Enterococcus faecium* (BDU7) from Ngari. *Int J Biol Macromol*. 2014; 70:450–4. Epub 2014/07/27. <https://doi.org/10.1016/j.ijbiomac.2014.07.026> PMID: 25062992.
44. Hofmann AF, Hagey LR. Bile acids: chemistry, pathochemistry, biology, pathobiology, and therapeutics. *Cell Mol Life Sci*. 2008; 65(16):2461–83. Epub 2008/05/20. <https://doi.org/10.1007/s00018-008-7568-6> PMID: 18488143.
45. Urdaneta V, Casadesus J. Interactions between Bacteria and Bile Salts in the Gastrointestinal and Hepatobiliary Tracts. *Front Med*. 2017; 4:163. Epub 2017/10/19. <https://doi.org/10.3389/fmed.2017.00163> PMID: 29043249; PubMed Central PMCID: PMC5632352.
46. Solheim M, La Rosa SL, Mathisen T, Snipen LG, Nes IF, Brede DA. Transcriptomic and functional analysis of NaCl-induced stress in *Enterococcus faecalis*. *PLoS ONE*. 2014; 9(4):e94571. Epub 2014/04/24. <https://doi.org/10.1371/journal.pone.0094571> PMID: 24755907; PubMed Central PMCID: PMC3995695.
47. Ho K, Huo W, Pas S, Dao R, Palmer KL. Loss-of-Function Mutations in *epaR* Confer Resistance to varphiNPV1 Infection in *Enterococcus faecalis* OG1RF. *Antimicrob Agents Chemother*. 2018; 62(10). Epub 2018/08/15. <https://doi.org/10.1128/AAC.00758-18> PMID: 30104266; PubMed Central PMCID: PMC6153818.
48. Arias CA, Murray BE. The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol*. 2012; 10(4):266–78. Epub 2012/03/17. <https://doi.org/10.1038/nrmicro2761> PMID: 22421879; PubMed Central PMCID: PMC3621121.

49. Rice LB. Mechanisms of resistance and clinical relevance of resistance to beta-lactams, glycopeptides, and fluoroquinolones. *Mayo Clin Proc.* 2012; 87(2):198–208. Epub 2012/02/07. <https://doi.org/10.1016/j.mayocp.2011.12.003> PMID: 22305032; PubMed Central PMCID: PMC3498059.
50. Bugg TD, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecular basis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry.* 1991; 30(43):10408–15. Epub 1991/10/29. <https://doi.org/10.1021/bi00107a007> PMID: 1931965.
51. Boyd DA, Willey BM, Fawcett D, Gillani N, Mulvey MR. Molecular characterization of *Enterococcus faecalis* N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, vanL. *Antimicrob Agents Chemother.* 2008; 52(7):2667–72. Epub 2008/05/07. <https://doi.org/10.1128/AAC.01516-07> PMID: 18458129; PubMed Central PMCID: PMC2443935.
52. Billot-Klein D, Blanot D, Gutmann L, van Heijenoort J. Association constants for the binding of vancomycin and teicoplanin to N-acetyl-D-alanyl-D-alanine and N-acetyl-D-alanyl-D-serine. *Biochem J.* 1994; 304(Pt 3):1021–2. Epub 1994/12/15. <https://doi.org/10.1042/bj3041021> PMID: 7818465; PubMed Central PMCID: PMC1137434.
53. Abadia Patino L, Courvalin P, Perichon B. vanE gene cluster of vancomycin-resistant *Enterococcus faecalis* BM4405. *J Bacteriol.* 2002; 184(23):6457–64. Epub 2002/11/12. <https://doi.org/10.1128/JB.184.23.6457-6464.2002> PMID: 12426332; PubMed Central PMCID: PMC135418.
54. Canfield GS, Chatterjee A, Espinosa J, Mangalea MR, Sheriff EK, Keidan M, et al. Lytic bacteriophages facilitate antibiotic sensitization of *Enterococcus faecium*. *Antimicrob Agents Chemother.* 2021. Epub 2021/03/03. <https://doi.org/10.1128/AAC.00143-21> PMID: 33649110.
55. Singh KV, Murray BE. Loss of a Major Enterococcal Polysaccharide Antigen (Epa) by *Enterococcus faecalis* Is Associated with Increased Resistance to Ceftriaxone and Carbapenems. *Antimicrob Agents Chemother.* 2019; 63(5). Epub 2019/03/13. <https://doi.org/10.1128/AAC.00481-19> PMID: 30858216; PubMed Central PMCID: PMC6496094.
56. Kurushima J, Tomita H. Inactivation of GalU Leads to a Cell Wall-Associated Polysaccharide Defect That Reduces the Susceptibility of *Enterococcus faecalis* to Bacteriolytic Agents. *Appl Environ Microbiol.* 2021; 87(7). Epub 2021/01/24. <https://doi.org/10.1128/AEM.02875-20> PMID: 33483312.
57. Rigottier-Gois L, Alberti A, Houel A, Taly JF, Palcy P, Manson J, et al. Large-scale screening of a targeted *Enterococcus faecalis* mutant library identifies envelope fitness factors. *PLoS ONE.* 2011; 6(12):e29023. <https://doi.org/10.1371/journal.pone.0029023> PMID: 22194979; PubMed Central PMCID: PMC3240637.
58. Di Rosa R, Creti R, Venditti M, D'Amelio R, Arciola CR, Montanaro L, et al. Relationship between biofilm formation, the enterococcal surface protein (Esp) and gelatinase in clinical isolates of *Enterococcus faecalis* and *Enterococcus faecium*. *FEMS Microbiol Lett.* 2006; 256(1):145–50. Epub 2006/02/21. <https://doi.org/10.1111/j.1574-6968.2006.00112.x> PMID: 16487332.
59. Komiyama EY, Lepesqueur LS, Yassuda CG, Samaranyake LP, Parahitiyawa NB, Balducci I, et al. *Enterococcus* Species in the Oral Cavity: Prevalence, Virulence Factors and Antimicrobial Susceptibility. *PLoS ONE.* 2016; 11(9):e0163001. Epub 2016/09/16. <https://doi.org/10.1371/journal.pone.0163001> PMID: 27631785; PubMed Central PMCID: PMC5025163.
60. Comenge Y, Quintiliani R Jr, Li L, Dubost L, Brouard JP, Hugonnet JE, et al. The CroRS two-component regulatory system is required for intrinsic beta-lactam resistance in *Enterococcus faecalis*. *J Bacteriol.* 2003; 185(24):7184–92. Epub 2003/12/03. <https://doi.org/10.1128/JB.185.24.7184-7192.2003> PMID: 14645279; PubMed Central PMCID: PMC296236.
61. Kristich CJ, Wells CL, Dunny GM. A eukaryotic-type Ser/Thr kinase in *Enterococcus faecalis* mediates antimicrobial resistance and intestinal persistence. *Proc Natl Acad Sci U S A.* 2007; 104(9):3508–13. Epub 2007/03/16. <https://doi.org/10.1073/pnas.0608742104> PMID: 17360674; PubMed Central PMCID: PMC1805595.
62. Kellogg SL, Kristich CJ. Convergence of PASTA Kinase and Two-Component Signaling in Response to Cell Wall Stress in *Enterococcus faecalis*. *J Bacteriol.* 2018; 200(12). Epub 2018/04/11. <https://doi.org/10.1128/JB.00086-18> PMID: 29632091; PubMed Central PMCID: PMC5971478.
63. Labbe BD, Kristich CJ. Growth- and Stress-Induced PASTA Kinase Phosphorylation in *Enterococcus faecalis*. *J Bacteriol.* 2017; 199(21). Epub 2017/08/16. <https://doi.org/10.1128/JB.00363-17> PMID: 28808126; PubMed Central PMCID: PMC5626955.
64. Kellogg SL, Kristich CJ. Functional Dissection of the CroRS Two-Component System Required for Resistance to Cell Wall Stressors in *Enterococcus faecalis*. *J Bacteriol.* 2016; 198(8):1326–36. Epub 2016/02/18. <https://doi.org/10.1128/JB.00995-15> PMID: 26883822; PubMed Central PMCID: PMC4859583.

65. Kristich CJ, Little JL, Hall CL, Hoff JS. Reciprocal regulation of cephalosporin resistance in *Enterococcus faecalis*. *mBio*. 2011; 2(6):e00199–11. Epub 2011/11/03. <https://doi.org/10.1128/mBio.00199-11> PMID: 22045988; PubMed Central PMCID: PMC3202758.
66. Moldovan R, Chapman-McQuiston E, Wu XL. On kinetics of phage adsorption. *Biophys J*. 2007; 93(1):303–15. Epub 2007/04/17. <https://doi.org/10.1529/biophysj.106.102962> PMID: 17434950; PubMed Central PMCID: PMC1914437.
67. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2016; 14(1):20–32. Epub 2015/10/27. <https://doi.org/10.1038/nrmicro3552> PMID: 26499895; PubMed Central PMCID: PMC4837114.
68. Swidsinski A, Loening-Baucke V, Lochs H, Hale LP. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J Gastroenterol*. 2005; 11(8):1131–40. Epub 2005/03/09. <https://doi.org/10.3748/wjg.v11.i8.1131> PMID: 15754393; PubMed Central PMCID: PMC4250702.
69. McKenney PT, Yan J, Vaubourgeix J, Becattini S, Lampen N, Motzer A, et al. Intestinal Bile Acids Induce a Morphotype Switch in Vancomycin-Resistant *Enterococcus* that Facilitates Intestinal Colonization. *Cell Host Microbe*. 2019; 25(5):695–705.e5. Epub 2019/04/30. <https://doi.org/10.1016/j.chom.2019.03.008> PMID: 31031170; PubMed Central PMCID: PMC6939634.
70. Ainsworth S, Sadovskaya I, Vinogradov E, Courtin P, Guerardel Y, Mahony J, et al. Differences in lactococcal cell wall polysaccharide structure are major determining factors in bacteriophage sensitivity. *mBio*. 2014; 5(3):e00880–14. Epub 2014/05/08. <https://doi.org/10.1128/mBio.00880-14> PMID: 24803515; PubMed Central PMCID: PMC4010823.
71. Archambaud C, Derre-Bobillot A, Lapaque N, Rigottier-Gois L, Serron P. Intestinal translocation of enterococci requires a threshold level of enterococcal overgrowth in the lumen. *Sci Rep*. 2019; 9(1):8926. Epub 2019/06/22. <https://doi.org/10.1038/s41598-019-45441-3> PMID: 31222056; PubMed Central PMCID: PMC6586816.
72. Chakraborty R, Lam V, Kommineni S, Stromich J, Hayward M, Kristich CJ, et al. Ceftriaxone Administration Disrupts Intestinal Homeostasis, Mediating Noninflammatory Proliferation and Dissemination of Commensal Enterococci. *Infect Immun*. 2018; 86(12). Epub 2018/09/19. <https://doi.org/10.1128/IAI.00674-18> PMID: 30224553; PubMed Central PMCID: PMC6246901.
73. Maharshak N, Huh EY, Paiboonrungruang C, Shanahan M, Thurlow L, Herzog J, et al. *Enterococcus faecalis* Gelatinase Mediates Intestinal Permeability via Protease-Activated Receptor 2. *Infect Immun*. 2015; 83(7):2762–70. Epub 2015/04/29. <https://doi.org/10.1128/IAI.00425-15> PMID: 25916983; PubMed Central PMCID: PMC4468563.
74. Shogan BD, Belogortseva N, Luong PM, Zaborin A, Lax S, Bethel C, et al. Collagen degradation and MMP9 activation by *Enterococcus faecalis* contribute to intestinal anastomotic leak. *Sci Transl Med*. 2015; 7(286):286ra68. Epub 2015/05/08. <https://doi.org/10.1126/scitranslmed.3010658> PMID: 25947163; PubMed Central PMCID: PMC5027898.
75. Chong KKL, Tay WH, Janela B, Yong AMH, Liew TH, Madden L, et al. *Enterococcus faecalis* Modulates Immune Activation and Slows Healing During Wound Infection. *J Infect Dis*. 2017; 216(12):1644–54. Epub 2017/10/19. <https://doi.org/10.1093/infdis/jix541> PMID: 29045678; PubMed Central PMCID: PMC5854026.
76. Daw K, Baghdayan AS, Awasthi S, Shankar N. Biofilm and planktonic *Enterococcus faecalis* elicit different responses from host phagocytes in vitro. *FEMS Immunol Med Microbiol*. 2012; 65(2):270–82. <https://doi.org/10.1111/j.1574-695X.2012.00944.x> PMID: 22333034; PubMed Central PMCID: PMC3366019.
77. Mathew S, Yaw-Chyn L, Kishen A. Immunogenic potential of *Enterococcus faecalis* biofilm under simulated growth conditions. *J Endod*. 2010; 36(5):832–6. <https://doi.org/10.1016/j.joen.2010.02.022> PMID: 20416428.
78. Baldassarri L, Cecchini R, Bertuccini L, Ammendolia MG, Iosi F, Arciola CR, et al. *Enterococcus* spp. produces slime and survives in rat peritoneal macrophages. *Med Microbiol Immunol*. 2001; 190(3):113–20. Epub 2002/02/06. <https://doi.org/10.1007/s00430-001-0096-8> PMID: 11827199.
79. Abrantes MC, Kok J, Lopes Mde F. EfaR is a major regulator of *Enterococcus faecalis* manganese transporters and influences processes involved in host colonization and infection. *Infect Immun*. 2013; 81(3):935–44. Epub 2013/01/09. <https://doi.org/10.1128/IAI.06377-11> PMID: 23297382; PubMed Central PMCID: PMC3584879.
80. Weidenmaier C, Peschel A. Teichoic acids and related cell-wall glycopolymers in Gram-positive physiology and host interactions. *Nat Rev Microbiol*. 2008; 6(4):276–87. Epub 2008/03/11. <https://doi.org/10.1038/nrmicro1861> PMID: 18327271.
81. Fabretti F, Theilacker C, Baldassarri L, Kaczynski Z, Kropec A, Holst O, et al. Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides. *Infect*

- Immun. 2006; 74(7):4164–71. Epub 2006/06/23. <https://doi.org/10.1128/IAI.00111-06> PMID: 16790791; PubMed Central PMCID: PMC1489678.
82. Lin D, Gao Y, Zhao L, Chen Y, An S, Peng Z. *Enterococcus faecalis* lipoteichoic acid regulates macrophages autophagy via PI3K/Akt/mTOR pathway. *Biochem Biophys Res Commun*. 2018; 498(4):1028–36. Epub 2018/03/20. <https://doi.org/10.1016/j.bbrc.2018.03.109> PMID: 29551680.
 83. Im J, Baik JE, Kim KW, Kang SS, Jeon JH, Park OJ, et al. *Enterococcus faecalis* lipoteichoic acid suppresses *Aggregatibacter actinomycetemcomitans* lipopolysaccharide-induced IL-8 expression in human periodontal ligament cells. *Int Immunol*. 2015; 27(8):381–91. Epub 2015/04/04. <https://doi.org/10.1093/intimm/dxv016> PMID: 25840438.
 84. Teng F, Jacques-Palaz KD, Weinstock GM, Murray BE. Evidence that the enterococcal polysaccharide antigen gene (epa) cluster is widespread in *Enterococcus faecalis* and influences resistance to phagocytic killing of *E. faecalis*. *Infect Immun*. 2002; 70(4):2010–5. Epub 2002/03/16. <https://doi.org/10.1128/IAI.70.4.2010-2015.2002> PMID: 11895965; PubMed Central PMCID: PMC127866.
 85. Geiss-Liebisch S, Rooijackers SH, Beczala A, Sanchez-Carballo P, Kruszynska K, Repp C, et al. Secondary cell wall polymers of *Enterococcus faecalis* are critical for resistance to complement activation via mannose-binding lectin. *J Biol Chem*. 2012; 287(45):37769–77. Epub 2012/08/22. <https://doi.org/10.1074/jbc.M112.358283> PMID: 22908219; PubMed Central PMCID: PMC3488052.
 86. Rajagopal M, Walker S. Envelope Structures of Gram-Positive Bacteria. *Curr Top Microbiol Immunol*. 2017; 404:1–44. Epub 2016/02/28. https://doi.org/10.1007/82_2015_5021 PubMed Central PMCID: PMC5002265. PMID: 26919863
 87. Neuhaus FC, Baddiley J. A continuum of anionic charge: structures and functions of D-alanyl-teichoic acids in Gram-positive bacteria. *Microbiol Mol Biol Rev*. 2003; 67(4):686–723. Epub 2003/12/11. <https://doi.org/10.1128/MMBR.67.4.686-723.2003> PMID: 14665680; PubMed Central PMCID: PMC309049.
 88. Araki Y, Ito E. Linkage units in cell walls of gram-positive bacteria. *Crit Rev Microbiol*. 1989; 17(2):121–35. Epub 1989/01/01. <https://doi.org/10.3109/10408418909105745> PMID: 2692601.
 89. Ragland SA, Criss AK. From bacterial killing to immune modulation: Recent insights into the functions of lysozyme. *PLoS Pathog*. 2017; 13(9):e1006512. Epub 2017/09/22. <https://doi.org/10.1371/journal.ppat.1006512> PMID: 28934357; PubMed Central PMCID: PMC5608400.
 90. Faurschou M, Borregaard N. Neutrophil granules and secretory vesicles in inflammation. *Microbes Infect*. 2003; 5(14):1317–27. Epub 2003/11/14. <https://doi.org/10.1016/j.micinf.2003.09.008> PMID: 14613775.
 91. Brouwer J, van Leeuwen-Herberts T, Otting-van de Ruit M. Determination of lysozyme in serum, urine, cerebrospinal fluid and feces by enzyme immunoassay. *Clin Chim Acta*. 1984; 142(1):21–30. Epub 1984/09/15. [https://doi.org/10.1016/0009-8981\(84\)90097-4](https://doi.org/10.1016/0009-8981(84)90097-4) PMID: 6383662.
 92. Lelouard H, Henri S, De Bovis B, Mugnier B, Chollat-Namy A, Malissen B, et al. Pathogenic bacteria and dead cells are internalized by a unique subset of Peyer's patch dendritic cells that express lysozyme. *Gastroenterology*. 2010; 138(1):173–84.e1–3. Epub 2009/10/06. <https://doi.org/10.1053/j.gastro.2009.09.051> PMID: 19800337.
 93. Schindler M, Assaf Y, Sharon N, Chipman DM. Mechanism of lysozyme catalysis: role of ground-state strain in subsite D in hen egg-white and human lysozymes. *Biochemistry*. 1977; 16(3):423–31. Epub 1977/02/08. <https://doi.org/10.1021/bi00622a013> PMID: 13816.
 94. Derde M, Lechevalier V, Guerin-Dubiard C, Cochet MF, Jan S, Baron F, et al. Hen egg white lysozyme permeabilizes *Escherichia coli* outer and inner membranes. *J Agric Food Chem*. 2013; 61(41):9922–9. Epub 2013/09/21. <https://doi.org/10.1021/jf4029199> PMID: 24047287.
 95. Hebert L, Courtin P, Torelli R, Sanguinetti M, Chapot-Chartier MP, Auffray Y, et al. *Enterococcus faecalis* constitutes an unusual bacterial model in lysozyme resistance. *Infect Immun*. 2007; 75(11):5390–8. Epub 2007/09/06. <https://doi.org/10.1128/IAI.00571-07> PMID: 17785473; PubMed Central PMCID: PMC2168276.
 96. Le Jeune A, Torelli R, Sanguinetti M, Giard JC, Hartke A, Auffray Y, et al. The extracytoplasmic function sigma factor SigV plays a key role in the original model of lysozyme resistance and virulence of *Enterococcus faecalis*. *PLoS ONE*. 2010; 5(3):e9658. Epub 2010/03/20. <https://doi.org/10.1371/journal.pone.0009658> PMID: 20300180; PubMed Central PMCID: PMC2836378.
 97. Benachour A, Ladjouzi R, Le Jeune A, Hebert L, Thorpe S, Courtin P, et al. The lysozyme-induced peptidoglycan N-acetylglucosamine deacetylase PgdA (EF1843) is required for *Enterococcus faecalis* virulence. *J Bacteriol*. 2012; 194(22):6066–73. Epub 2012/09/11. <https://doi.org/10.1128/JB.00981-12> PMID: 22961856; PubMed Central PMCID: PMC3486378.
 98. Corbett D, Roberts IS. The role of microbial polysaccharides in host-pathogen interaction. *F1000 Biol Rep*. 2009; 1:30. Epub 2009/01/01. <https://doi.org/10.3410/B1-30> PMID: 20948655; PubMed Central PMCID: PMC2924690.

99. Ali L, Spiess M, Wobser D, Rodriguez M, Blum HE, Sakiotanc T. Identification and functional characterization of the putative polysaccharide biosynthesis protein (CapD) of *Enterococcus faecium* U0317. *Infect Genet Evol.* 2016; 37:215–24. Epub 2015/11/28. <https://doi.org/10.1016/j.meegid.2015.11.020> PMID: 26611826.
100. Hancock LE, Gilmore MS. The capsular polysaccharide of *Enterococcus faecalis* and its relationship to other polysaccharides in the cell wall. *Proc Natl Acad Sci U S A.* 2002; 99(3):1574–9. <https://doi.org/10.1073/pnas.032448299> PMID: 11830672; PubMed Central PMCID: PMC122232.
101. Thurlow LR, Thomas VC, Hancock LE. Capsular polysaccharide production in *Enterococcus faecalis* and contribution of CpsF to capsule serospecificity. *J Bacteriol.* 2009; 191(20):6203–10. <https://doi.org/10.1128/JB.00592-09> PMID: 19684130; PubMed Central PMCID: PMC2753019.
102. McBride SM, Fischetti VA, Leblanc DJ, Moellering RC Jr, Gilmore MS. Genetic diversity among *Enterococcus faecalis*. *PLoS ONE.* 2007; 2(7):e582. Epub 2007/07/06. <https://doi.org/10.1371/journal.pone.0000582> PMID: 17611618; PubMed Central PMCID: PMC1899230.
103. Wang S, Liu K, Seneviratne CJ, Li X, Cheung GS, Jin L, et al. Lipoteichoic acid from an *Enterococcus faecalis* clinical strain promotes TNF-alpha expression through the NF-kappaB and p38 MAPK signaling pathways in differentiated THP-1 macrophages. *Biomed Rep.* 2015; 3(5):697–702. <https://doi.org/10.3892/br.2015.495> PMID: 26405548; PubMed Central PMCID: PMC4576493.
104. Qin X, Galloway-Pena JR, Sillanpaa J, Roh JH, Nallapareddy SR, Chowdhury S, et al. Complete genome sequence of *Enterococcus faecium* strain TX16 and comparative genomic analysis of *Enterococcus faecium* genomes. *BMC Microbiol.* 2012; 12:135. Epub 2012/07/10. <https://doi.org/10.1186/1471-2180-12-135> PMID: 22769602; PubMed Central PMCID: PMC3433357.
105. Palmer KL, Godfrey P, Griggs A, Kos VN, Zucker J, Desjardins C, et al. Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. *mBio.* 2012; 3(1):e00318–11. <https://doi.org/10.1128/mBio.00318-11> PMID: 22354958; PubMed Central PMCID: PMC3374389.
106. Chilambi GS, Nordstrom HR, Evans DR, Ferrolino JA, Hayden RT, Maron GM, et al. Evolution of vancomycin-resistant *Enterococcus faecium* during colonization and infection in immunocompromised pediatric patients. *Proc Natl Acad Sci U S A.* 2020; 117(21):11703–14. Epub 2020/05/13. <https://doi.org/10.1073/pnas.1917130117> PMID: 32393645; PubMed Central PMCID: PMC7261057.
107. de Been M, van Schaik W, Cheng L, Corander J, Willems RJ. Recent recombination events in the core genome are associated with adaptive evolution in *Enterococcus faecium*. *Genome Biol Evol.* 2013; 5(8):1524–35. Epub 2013/07/25. <https://doi.org/10.1093/gbe/evt111> PMID: 23882129; PubMed Central PMCID: PMC3762198.