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Citation

Van Tyne D, Gilmore MS. 2014. Virulence Plasmids of Nonsporulating Gram-Positive Pathogens. Microbiol Spectr. 2, no. 5. doi: 10.1128/microbiolspec.PLAS-0002-2013.

Published Version

doi:10.1128/microbiolspec.PLAS-0002-2013

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Published in final edited form as:

Microbiol Spectr. 2014 ; 2(5): . doi:10.1128/microbiolspec.PLAS-0002-2013.

Virulence Plasmids of Nonsporulating Gram-Positive Pathogens

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SUMMARY

Gram-positive bacteria are leading causes of many types of human infection, including pneumonia, skin and nasopharyngeal infections, as well as urinary tract and surgical wound infections among hospitalized patients. These infections have become particularly problematic because many of the species causing them have become highly resistant to antibiotics. The role of mobile genetic elements, such as plasmids, in the dissemination of antibiotic resistance among Gram-positive bacteria has been well studied; less well understood is the role of mobile elements in the evolution and spread of virulence traits among these pathogens. While these organisms are leading agents of infection, they are also prominent members of the human commensal ecology. It appears that these bacteria are able to take advantage of the intimate association between host and commensal, via virulence traits that exacerbate infection and cause disease. However, evolution into an obligate pathogen has not occurred, presumably because it would lead to rejection of pathogenic organisms from the host ecology. Instead, in organisms that exist as both commensal and pathogen, selection has favored the development of mechanisms for variability. As a result, many virulence traits are localized on mobile genetic elements, such as virulence plasmids and pathogenicity islands. Virulence traits may occur within a minority of isolates of a given species, but these minority populations have nonetheless emerged as a leading problem in infectious disease. This chapter reviews virulence plasmids in nonsporulating Gram-positive bacteria, and examines their contribution to disease pathogenesis.

VIRULENCE PLASMIDS IN *STAPHYLOCOCCUS AUREUS*

S. aureus – virulence and pathogenesis

Infection with *S. aureus* can result in a wide variety of diseases, including wound infections, toxic shock, food poisoning, endocarditis, pneumonia, and septicemia (1). Virulence and drug resistance often occur together, as recent outbreak strains of methicillin-resistant *S. aureus* (MRSA) also produce a number of different virulence factors (2). It is perhaps not surprising that a bacterium capable of causing such a wide array of diseases possesses a diverse repertoire of virulence factors. A consequence of this versatility is that the pathogenesis of *S. aureus* is usually multifactorial (3).

S. aureus is capable of producing a number of extracellular toxins, including cytolytic toxins (α -toxin, β -toxin, γ -toxin), enterotoxins, toxic shock syndrome toxin (TSST-1), and exfoliative toxins. Although staphylococcal virulence is seldom attributable to one factor alone, different toxins have been linked to different types of staphylococcal infection. For example, staphylococcal enterotoxins are associated with food poisoning (4, 5), and

exfoliative toxins have been linked to staphylococcal scalded-skin syndrome (SSSS) (6–9). Many of these toxins function as superantigens, causing widespread T-lymphocyte activation and resulting in systemic shock (10–15). Cytolytic toxins, enterotoxins, and TSST-1 have all been shown to function as superantigens, while the association of superantigenic activity with exfoliative toxins is less clear (9, 16). Several staphylococcal antigens have been found to occur on mobile genetic elements, including the plasmid-encoded staphylococcal enterotoxin D (SED), staphylococcal enterotoxin J (SEJ), and exfoliative toxin B (ETB), which are discussed in greater detail below.

Enterotoxin

Staphylococcal enterotoxins (SEs) comprise a large family of related proteins similar to streptococcal pyrogenic exotoxins (15). These heat stable, pepsin resistant proteins include SEA-SEE, SEG-SEI, and SER-SET (4, 5). Staphylococcal food poisoning results primarily from the ingestion of contaminated meat or poultry, and to a lesser degree from contaminated fish, shellfish, and milk (4, 5, 17). Enterotoxin production within different *S. aureus* strains may vary, but food poisoning often results from the ingestion of any one or a combination of preformed enterotoxins (18). Historically, the most common enterotoxin associated with staphylococcal food poisoning is staphylococcal enterotoxin A (SEA), followed by SED and SEB (19, 20).

The gene encoding SED, *sed*, was determined to be present on a 27.6-kilobase penicillinase plasmid designated pIB485, which was isolated from strain KSI1410 and also contains genes for resistance to penicillin and cadmium sulfate (Figure 1) (21). Upon curing the strain of this plasmid, the ability to produce SED was lost. Cloning and expression of SED in *E. coli* from a restriction fragment of pIB485 proved that *sed* was encoded by pIB485. Transcription of *sed* was found to be regulated by the accessory gene regulator system (*agr*), a two-component system which regulates the transcription of virulence factors in a cell density-dependent manner (22–24). Regulation of *sed* by *agr* results in its induction during post-exponential growth (23). A study by Zhang et al. (23) identified a second enterotoxin-like gene within pIB485. This gene was termed *selj* and encodes staphylococcal enterotoxin-like protein J (SEJ) (Figure 1).

More recently, additional plasmid-encoded enterotoxins have been found in *S. aureus*. These include the enterotoxin-like protein SER, which shows sequence similarity to SEG and is encoded by a plasmid similar to pIB485 named pF5, for the Fukuoka 5 strain from which it was isolated (25). pF5 was also found to encode staphylococcal enterotoxins S and T (SES and SET), as well as several accessory genes. The contributions of SEJ, SER, SES, and SET to the pathogenesis of staphylococcal food poisoning, either singularly or in combination with SED, have not yet been addressed.

Exfoliative toxin

S. aureus is capable of producing four exfoliative toxins (ETs): exfoliative toxin A (ETA), ETB, ETC, and ETD. ETs are most common among phage group II isolates of *S. aureus* (26, 27), and cause peeling and blistering lesions of the skin (28). ETA, ETB, and ETD are glutamate-specific serine proteases that cleave desmoglein I, a desmosomal intracellular

adhesion molecule expressed in the superficial layers of the epidermis (29). ETC was first isolated from a horse lesion and appears to affect horses, chickens and mice (30), but its role in human disease, if any, has not been characterized. ETs have been associated with staphylococcal scalded skin syndrome (SSSS), a group of diseases including Ritter's disease, toxic epidermal necrosis, bullous impetigo, and erythema (7, 31). It has been suggested that high nasal carriage rates of *S. aureus* in adults, as well as the apparent protective nature of ET antibodies in SSSS (32–35), account for why SSSS is seen primarily in young children and the immunocompromised (36, 37). Finally, fewer than 5% of *S. aureus* clinical isolates harbor genes for ETs (38, 39), suggesting that these genes are acquired by horizontal gene transfer and can be passed between bacterial strains on mobile genetic elements.

While ETA, ETB, and ETD share similar gene sequences and modes of action, they are found in distinct genetic contexts. The gene encoding ETA is normally localized to the *S. aureus* chromosome (40), however it has also been found within the genome of an integrated temperate phage (41). ETD was identified on a novel *S. aureus* chromosomal pathogenicity island along with a gene for the epidermal cell differentiation inhibitor-B (EDIN-B) (42). ETD appears to act similarly to ETA, but it has also been found in patients with infections other than bullous impetigo or SSSS. Additionally, ETD was found to be present in approximately 10% of *S. aureus* clinical isolates in France (43), suggesting that it may play a role in a wider array of staphylococcal infections than ETA and ETB.

In contrast to ETA and ETD, the locus for ETB is typically plasmid-encoded (Figure 2) (44, 45). Formal evidence that the structural gene for ETB, designated *etb*, occurred on a plasmid was provided by Jackson and Iandolo (46). It was also discovered that the plasmid carries a cadmium sulfate resistance gene, which is part of a putative transposon (47). ETB is found on plasmids that vary in size from approximately 35 to 60 kilobases, yet the genetic organization of these plasmids appears to be conserved (Figure 2B) (48). Interestingly, the exfoliative toxins of *Staphylococcus hyicus*, SHETA and SHETB, which are associated with exudative epidermitis in pigs and chickens, are similar to ETA and ETB – SHETA is produced by plasmid-free strains, while SHETB production is dependent on the presence of a large plasmid (49, 50).

A staphylococcal bacteriocin two-component lantibiotic system, named BacR1/C55, is also located on various ETB-producing plasmids of *S. aureus* (51). BacR1 was initially characterized by Rogolsky (52), and was more extensively examined by Crupper et al. (53). Staphylococcin activity was attributed to the synergistic activity of two peptides, called C55 α and C55 β . The respective structural genes *sacA* and *sac β A*, as well as potential processing genes *sacM1* and *sacT*, are all organized within the lantibiotic operon shown in Figure 2 (51). The *sacA* and *sac β A* genes were detected by PCR only in strains that were also positive for ETB. Although found on different plasmids, C55 and BacR1 appear to constitute the same lantibiotic system, which also resembles the two-component bacteriocin lactacin 3147 from *Lactococcus lactis* (51).

In addition to bacteriocin activity attributable to either BacR1/C55, a novel virulence factor has also been linked to ETB-encoding plasmids. Through sequencing, Yamaguchi et al. (48)

identified a protein potentially capable of ADP-ribosylating Rho GTPases, which are members of the Ras superfamily of proteins involved in cytoskeletal network regulation within eukaryotic cells. Inactivation of Rho GTPases has been shown to inhibit the chemotactic and phagocytic activities of immune cells during infection (54), inhibit the differentiation of structural cells (55), and contribute to the dissemination of bacteria through the vasculature and tissues (56). Inactivation of Rho GTPases by *S. aureus* is accomplished by exotoxins of the epidermal cell differentiation inhibitor (EDIN) family (48, 55, 57, 58). The EDIN determinant on ETB-containing plasmids was designated EDIN-C (*ednC*) (Figure 2). EDIN-C has been found in the vast majority of EDIN-carrying *S. aureus* isolates, and was enriched among isolates recovered from deep-seated infections (59). This further suggests that EDIN-C is an important contributor to virulence of *S. aureus* infections in humans.

More recently, next-generation DNA sequencing has allowed for a systematic analysis of virulence factor-encoding plasmids in *S. aureus* (60). This effort has more than doubled the number of known plasmid groups, giving a fuller picture of the dissemination of plasmids among different strains of *S. aureus*, as well as the drug resistance and virulence traits that are associated with particular plasmid groups. The diversity among *S. aureus* plasmids is so great that plasmids can be used to track the spread of drug resistant isolates within a hospital setting (61). Importantly, this effort has identified many new *S. aureus* plasmids, which likely contain additional virulence factors that have yet to be described.

VIRULENCE PLASMIDS IN *ENTEROCOCCUS FAECALIS*

E. faecalis – virulence and pathogenesis

Enterococcus faecalis and *E. faecium* are members of the normal commensal flora of the gastrointestinal tracts of humans and other animals (62). However, *E. faecalis* and *E. faecium* are also leading causes of hospital acquired infection, including urinary tract infections, bacteremia, endocarditis, and intra-abdominal infections (63–68). The treatment of enterococcal infections is particularly challenging because antibiotic resistances enable the bacteria to survive standard therapies (69). Furthermore, the ability of enterococci to survive harsh environments, such as low pH, detergents, and bile salts, enables them to persist in the hospital environment (70–72). Vancomycin has been considered a drug of last resort for many enterococcal infections, but vancomycin-resistant enterococci (VRE), particularly *E. faecium*, are now commonly found in hospital settings (73). Although the majority of VRE infections are due to *E. faecium*, nearly 75% of all enterococcal infections are caused by *E. faecalis* (66, 74). This may be attributable to the additional virulence determinants that *E. faecalis* possesses, some of which are described below.

E. faecalis produces a number of factors that are presumably involved in adhesion to host tissues, and thereby contribute to host-pathogen interactions, as well as to the virulence and pathogenesis of enterococcal infections. These factors include enterococcal surface protein (Esp), aggregation substance, and the cytolysin. *E. faecalis* also expresses a capsular polysaccharide, which helps form the physical interface between bacteria and host. The capsular polysaccharide varies in structure, possibly because of immune pressure (75, 76). Because it is easily detectable on the surface of the bacterial cell and is the target of

complement-mediated opsonophagocytosis, the capsular polysaccharide has been used in typing schemes to classify different serotypes of *E. faecalis* (77). While the structure of the capsular polysaccharide is variable among different *E. faecalis* strains, the genes encoding the capsule have thus far been localized to the bacterial chromosome (78, 79).

The enterococcal surface protein Esp is an adhesin that has been localized to a 150-kilobase pathogenicity island in strains of *E. faecalis* (80, 81). Esp has been associated with outbreaks of VRE in hospitals (82), antibiotic resistance and biofilm formation (83, 84), and adherence of *E. faecalis* in the pathogenesis of urinary tract infections (80, 85). Aggregation substance is a surface protein that has similarly been shown to contribute to disease pathogenesis, also via enhanced adhesion and biofilm formation (86–92). Finally, some *E. faecalis* strains produce a cytolysin with both bacteriocidal and toxin activities (93, 94). The cytolysin operon is found, along with aggregation substance, on both pheromone-responsive plasmids (95), as well as within the chromosomal pathogenicity island on which Esp and is also found (81). Pheromone-responsive plasmids in enterococci are briefly reviewed below, followed by a discussion of the *E. faecalis* pheromone-responsive virulence plasmids that encode for aggregation substance and the cytolysin.

Pheromone-responsive plasmids in enterococci

Plasmid-free enterococci secrete over a dozen distinct peptide pheromones of 7–8 amino acids in length, which induce a mating response from donors carrying plasmids that specifically respond to each of these peptides (96–101) (Figure 3). Upon pheromone peptide secretion by recipient cells, donor cells containing the corresponding pheromone-responsive plasmid will express factors that promote physical interaction between donor and recipient cells, and thereby allow for conjugal DNA transfer. Aggregation substance on the surface of donor cells mediates clumping between donors and recipients via binding substance, a constituent of the cell wall of both plasmid containing and plasmid-free enterococci (102). It is believed that binding substance consists in part of lipoteichoic acid (103–105). Aggregation provides the initial cell-cell contact required for conjugal transfer of plasmid from donor to recipient. In broth, the mating potential of donor cells upon exposure to peptide pheromones is at least 100,000 times greater than that exhibited by non-induced donor cells (106). Transfer dynamics likely differ between bacteria growing in biofilms as compared to broth (107), but in both cases plasmid transfer depends upon cells sensing that they are in close proximity to one another. More than a dozen distinct pheromone-responsive plasmids have been identified within *E. faecalis* (108); the most well characterized of these include pAD1, pCF10, and pPD1. The mechanistic details of pheromone-responsive plasmid transfer in enterococci is covered in considerable detail elsewhere. However, the mechanism underlying transfer of pAD1 is also covered below.

Aggregation substance

The gene encoding aggregation substance (*asa1*) is often found on pAD1, a 60-kilobase, pheromone-responsive, transmissible plasmid (98, 109). Aggregation substances of other pheromone-responsive plasmids are homologous to the aggregation substance of pAD1 (108), although these other plasmids respond to different pheromones. The aggregation substance found on pAD1 is a 137-kDa surface protein, and has often been implicated in the

adherence and virulence of enterococci (86, 87, 89, 110, 111). Aggregation substance also appears to contribute to the spread of cytotoxin and antibiotic resistance determinants, via its role in the conjugal transfer of pheromone-responsive plasmids (112). The pheromone eliciting a response from pAD1-containing donor cells is designated cAD1 (113).

Genes encoding aggregation substance, functions involved in pheromone sensing and response, maintenance of plasmid pAD1, and the cytotoxin operon, are shown in Figure 4. The *asa1* gene is located adjacent to *seal*, which encodes a surface exclusion protein that inhibits transfer between cells containing similar plasmids and thereby limits redundant plasmid transfer (Figure 4A) (108). The *asa1* and *seal* genes are adjacent to the Tra regulon, which encodes a number of ORFs involved in pheromone sensing and regulation. Products of the *traA*, *traB*, *traD*, and *traE1* genes are involved in the regulation of the pheromone response (106, 114–118), while TraC is a surface protein involved in pheromone sensing, and functions as a specific binding factor (119). The *iad* gene encodes the pheromone inhibitor iAD1, which prevents self-induction of the pheromone response by inhibiting it at lower pheromone concentrations (120). Low concentrations of pheromone may arise from plasmid-containing or plasmid-free cells, at such a distance that an interaction allowing for conjugation is unlikely to occur.

Adjacent to genes associated with the transfer of pAD1 lies the *par* locus, a post-segregational killing mechanism (PSK, Figure 4B) (121). A region exhibiting a high degree of homology to the *par* determinant of pAD1 has also been identified in pCF10, another pheromone-responsive plasmid of *E. faecalis* (121). The primary function of PSKs is to maintain low-copy plasmids within a population of bacteria by killing plasmid-free segregants. The *par* locus within pAD1 encodes a toxin, Fst, which is believed to target chromosomal separation and/or cell division. Transcript RNA I contains the ORF believed to encode Fst toxin, a 33 amino acid peptide (122). Transcript RNA II is capable of occluding the 5' end of RNA I via antisense transcription of direct repeats within RNA I, as well as the 3' end of RNA I via the interaction of complementary transcriptional terminator stem-loops (122). RNA II is less stable than RNA I, therefore cells that do not maintain plasmid will not be able to inhibit toxin activity through the synthesis of antidote RNA II. PSKs are discussed in more detail elsewhere. The gene products of *repA* and *repB* are also involved in plasmid replication and maintenance (123, 124). RepA is responsible for initiation of replication, while RepBC, along with flanking repeat sequences, most probably represents a ParAB/SopAB partitioning system, which is covered in detail elsewhere.

Although the exact occurrence of events, as well as the interplay of the various Tra regulon factors in the coordination of the pheromone response, continue to be somewhat controversial topics, a generalized scheme is presented in Figure 5. In the uninduced state, TraA and a small RNA molecule resulting from the transcription of *traD* both negatively regulate levels of TraE1 (125). TraA accomplishes this negative regulation through DNA binding upstream of *iad* and *traE1*. When levels of the pheromone cAD1 surpass inhibition by iAD1, cAD1 binds directly to TraA, which loses its affinity for DNA and allows for transcription of *traE1* (126). TraE1 then goes on to activate production of other factors involved in conjugation (127).

Apart from its role in conjugal plasmid transfer, aggregation substance has also been linked to the virulence of *E. faecalis*. Aggregation substance was postulated to have a role in the adherence of enterococci to host cells, based on the presence of two Arg-Gly-Asp (RGD) motifs within its protein sequence. These motifs were hypothesized to mediate interactions between fibronectin and integrins on mammalian cell surfaces (128). *E. faecalis* expressing aggregation substance was shown to more readily bind renal epithelial cells in culture, and this enhanced adherence was blocked by the addition of a synthetic RGD peptide, but not by a peptide of divergent sequence (86). Mutating the RGD motifs in the pCF10-encoded aggregation substance gene (*prgB*) affected the virulence of *E. faecalis* in a rabbit endocarditis model (129), but not the invasion of human intestinal epithelial cells *in vitro* (130). The latter study focused instead on the aggregation domain as being critical for internalization. Finally, expression of aggregation substance has been reported to be induced *in vivo*, via an interaction between plasma components and the inhibitor peptide pheromone, which renders the latter unable to inhibit endogenous pheromone (91).

Few studies of the role of aggregation substance in the pathogenesis of infection have distinguished between the ability of aggregation substance to promote bacterial clumping, versus its potential contribution to the direct interaction between bacteria and specific types of human or animal cells (131). The aggregation domain of aggregation substance has been shown to mediate internalization of bacteria by enterocytes (130, 132). Expression of aggregation substance also correlates with enhanced uptake of enterococci by intestinal epithelial cells, but this increase in uptake did not result in an increase in bacterial translocation across the intestinal epithelium *in vitro* (89). Expression of aggregation substance was also found to affect the function of polymorphonuclear neutrophils (PMNs) and macrophages, by promoting the phagocytosis of enterococci by these cells (133, 134). Interestingly, aggregation substance was observed to promote the intracellular survival of enterococci within PMNs by inhibiting acidification of the phagolysosome (110) and within macrophages by inhibiting respiratory burst (134). In addition, the peptide pheromones involved in the induction of AS expression are also potentially able to alter the host immune response, in that the pheromones appear to be chemotactic for PMNs (135).

Aggregation substance does not appear to contribute to the virulence of *E. faecalis* in either a rabbit model of endophthalmitis (136), nor in *Caenorhabditis elegans* infection (137). However, in a rabbit model of endocarditis, a disease in which vegetations of platelets and fibrin resulting from bacterial infection are associated with inflammation of the heart valves and lining, aggregation substance from both pAD1 and pCF10 caused an increase in the size of cardiac vegetations (88, 90, 129). No effect of aggregation substance on vegetation size was observed in a rat endocarditis model (138), suggesting that the effects of aggregation substance on pathogenesis may be species or model-specific. The role of aggregation substance in an infection may be to promote the formation of a quorum of cells in a localized microenvironment through clumping, which could affect the expression of factors now known to be quorum regulated, such as the cytolysin (139). Aggregation substance in the absence of cytolysin expression was shown to increase the size of vegetations in endocarditis (88). When expressed from a plasmid that also encodes the cytolysin, such as pAD1, vegetations were observed to be smaller in mass but much more toxic. Thus

aggregation substance, by increasing the number of bacteria at a site of infection, could enhance production of cytolysin, though this has not yet been proven directly.

Apart from its potential to aid in achieving a quorum, the ability of aggregation substance to spread pheromone-responsive plasmids throughout a bacterial population could also enhance the virulence of *E. faecalis*, via the concomitant spread of cytolysin and antibiotic resistance determinants. In a study by Huycke et al. (140), an *in vivo* model was used to examine pheromone-responsive plasmid transfer between *E. faecalis* in the intestine. This study found that pAD1 and its derivatives were transferred between bacteria at high frequency, independent of antibiotic selection. While investigating the contribution of aggregation substance to endocarditis in a rabbit model, Hirt et al. (91) found that pCF10 was also transferred at high frequency *in vivo*. Although the conjugative spread of pheromone-responsive plasmids *in vivo* has been well documented, the precise consequence of the spread of these plasmids and their determinants during infection is not well understood.

Cytolysin

The cytolysin produced by *E. faecalis* is structurally and functionally unique (141, 142). It is capable of lysing erythrocytes, eukaryotic cells, and bacteria, and may also function as a signaling molecule to communicate with other bacteria and probe the environment (143, 144). Since its identification by Todd in 1934 from group D streptococci (*Streptococcus faecalis* subspecies *zymogenes* has since been reclassified as *E. faecalis*) (93), it has been characterized genetically and shown to contribute to virulence in virtually all models of *E. faecalis* infection tested (64, 88, 137, 145, 146). The cytolytic phenotype has also been reported to be more prevalent among infection-derived isolates of *E. faecalis* compared to non-infection isolates (147–149).

The determinant for cytolysin was originally suspected to be plasmid-encoded, because the hemolytic phenotype was observed to be a variable trait of *E. faecalis* and this trait could be transferred to plasmid-free recipients (150, 151). Clewell noted that a transposon insertion into pAD1 correlated with the loss of hemolysin/bacteriocin activity (152). Since then, a highly conserved cytolysin determinant has been found within plasmids of various *E. faecalis* isolates, including the pheromone-responsive plasmids pAD1 (152–154), pJH2 (151, 155), pOB1 (155, 156), pAM81 (157), pX98 (158), and pIP964 (159). Plasmids encoding cytolysin have been determined to fall within the same plasmid incompatibility group (160).

The organization of the cytolysin operon was elucidated through transposon mutagenesis of pAD1 and extracellular complementation, showing that eight genes are involved in the production of the toxin (Figure 4C) (95, 139, 161–163). A schematic of cytolysin expression, including posttranslational modifications, is depicted in Figure 6. Cytolysin activity is attributed to two structural components, designated CylL_L and CylL_S. Both peptides are post-translationally modified within *E. faecalis* by CylM (164), resulting in CylL_L* and CylL_S*. After modification, the subunits are secreted by CylB, an ATP-binding transporter (161, 164). While both CylL_L* and CylL_S* require CylB for secretion, only CylL_L* secretion is dependent upon ATP hydrolysis (161, 164). During secretion, the serine protease domain of CylB removes the leader sequences associated with the modified CylL_L*

and CylL_S* peptides (165), resulting in extracellular CyL_L' and CylL_S'. Finally, CyL_L' and CylL_S' are further cleaved by CylA, an extracellular serine protease, to generate the active toxin subunits CyL_L'' and CylL_S'' (162, 165). Expression of cytolysin is tightly regulated within *E. faecalis*. Its production was determined to be density dependent, with CylL_S'' serving as a signaling molecule for the induction of cytolysin expression (139, 166). Two genes involved in autoinduction by CylL_S'', *cylR1* and *cylR2*, have also been identified (Figure 4C) (139). CylR2 appears to repress expression of the operon in the absence of autoinducer via direct binding to the cytolysin promoter region (167, 168). The precise role of CylR1 is still undetermined.

Although the exact mechanism of action of the cytolysin bacteriocin has yet to be determined, cytolysin structural components resemble lantibiotics, a class of bactericidal peptides produced by Gram-positive bacteria (164, 165, 169). The composition, size, and presence of lanthionine linkages (post-translational condensation of cysteine and serine or threonine side chains) are characteristics shared with other lantibiotics (170–173). Cytolysin was found to kill a wide variety of Gram-positive species including staphylococci, streptococci, clostridia and enterococci (174, 175). It has been shown that CylII, a putative membrane metalloprotease encoded within the cytolysin operon, confers immunity to the cytolysin-producing cell (163), although the precise mechanism of immunity conferred by CylII has not yet been determined.

The contribution of the cytolysin to the virulence of *E. faecalis* has been well demonstrated in a number of infection models. Cytolysin-expressing bacterial strains exhibit a significantly lower LD₅₀ in a murine intraperitoneal challenge model than strains deficient in cytolysin (145, 146). In a rabbit model of endocarditis, lethality attributable to *E. faecalis* expressing aggregation substance in the absence of cytolysin was only 7% (88). However, 55% lethality was observed in *E. faecalis* infections where the bacteria expressed both aggregation substance and cytolysin (88). Cytolysin was also determined to contribute to the virulence of *E. faecalis* in a rabbit model of endophthalmitis, in which significant retinal damage and loss of vision was attributable to cytolysin expression (176, 177). Furthermore, cytolysin was shown to increase the lethality of *E. faecalis* in *C. elegans* infection (137), a model currently used as an initial rapid screen for potential virulence factors.

The ability of cytolysin to contribute to enhanced colonization of the intestine by pathogenic *E. faecalis*, as well as the spread of enterococci into the bloodstream, has been addressed in several studies. The potential role of cytolysin in competition with other enterococci within the intestinal niche was examined by Huycke et al. (178). In the same study, cytolytic strains were also able to outcompete non-cytolytic strains *in vitro*. However, in mice that were fed a mixture of cytolytic and non-cytolytic *E. faecalis*, no significant difference in the proportion of cytolytic and non-cytolytic isolates was observed in stool samples. Although a cytolytic strain has been shown to be capable of translocation across the intestinal epithelium (179), this activity has not been conclusively determined to be dependent upon expression of cytolysin. The exact contribution of cytolysin to *E. faecalis* bacteremia has yet to be determined, but cytolytic *E. faecalis* were found to spread from the peritoneum into the bloodstream of mice more readily than non-cytolytic isogenic mutants (66). It has also been suggested that cytolysin-expressing bacteria may be able to acquire exogenous heme,

resulting in enhanced growth within the bloodstream, although this hypothesis has yet to be tested (180).

It is clear that plasmids encoding cytolysin contribute to the virulence of *E. faecalis*. However, the precise contributions of cytolysin to the pathogenesis of infection remain to be described.

VIRULENCE PLASMIDS IN OTHER NONSPORULATING, GRAM-POSITIVE PATHOGENS

Rhodococcus equi

R. equi, a facultative intracellular pathogen which can persist and multiply within macrophages, is associated with severe equine pneumonia in foals, and is also capable of causing pneumonia in humans (181). It has been shown that equine and human clinical isolates of *R. equi* contain large plasmids (182, 183), and these plasmids have been determined to be essential for the ability of *R. equi* to survive within macrophages (184). In the absence of these plasmids, the virulence of *R. equi* is significantly attenuated (184–186).

Virulence-associated proteins (VapA-M) encoded by large plasmids (>50 kb) from *R. equi* clinical isolates are localized to the bacterial cell surface and appear to be critical for virulence (182, 185, 187–190). VapA in particular appears to play a major role in *R. equi* pathogenesis (184, 191). Expression of VapA was found to be dependent on both temperature and pH (192, 193), a feature shared with proteins encoded by virulence plasmids of *Yersinia pestis* and *Shigella flexneri* (194–196). VapB is encoded by a separate plasmid from the one encoding VapA (197, 198), and is associated with less virulent *R. equi* infections in humans (199). VapA-containing plasmids also encode additional Vap proteins VapC-H (200), while VapB-containing plasmids encode Vap proteins VapI-M (198). A map of the VapA-containing plasmid p33701 is shown in Figure 7 (200). The plasmid also contains genes that are associated with conjugation and maintenance functions (Figure 7; red ORFs). The region containing the genes encoding VapC-H (Figure 7; blue ORFs) is bounded by two transposon resolvases. p33701 also contains a putative two-component regulator (*tcr*) and a putative lysyl-tRNA synthetase (Figure 7; yellow ORFs) that both appear from G+C content to be foreign to *R. equi* (200). All Vaps resemble each other in sequence, and Vap homologs have not yet been identified in other organisms. The specific roles of the different Vap proteins in *R. equi* infection remain unknown.

Lactococcus garvieae

Lactococcus garvieae is a widely distributed lactic acid bacteria that is a well-known pathogen of fish, but is also occasionally found as an opportunistic pathogen of humans (201, 202). *L. garvieae* is considered an emerging zoonotic pathogen, and the number of human cases of infection has increased in recent years (203). A recently isolated human strain of *L. garvieae* was found to contain five plasmids, one of which appears to encode putative virulence factors such as toxins and possible surface proteins (204). These proteins may aid the bacteria in adhering to host tissue; future studies should aim to determine if these genes do in fact play a role in *L. garvieae* virulence.

CONCLUDING REMARKS

In general, virulence plasmids are uncommon among nonsporulating Gram-positive pathogens, with notable exceptions in staphylococci and enterococci, as well as the opportunistic pathogens *R. equi* and *L. garvieae*. Staphylococci and enterococci are leading causes of antibiotic resistant, hospital-acquired infections (205). Although these organisms are major causes of nosocomial infection, numerically only a miniscule population of cells is involved in infection, with the vast majority of bacteria occurring in peaceful coexistence with the host as part of the commensal flora. Virulence plasmids may therefore represent “selfish DNA” (206), taking advantage of the otherwise stable association between commensal and host to propagate throughout a population. Alternatively, virulence plasmids may represent a recent acquisition by these species, which at this point has failed to reach a deep penetrance in the population, potentially because of niche sub-specialization and isolation. The occurrence of clonal lineages possessing virulence traits in these species argues for the latter (207, 208), but these two prospects are not mutually exclusive.

Acknowledgments

The authors thank Chris Pillar, Philip Coburn, and Wolfgang Haas for their contributions to the prior edition of this chapter. Portions of this work were funded by NIH grant AI072360, and the Harvard-wide Program on Antibiotic Resistance (NIH grant AI083214). D.V.T. is supported by Grant EY007145 from the National Institutes of Health.

References

1. Novick, RP. Pathogenicity factors and their regulation. In: Fischetti, V., editor. Gram-positive pathogens. American Society for Microbiology; Washington, D.C: 2000. p. 392-407.
2. Otto M. MRSA virulence and spread. Cell Microbiol. 2012; 14:1513–1521. [PubMed: 22747834]
3. Projan, SJ.; Novick, RP. The molecular basis of virulence. In: Archer, G.; Crossley, K., editors. Staphylococci in human disease. Churchill Livingstone; New York, NY: 1997. p. 55-81.
4. Balaban N, Rasooly A. Staphylococcal enterotoxins. Int J Food Microbiol. 2000; 61:1–10. [PubMed: 11028954]
5. Argudin MA, Mendoza MC, Rodicio MR. Food poisoning and Staphylococcus aureus enterotoxins. Toxins (Basel). 2010; 2:1751–1773. [PubMed: 22069659]
6. Lowney ED, Baublis JV, Kreye GM, Harrell ER, McKenzie AR. The scalded skin syndrome in small children. Arch Dermatol. 1967; 95:359–369. [PubMed: 6023040]
7. Melish ME, Glasgow LA. The staphylococcal scalded-skin syndrome. N Engl J Med. 1970; 282:1114–1119. [PubMed: 4245327]
8. Melish ME, Glasgow LA, Turner MD, Lillibridge CB. The staphylococcal epidermolytic toxin: its isolation, characterization, and site of action. Ann N Y Acad Sci. 1974; 236:317–342. [PubMed: 4278991]
9. Bukowski M, Wladyka B, Dubin G. Exfoliative toxins of Staphylococcus aureus. Toxins (Basel). 2010; 2:1148–1165. [PubMed: 22069631]
10. Choi YW, Herman A, DiGiusto D, Wade T, Marrack P, Kappler J. Residues of the variable region of the T-cell-receptor beta-chain that interact with S. aureus toxin superantigens. Nature. 1990; 346:471–473. [PubMed: 2377208]
11. Chintagumpala MM, Mollick JA, Rich RR. Staphylococcal toxins bind to different sites on HLA-DR. J Immunol. 1991; 147:3876–3881. [PubMed: 1940373]
12. Mollick JA, Chintagumpala M, Cook RG, Rich RR. Staphylococcal exotoxin activation of T cells. Role of exotoxin-MHC class II binding affinity and class II isotype. J Immunol. 1991; 146:463–468. [PubMed: 1987273]

13. Monday, SR.; Bohach, GA. Properties of *Staphylococcus aureus* enterotoxins and toxic shock syndrome toxin-1. In: Alouf, J.; Freer, J., editors. The Comprehensive Sourcebook of Bacterial Protein Toxins. Academic Press; London, England: 1999. p. 589-610.
14. Rajagopalan G, Sen MM, Singh M, Murali NS, Nath KA, Iijima K, Kita H, Leontovich AA, Gopinathan U, Patel R, David CS. Intranasal exposure to staphylococcal enterotoxin B elicits an acute systemic inflammatory response. Shock. 2006; 25:647–656. [PubMed: 16721274]
15. Spaulding AR, Salgado-Pabon W, Kohler PL, Horswill AR, Leung DY, Schlievert PM. Staphylococcal and streptococcal superantigen exotoxins. Clin Microbiol Rev. 2013; 26:422–447. [PubMed: 23824366]
16. Ladhani S, Joannou CL, Lochrie DP, Evans RW, Poston SM. Clinical, microbial, and biochemical aspects of the exfoliative toxins causing staphylococcal scalded-skin syndrome. Clin Microbiol Rev. 1999; 12:224–242. [PubMed: 10194458]
17. Wieneke AA, Roberts D, Gilbert RJ. Staphylococcal food poisoning in the United Kingdom, 1969–90. Epidemiol Infect. 1993; 110:519–531. [PubMed: 8519317]
18. Holmberg SD, Blake PA. Staphylococcal food poisoning in the United States. New facts and old misconceptions. JAMA. 1984; 251:487–489. [PubMed: 6690814]
19. Casman EP. Staphylococcal enterotoxin. Ann N Y Acad Sci. 1965; 128:124–131. [PubMed: 4221871]
20. Bergdoll MS, Czop JK, Gould SS. Enterotoxin synthesis by the staphylococci. Ann N Y Acad Sci. 1974; 236:307–316. [PubMed: 4529481]
21. Bayles KW, Iandolo JJ. Genetic and molecular analyses of the gene encoding staphylococcal enterotoxin D. J Bacteriol. 1989; 171:4799–4806. [PubMed: 2549000]
22. Kornblum, J.; Kreiswirth, BN.; Projan, SJ.; Ross, H.; Novick, RP. Agr: A polycistronic locus regulating exoprotein synthesis in *Staphylococcus aureus*. In: Novick, RP., editor. Molecular Biology of the Staphylococci. VCH Publishers; New York, NY: 1991. p. 373-402.
23. Zhang S, Iandolo JJ, Stewart GC. The enterotoxin D plasmid of *Staphylococcus aureus* encodes a second enterotoxin determinant (sej). FEMS Microbiol Lett. 1998; 168:227–233. [PubMed: 9835033]
24. Tseng CW, Zhang S, Stewart GC. Accessory gene regulator control of staphylococcal enterotoxin d gene expression. J Bacteriol. 2004; 186:1793–1801. [PubMed: 14996810]
25. Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K. Identification and characterization of a new staphylococcal enterotoxin-related putative toxin encoded by two kinds of plasmids. Infect Immun. 2003; 71:6088–6094. [PubMed: 14500536]
26. Dajani AS. The scalded-skin syndrome: relation to phage-group II staphylococci. J Infect Dis. 1972; 125:548–551. [PubMed: 4260083]
27. Kondo I, Sakurai S, Sarai Y. Purification of exfoliatin produced by *Staphylococcus aureus* of bacteriophage group 2 and its physicochemical properties. Infect Immun. 1973; 8:156–164. [PubMed: 4269383]
28. Iwatsuki K, Yamasaki O, Morizane S, Oono T. Staphylococcal cutaneous infections: invasion, evasion and aggression. J Dermatol Sci. 2006; 42:203–214. [PubMed: 16679003]
29. Nishifuji K, Sugai M, Amagai M. Staphylococcal exfoliative toxins: “molecular scissors” of bacteria that attack the cutaneous defense barrier in mammals. J Dermatol Sci. 2008; 49:21–31. [PubMed: 17582744]
30. Sato H, Matsumori Y, Tanabe T, Saito H, Shimizu A, Kawano J. A new type of staphylococcal exfoliative toxin from a *Staphylococcus aureus* strain isolated from a horse with phlegmon. Infect Immun. 1994; 62:3780–3785. [PubMed: 8063394]
31. Melish ME, Glasgow LA. Staphylococcal scalded skin syndrome: the expanded clinical syndrome. J Pediatr. 1971; 78:958–967. [PubMed: 4252715]
32. Miller MM, Kapral FA. Neutralization of *Staphylococcus aureus* exfoliatin by antibody. Infect Immun. 1972; 6:561–563. [PubMed: 4634954]
33. Elias PM, Fritsch P, Tappeiner G, Mittermayer H, Wolff K. Experimental staphylococcal toxic epidermal necrolysis (TEN) in adult humans and mice. J Lab Clin Med. 1974; 84:414–424. [PubMed: 4851979]

34. McLay AL, Arbuthnott JP, Lyell A. Action of staphylococcal epidermolytic toxin on mouse skin: an electron microscopic study. *J Invest Dermatol.* 1975; 65:423–428. [PubMed: 1081558]
35. Wiley BB, Glasgow LA, Rogolsky M. Staphylococcal scalded-skin syndrome: development of a primary binding assay for human antibody to the exfoliative toxin. *Infect Immun.* 1976; 13:513–520. [PubMed: 131109]
36. Kapral FA. Staphylococcus aureus: some host-parasite interactions. *Ann N Y Acad Sci.* 1974; 236:267–276. [PubMed: 4278721]
37. Todd JK. Staphylococcal toxin syndromes. *Annu Rev Med.* 1985; 36:337–347. [PubMed: 3158268]
38. Dancer SJ, Noble WC. Nasal, axillary, and perineal carriage of Staphylococcus aureus among women: identification of strains producing epidermolytic toxin. *J Clin Pathol.* 1991; 44:681–684. [PubMed: 1890203]
39. Becker K, Friedrich AW, Lubritz G, Weilert M, Peters G, Von Eiff C. Prevalence of genes encoding pyrogenic toxin superantigens and exfoliative toxins among strains of Staphylococcus aureus isolated from blood and nasal specimens. *J Clin Microbiol.* 2003; 41:1434–1439. [PubMed: 12682126]
40. Lee CY, Schmidt JJ, Johnson-Winegar AD, Spero L, Iandolo JJ. Sequence determination and comparison of the exfoliative toxin A and toxin B genes from Staphylococcus aureus. *J Bacteriol.* 1987; 169:3904–3909. [PubMed: 3040666]
41. Yamaguchi T, Hayashi T, Takami H, Nakasone K, Ohnishi M, Nakayama K, Yamada S, Komatsuzawa H, Sugai M. Phage conversion of exfoliative toxin A production in Staphylococcus aureus. *Mol Microbiol.* 2000; 38:694–705. [PubMed: 11115106]
42. Yamaguchi T, Nishifuji K, Sasaki M, Fudaba Y, Aepfelbacher M, Takata T, Ohara M, Komatsuzawa H, Amagai M, Sugai M. Identification of the Staphylococcus aureus etd pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. *Infect Immun.* 2002; 70:5835–5845. [PubMed: 12228315]
43. Yamasaki O, Tristan A, Yamaguchi T, Sugai M, Lina G, Bes M, Vandenesch F, Etienne J. Distribution of the exfoliative toxin D gene in clinical Staphylococcus aureus isolates in France. *Clin Microbiol Infect.* 2006; 12:585–588. [PubMed: 16700711]
44. Warren R, Rogolsky M, Wiley BB, Glasgow LA. Effect of ethidium bromide on elimination of exfoliative toxin and bacteriocin production in Staphylococcus aureus. *J Bacteriol.* 1974; 118:980–985. [PubMed: 4829933]
45. Rogolsky M, Warren R, Wiley BB, Nakamura HT, Glasgow LA. Nature of the genetic determinant controlling exfoliative toxin production in Staphylococcus aureus. *J Bacteriol.* 1974; 117:157–165. [PubMed: 4271807]
46. Jackson MP, Iandolo JJ. Cloning and expression of the exfoliative toxin B gene from Staphylococcus aureus. *J Bacteriol.* 1986; 166:574–580. [PubMed: 3009410]
47. Jackson MP, Iandolo JJ. Sequence of the exfoliative toxin B gene of Staphylococcus aureus. *J Bacteriol.* 1986; 167:726–728. [PubMed: 3733674]
48. Yamaguchi T, Hayashi T, Takami H, Ohnishi M, Murata T, Nakayama K, Asakawa K, Ohara M, Komatsuzawa H, Sugai M. Complete nucleotide sequence of a Staphylococcus aureus exfoliative toxin B plasmid and identification of a novel ADP-ribosyltransferase, EDIN-C. *Infect Immun.* 2001; 69:7760–7771. [PubMed: 11705958]
49. Sato H, Tanabe T, Kuramoto M, Tanaka K, Hashimoto T, Saito H. Isolation of exfoliative toxin from Staphylococcus hyicus subsp. hyicus and its exfoliative activity in the piglet. *Vet Microbiol.* 1991; 27:263–275. [PubMed: 1882504]
50. Sato H, Watanabe T, Murata Y, Ohtake A, Nakamura M, Aizawa C, Saito H, Maehara N. New exfoliative toxin produced by a plasmid-carrying strain of Staphylococcus hyicus. *Infect Immun.* 1999; 67:4014–4018. [PubMed: 10417168]
51. Navaratna MA, Sahl HG, Tagg JR. Identification of genes encoding two-component lantibiotic production in Staphylococcus aureus C55 and other phage group II S. aureus strains and demonstration of an association with the exfoliative toxin B gene. *Infect Immun.* 1999; 67:4268–4271. [PubMed: 10417203]

52. Rogolsky M, Wiley BB. Production and properties of a staphylococcal genetically controlled by the staphylococcal plasmid for exfoliative toxin synthesis. *Infect Immun*. 1977; 15:726–732. [PubMed: 870429]
53. Crupper SS, Gies AJ, Iandolo JJ. Purification and characterization of staphylococcal BacR1, a broad-spectrum bacteriocin. *Appl Environ Microbiol*. 1997; 63:4185–4190. [PubMed: 9361402]
54. Aktories K. Rho proteins: targets for bacterial toxins. *Trends Microbiol*. 1997; 5:282–288. [PubMed: 9234511]
55. Sugai M, Enomoto T, Hashimoto K, Matsumoto K, Matsuo Y, Ohgai H, Hong YM, Inoue S, Yoshikawa K, Suganaka H. A novel epidermal cell differentiation inhibitor (EDIN): purification and characterization from *Staphylococcus aureus*. *Biochem Biophys Res Commun*. 1990; 173:92–98. [PubMed: 2256941]
56. Munro P, Benchetrit M, Nahori MA, Stefani C, Clement R, Michiels JF, Landraud L, Dussurget O, Lemichez E. The *Staphylococcus aureus* epidermal cell differentiation inhibitor toxin promotes formation of infection foci in a mouse model of bacteremia. *Infect Immun*. 2010; 78:3404–3411. [PubMed: 20479081]
57. Inoue S, Sugai M, Murooka Y, Paik SY, Hong YM, Ohgai H, Suganaka H. Molecular cloning and sequencing of the epidermal cell differentiation inhibitor gene from *Staphylococcus aureus*. *Biochem Biophys Res Commun*. 1991; 174:459–464. [PubMed: 1993048]
58. Aktories K. Bacterial protein toxins that modify host regulatory GTPases. *Nat Rev Microbiol*. 2011; 9:487–498. [PubMed: 21677684]
59. Munro P, Clement R, Lavigne JP, Pulcini C, Lemichez E, Landraud L. High prevalence of edin-C encoding RhoA-targeting toxin in clinical isolates of *Staphylococcus aureus*. *Eur J Clin Microbiol Infect Dis*. 2011; 30:965–972. [PubMed: 21311940]
60. McCarthy AJ, Lindsay JA. The distribution of plasmids that carry virulence and resistance genes in *Staphylococcus aureus* is lineage associated. *BMC Microbiol*. 2012; 12:104. [PubMed: 22691167]
61. Lindsay JA, Knight GM, Budd EL, McCarthy AJ. Shuffling of mobile genetic elements (MGEs) in successful healthcare-associated MRSA (HA-MRSA). *Mob Genet Elements*. 2012; 2:239–243. [PubMed: 23446976]
62. Mundt JO. Occurrence of enterococci in animals in a wild environment. *Appl Microbiol*. 1963; 11:136–140. [PubMed: 13936610]
63. Murray BE. The life and times of the *Enterococcus*. *Clin Microbiol Rev*. 1990; 3:46–65. [PubMed: 2404568]
64. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. *Clin Microbiol Rev*. 1994; 7:462–478. [PubMed: 7834601]
65. Moellering, R. *Enterococcus* species, *Streptococcus bovis*, and *Leuconostoc* species. In: Mandell, G.; Bennett, J.; Dolin, R., editors. *Principles and Practices of Infectious Diseases*. 4. Churchill Livingstone; New York, NY: 1995. p. 1826–1835.
66. Huycke MM, Sahm DF, Gilmore MS. Multiple-drug resistant enterococci: the nature of the problem and an agenda for the future. *Emerg Infect Dis*. 1998; 4:239–249. [PubMed: 9621194]
67. Willems RJ, van Schaik W. Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. *Future Microbiol*. 2009; 4:1125–1135. [PubMed: 19895216]
68. Gilmore MS, Lebreton F, van Schaik W. Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. *Curr Opin Microbiol*. 2013; 16:10–16. [PubMed: 23395351]
69. Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in enterococcus. *Virulence*. 2012; 3:421–433. [PubMed: 23076243]
70. Flahaut S, Frere J, Boutibonnes P, Auffray Y. Comparison of the bile salts and sodium dodecyl sulfate stress responses in *Enterococcus faecalis*. *Appl Environ Microbiol*. 1996; 62:2416–2420. [PubMed: 8779581]
71. Flahaut S, Hartke A, Giard JC, Benachour A, Boutibonnes P, Auffray Y. Relationship between stress response toward bile salts, acid and heat treatment in *Enterococcus faecalis*. *FEMS Microbiol Lett*. 1996; 138:49–54. [PubMed: 8674969]

72. Flahaut S, Hartke A, Giard JC, Auffray Y. Alkaline stress response in *Enterococcus faecalis*: adaptation, cross-protection, and changes in protein synthesis. *Appl Environ Microbiol.* 1997; 63:812–814. [PubMed: 9023964]
73. Arias CA, Murray BE. The rise of the *Enterococcus*: beyond vancomycin resistance. *Nat Rev Microbiol.* 2012; 10:266–278. [PubMed: 22421879]
74. Mundy LM, Sahm DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. *Clin Microbiol Rev.* 2000; 13:513–522. [PubMed: 11023953]
75. Maekawa S, Yoshioka M, Kumamoto Y. Proposal of a new scheme for the serological typing of *Enterococcus faecalis* strains. *Microbiol Immunol.* 1992; 36:671–681. [PubMed: 1406370]
76. Thurlow LR, Thomas VC, Fleming SD, Hancock LE. *Enterococcus faecalis* capsular polysaccharide serotypes C and D and their contributions to host innate immune evasion. *Infect Immun.* 2009; 77:5551–5557. [PubMed: 19805541]
77. Hufnagel M, Hancock LE, Koch S, Theilacker C, Gilmore MS, Huebner J. Serological and genetic diversity of capsular polysaccharides in *Enterococcus faecalis*. *J Clin Microbiol.* 2004; 42:2548–2557. [PubMed: 15184433]
78. 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; 99:1574–1579. [PubMed: 11830672]
79. 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; 77:3759–3767. [PubMed: 19581393]
80. Shankar N, Lockatell CV, Baghdayan AS, Drachenberg C, Gilmore MS, Johnson DE. Role of *Enterococcus faecalis* surface protein *Esp* in the pathogenesis of ascending urinary tract infection. *Infect Immun.* 2001; 69:4366–4372. [PubMed: 11401975]
81. Shankar N, Baghdayan AS, Gilmore MS. Modulation of virulence within a pathogenicity island in vancomycin-resistant *Enterococcus faecalis*. *Nature.* 2002; 417:746–750. [PubMed: 12066186]
82. Willems RJ, Homan W, Top J, van Santen-Verheuve M, Tribe D, Manziros X, Gaillard C, Vandenbroucke-Grauls CM, Mascini EM, van Kregten E, van Embden JD, Bonten MJ. Variant *esp* gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. *Lancet.* 2001; 357:853–855. [PubMed: 11265956]
83. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, Amorena B, Leiva J, Penades JR, Lasa I. The enterococcal surface protein, *Esp*, is involved in *Enterococcus faecalis* biofilm formation. *Appl Environ Microbiol.* 2001; 67:4538–4545. [PubMed: 11571153]
84. Foulquie Moreno MR, Sarantinopoulos P, Tsakalidou E, De Vuyst L. The role and application of enterococci in food and health. *Int J Food Microbiol.* 2006; 106:1–24. [PubMed: 16216368]
85. Borgmann S, Niklas DM, Klare I, Zabel LT, Buchenau P, Autenrieth IB, Heeg P. Two episodes of vancomycin-resistant *Enterococcus faecium* outbreaks caused by two genetically different clones in a newborn intensive care unit. *Int J Hyg Environ Health.* 2004; 207:386–389. [PubMed: 15471103]
86. Kreft B, Marre R, Schramm U, Wirth R. Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured renal tubular cells. *Infect Immun.* 1992; 60:25–30. [PubMed: 1729187]
87. Clewell DB. Bacterial sex pheromone-induced plasmid transfer. *Cell.* 1993; 73:9–12. [PubMed: 8462105]
88. Chow JW, Thal LA, Perri MB, Vazquez JA, Donabedian SM, Clewell DB, Zervos MJ. Plasmid-associated hemolysin and aggregation substance production contribute to virulence in experimental enterococcal endocarditis. *Antimicrob Agents Chemother.* 1993; 37:2474–2477. [PubMed: 8285637]
89. Olmsted SB, Dunny GM, Erlandsen SL, Wells CL. A plasmid-encoded surface protein on *Enterococcus faecalis* augments its internalization by cultured intestinal epithelial cells. *J Infect Dis.* 1994; 170:1549–1556. [PubMed: 7995995]
90. Schlievert PM, Gahr PJ, Assimacopoulos AP, Dinges MM, Stoehr JA, Harmala JW, Hirt H, Dunny GM. Aggregation and binding substances enhance pathogenicity in rabbit models of *Enterococcus faecalis* endocarditis. *Infect Immun.* 1998; 66:218–223. [PubMed: 9423861]

91. Hirt H, Schlievert PM, Dunny GM. In vivo induction of virulence and antibiotic resistance transfer in *Enterococcus faecalis* mediated by the sex pheromone-sensing system of pCF10. *Infect Immun*. 2002; 70:716–723. [PubMed: 11796604]
92. Chuang-Smith ON, Wells CL, Henry-Stanley MJ, Dunny GM. Acceleration of *Enterococcus faecalis* biofilm formation by aggregation substance expression in an ex vivo model of cardiac valve colonization. *PLoS One*. 2010; 5:e15798. [PubMed: 21209892]
93. Todd E. A comparative serological study of streptolysins derived from human and from animal infections, with notes on pneumococcal haemolysin, tetanolysin and staphylococcus toxin. *J Pathol Bacteriol*. 1934; 39:299–321.
94. Sherwood N, Russell B, Jay A, Bowman K. Studies on streptococci. III. New antibiotic substances produced by beta hemolytic streptococci. *J Infect Dis*. 1949; 84:88–91. [PubMed: 18109256]
95. Ike Y, Clewell DB, Segarra RA, Gilmore MS. Genetic analysis of the pAD1 hemolysin/bacteriocin determinant in *Enterococcus faecalis*: Tn917 insertional mutagenesis and cloning. *J Bacteriol*. 1990; 172:155–163. [PubMed: 2152897]
96. Wirth R. The sex pheromone system of *Enterococcus faecalis*. More than just a plasmid-collection mechanism? *Eur J Biochem*. 1994; 222:235–246. [PubMed: 8020463]
97. Dunny GM, Leonard BA, Hedberg PJ. Pheromone-inducible conjugation in *Enterococcus faecalis*: interbacterial and host-parasite chemical communication. *J Bacteriol*. 1995; 177:871–876. [PubMed: 7860595]
98. Clewell, D. Sex pheromone systems in enterococci. In: Dunny, G.; Winans, S., editors. *Cell-Cell Signaling in Bacteria*. American Society for Microbiology; Washington, DC: 1999. p. 47-65.
99. Wirth R. Sex pheromones and gene transfer in *Enterococcus faecalis*. *Res Microbiol*. 2000; 151:493–496. [PubMed: 10961465]
100. Palmer KL, Kos VN, Gilmore MS. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. *Curr Opin Microbiol*. 2010; 13:632–639. [PubMed: 20837397]
101. Wardal E, Sadowy E, Hryniewicz W. Complex nature of enterococcal pheromone-responsive plasmids. *Pol J Microbiol*. 2010; 59:79–87. [PubMed: 20734751]
102. Galli D, Wirth R, Wanner G. Identification of aggregation substances of *Enterococcus faecalis* cells after induction by sex pheromones. An immunological and ultrastructural investigation. *Arch Microbiol*. 1989; 151:486–490. [PubMed: 2505719]
103. Ehrenfeld EE, Kessler RE, Clewell DB. Identification of pheromone-induced surface proteins in *Streptococcus faecalis* and evidence of a role for lipoteichoic acid in formation of mating aggregates. *J Bacteriol*. 1986; 168:6–12. [PubMed: 3093466]
104. Bensing BA, Dunny GM. Cloning and molecular analysis of genes affecting expression of binding substance, the recipient-encoded receptor(s) mediating mating aggregate formation in *Enterococcus faecalis*. *J Bacteriol*. 1993; 175:7421–7429. [PubMed: 8226689]
105. Waters CM, Hirt H, McCormick JK, Schlievert PM, Wells CL, Dunny GM. An amino-terminal domain of *Enterococcus faecalis* aggregation substance is required for aggregation, bacterial internalization by epithelial cells and binding to lipoteichoic acid. *Mol Microbiol*. 2004; 52:1159–1171. [PubMed: 15130132]
106. Ike Y, Clewell DB. Genetic analysis of the pAD1 pheromone response in *Streptococcus faecalis*, using transposon Tn917 as an insertional mutagen. *J Bacteriol*. 1984; 158:777–783. [PubMed: 6327637]
107. Cook L, Chatterjee A, Barnes A, Yarwood J, Hu WS, Dunny G. Biofilm growth alters regulation of conjugation by a bacterial pheromone. *Mol Microbiol*. 2011; 81:1499–1510. [PubMed: 21843206]
108. Hirt H, Wirth R, Muscholl A. Comparative analysis of 18 sex pheromone plasmids from *Enterococcus faecalis*: detection of a new insertion element on pPD1 and implications for the evolution of this plasmid family. *Mol Gen Genet*. 1996; 252:640–647. [PubMed: 8917306]
109. Francia MV, Haas W, Wirth R, Samberger E, Muscholl-Silberhorn A, Gilmore MS, Ike Y, Weaver KE, An FY, Clewell DB. Completion of the nucleotide sequence of the *Enterococcus faecalis* conjugative virulence plasmid pAD1 and identification of a second transfer origin. *Plasmid*. 2001; 46:117–127. [PubMed: 11591137]

110. Rakita RM, Vanek NN, Jacques-Palaz K, Mee M, Mariscalco MM, Dunny GM, Snuggs M, Van Winkle WB, Simon SI. Enterococcus faecalis bearing aggregation substance is resistant to killing by human neutrophils despite phagocytosis and neutrophil activation. *Infect Immun*. 1999; 67:6067–6075. [PubMed: 10531268]
111. Fisher K, Phillips C. The ecology, epidemiology and virulence of Enterococcus. *Microbiology*. 2009; 155:1749–1757. [PubMed: 19383684]
112. Clewell DB. Properties of Enterococcus faecalis plasmid pAD1, a member of a widely disseminated family of pheromone-responding, conjugative, virulence elements encoding cytotoxin. *Plasmid*. 2007; 58:205–227. [PubMed: 17590438]
113. Mori M, Sakagami Y, Narita M, Isogai A, Fujino M, Kitada C, Craig RA, Clewell DB, Suzuki A. Isolation and structure of the bacterial sex pheromone, cAD1, that induces plasmid transfer in Streptococcus faecalis. *FEBS Lett*. 1984; 178:97–100. [PubMed: 6437872]
114. de Freire Bastos MC, Tanimoto K, Clewell DB. Regulation of transfer of the Enterococcus faecalis pheromone-responding plasmid pAD1: temperature-sensitive transfer mutants and identification of a new regulatory determinant, traD. *J Bacteriol*. 1997; 179:3250–3259. [PubMed: 9150221]
115. Tanimoto K, Clewell DB. Regulation of the pAD1-encoded sex pheromone response in Enterococcus faecalis: expression of the positive regulator TraE1. *J Bacteriol*. 1993; 175:1008–1018. [PubMed: 8432694]
116. An FY, Clewell DB. Characterization of the determinant (traB) encoding sex pheromone shutdown by the hemolysin/bacteriocin plasmid pAD1 in Enterococcus faecalis. *Plasmid*. 1994; 31:215–221. [PubMed: 8029329]
117. Muscholl-Silberhorn AB. Pheromone-regulated expression of sex pheromone plasmid pAD1-encoded aggregation substance depends on at least six upstream genes and a cis-acting, orientation-dependent factor. *J Bacteriol*. 2000; 182:3816–3825. [PubMed: 10850999]
118. Weaver KE, Clewell DB. Regulation of the pAD1 sex pheromone response in Enterococcus faecalis: construction and characterization of lacZ transcriptional fusions in a key control region of the plasmid. *J Bacteriol*. 1988; 170:4343–4352. [PubMed: 2842316]
119. Tanimoto K, An FY, Clewell DB. Characterization of the traC determinant of the Enterococcus faecalis hemolysin-bacteriocin plasmid pAD1: binding of sex pheromone. *J Bacteriol*. 1993; 175:5260–5264. [PubMed: 8349566]
120. Mori M, Tanaka H, Sakagami Y, Isogai A, Fujino M, Kitada C, White BA, An FY, Clewell DB, Suzuki A. Isolation and structure of the Streptococcus faecalis sex pheromone, cAM373. *FEBS Lett*. 1986; 206:69–72. [PubMed: 3093276]
121. Weaver KE, Jensen KD, Colwell A, Sriram SI. Functional analysis of the Enterococcus faecalis plasmid pAD1-encoded stability determinant par. *Mol Microbiol*. 1996; 20:53–63. [PubMed: 8861204]
122. Weaver, K. Enterococcal genetics. In: Fischetti, V.; Novick, R.; Ferretti, J.; Portnoy, D.; Rood, J., editors. *Gram-Positive Pathogens*. American Society for Microbiology; Washington, DC: 2000. p. 259-271.
123. Weaver KE, Clewell DB. Construction of Enterococcus faecalis pAD1 miniplasmids: identification of a minimal pheromone response regulatory region and evaluation of a novel pheromone-dependent growth inhibition. *Plasmid*. 1989; 22:106–119. [PubMed: 2516332]
124. Weaver KE, Clewell DB, An F. Identification, characterization, and nucleotide sequence of a region of Enterococcus faecalis pheromone-responsive plasmid pAD1 capable of autonomous replication. *J Bacteriol*. 1993; 175:1900–1909. [PubMed: 8384618]
125. Tomita H, Clewell DB. A pAD1-encoded small RNA molecule, mD, negatively regulates Enterococcus faecalis pheromone response by enhancing transcription termination. *J Bacteriol*. 2000; 182:1062–1073. [PubMed: 10648533]
126. Fujimoto S, Clewell DB. Regulation of the pAD1 sex pheromone response of Enterococcus faecalis by direct interaction between the cAD1 peptide mating signal and the negatively regulating, DNA-binding TraA protein. *Proc Natl Acad Sci U S A*. 1998; 95:6430–6435. [PubMed: 9600983]

127. Pontius LT, Clewell DB. Conjugative transfer of *Enterococcus faecalis* plasmid pAD1: nucleotide sequence and transcriptional fusion analysis of a region involved in positive regulation. *J Bacteriol.* 1992; 174:3152–3160. [PubMed: 1315730]
128. Galli D, Lottspeich F, Wirth R. Sequence analysis of *Enterococcus faecalis* aggregation substance encoded by the sex pheromone plasmid pAD1. *Mol Microbiol.* 1990; 4:895–904. [PubMed: 2120541]
129. Chuang ON, Schlievert PM, Wells CL, Manias DA, Tripp TJ, Dunne GM. Multiple functional domains of *Enterococcus faecalis* aggregation substance Asc10 contribute to endocarditis virulence. *Infect Immun.* 2009; 77:539–548. [PubMed: 18955479]
130. Waters CM, Wells CL, Dunne GM. The aggregation domain of aggregation substance, not the RGD motifs, is critical for efficient internalization by HT-29 enterocytes. *Infect Immun.* 2003; 71:5682–5689. [PubMed: 14500489]
131. Muscholl-Silberhorn A. Analysis of the clumping-mediating domain(s) of sex pheromone plasmid pAD1-encoded aggregation substance. *Eur J Biochem.* 1998; 258:515–520. [PubMed: 9874218]
132. Waters CM, Dunne GM. Analysis of functional domains of the *Enterococcus faecalis* pheromone-induced surface protein aggregation substance. *J Bacteriol.* 2001; 183:5659–5667. [PubMed: 11544229]
133. Vanek NN, Simon SI, Jacques-Palaz K, Mariscalco MM, Dunne GM, Rakita RM. *Enterococcus faecalis* aggregation substance promotes opsonin-independent binding to human neutrophils via a complement receptor type 3-mediated mechanism. *FEMS Immunol Med Microbiol.* 1999; 26:49–60. [PubMed: 10518042]
134. Sussmuth SD, Muscholl-Silberhorn A, Wirth R, Susa M, Marre R, Rozdzinski E. Aggregation substance promotes adherence, phagocytosis, and intracellular survival of *Enterococcus faecalis* within human macrophages and suppresses respiratory burst. *Infect Immun.* 2000; 68:4900–4906. [PubMed: 10948103]
135. An FY, Clewell DB. Identification of the cAD1 sex pheromone precursor in *Enterococcus faecalis*. *J Bacteriol.* 2002; 184:1880–1887. [PubMed: 11889094]
136. Jett BD, Atkuri RV, Gilmore MS. *Enterococcus faecalis* localization in experimental endophthalmitis: role of plasmid-encoded aggregation substance. *Infect Immun.* 1998; 66:843–848. [PubMed: 9453653]
137. Garsin DA, Sifri CD, Mylonakis E, Qin X, Singh KV, Murray BE, Calderwood SB, Ausubel FM. A simple model host for identifying Gram-positive virulence factors. *Proc Natl Acad Sci U S A.* 2001; 98:10892–10897. [PubMed: 11535834]
138. Berti M, Candiani G, Kaufhold A, Muscholl A, Wirth R. Does aggregation substance of *Enterococcus faecalis* contribute to development of endocarditis? *Infection.* 1998; 26:48–53. [PubMed: 9505182]
139. Haas W, Shepard BD, Gilmore MS. Two-component regulator of *Enterococcus faecalis* cytolysin responds to quorum-sensing autoinduction. *Nature.* 2002; 415:84–87. [PubMed: 11780122]
140. Huycke MM, Gilmore MS, Jett BD, Booth JL. Transfer of pheromone-inducible plasmids between *Enterococcus faecalis* in the Syrian hamster gastrointestinal tract. *J Infect Dis.* 1992; 166:1188–1191. [PubMed: 1402034]
141. Van Tyne D, Martin MJ, Gilmore MS. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. *Toxins (Basel).* 2013; 5:895–911. [PubMed: 23628786]
142. Tang W, van der Donk WA. The sequence of the enterococcal cytolysin imparts unusual lanthionine stereochemistry. *Nat Chem Biol.* 2013; 9:157–159. [PubMed: 23314913]
143. Cox CR, Coburn PS, Gilmore MS. Enterococcal cytolysin: a novel two component peptide system that serves as a bacterial defense against eukaryotic and prokaryotic cells. *Curr Protein Pept Sci.* 2005; 6:77–84. [PubMed: 15638770]
144. Roux A, Payne SM, Gilmore MS. Microbial telesensing: probing the environment for friends, foes, and food. *Cell Host Microbe.* 2009; 6:115–124. [PubMed: 19683678]
145. Ike Y, Hashimoto H, Clewell DB. Hemolysin of *Streptococcus faecalis* subspecies *zymogenes* contributes to virulence in mice. *Infect Immun.* 1984; 45:528–530. [PubMed: 6086531]

146. Dupont H, Montravers P, Mohler J, Carbon C. Disparate findings on the role of virulence factors of *Enterococcus faecalis* in mouse and rat models of peritonitis. *Infect Immun*. 1998; 66:2570–2575. [PubMed: 9596718]
147. Ike Y, Hashimoto H, Clewell DB. High incidence of hemolysin production by *Enterococcus* (*Streptococcus*) *faecalis* strains associated with human parenteral infections. *J Clin Microbiol*. 1987; 25:1524–1528. [PubMed: 3114322]
148. Huycke MM, Gilmore MS. Frequency of aggregation substance and cytolysin genes among enterococcal endocarditis isolates. *Plasmid*. 1995; 34:152–156. [PubMed: 8559804]
149. Booth MC, Hatter KL, Miller D, Davis J, Kowalski R, Parke DW, Chodosh J, Jett BD, Callegan MC, Penland R, Gilmore MS. Molecular epidemiology of *Staphylococcus aureus* and *Enterococcus faecalis* in endophthalmitis. *Infect Immun*. 1998; 66:356–360. [PubMed: 9423880]
150. Dunny GM, Clewell DB. Transmissible toxin (hemolysin) plasmid in *Streptococcus faecalis* and its mobilization of a noninfectious drug resistance plasmid. *J Bacteriol*. 1975; 124:784–790. [PubMed: 810482]
151. Jacob AE, Douglas GJ, Hobbs SJ. Self-transferable plasmids determining the hemolysin and bacteriocin of *Streptococcus faecalis* var. *zymogenes*. *J Bacteriol*. 1975; 121:863–872. [PubMed: 803965]
152. Clewell DB, Tomich PK, Gawron-Burke MC, Franke AE, Yagi Y, An FY. Mapping of *Streptococcus faecalis* plasmids pAD1 and pAD2 and studies relating to transposition of Tn917. *J Bacteriol*. 1982; 152:1220–1230. [PubMed: 6292164]
153. Dunny GM, Craig RA, Carron RL, Clewell DB. Plasmid transfer in *Streptococcus faecalis*: production of multiple sex pheromones by recipients. *Plasmid*. 1979; 2:454–465. [PubMed: 113798]
154. Tomich PK, An FY, Damle SP, Clewell DB. Plasmid-related transmissibility and multiple drug resistance in *Streptococcus faecalis* subsp. *zymogenes* strain DS16. *Antimicrob Agents Chemother*. 1979; 15:828–830. [PubMed: 112916]
155. Clewell DB. Plasmids, drug resistance, and gene transfer in the genus *Streptococcus*. *Microbiol Rev*. 1981; 45:409–436. [PubMed: 6272080]
156. Oliver DR, Brown BL, Clewell DB. Characterization of plasmids determining hemolysin and bacteriocin production in *Streptococcus faecalis* 5952. *J Bacteriol*. 1977; 130:948–950. [PubMed: 122514]
157. Clewell, D.; Yagi, Y.; Ike, Y.; Craig, R.; Brown, B.; An, F. Sex pheromones in *Streptococcus faecalis*: multiple pheromone systems in strain DS5, similarities of pAD1 and pAMd1, and mutants of pAD1 altered in conjugative properties. In: Schlessinger, D., editor. *Microbiology*. American Society for Microbiology; Washington, DC: 1982. p. 97-100.
158. Jett BD, Gilmore MS. The growth-inhibitory effect of the *Enterococcus faecalis* bacteriocin encoded by pAD1 extends to the oral streptococci. *J Dent Res*. 1990; 69:1640–1645. [PubMed: 2120304]
159. Le Bouguenec C, de Cespedes G, Horaud T. Molecular analysis of a composite chromosomal conjugative element (Tn3701) of *Streptococcus pyogenes*. *J Bacteriol*. 1988; 170:3930–3936. [PubMed: 2842292]
160. Colmar I, Horaud T. *Enterococcus faecalis* hemolysin-bacteriocin plasmids belong to the same incompatibility group. *Appl Environ Microbiol*. 1987; 53:567–570. [PubMed: 3107464]
161. Gilmore MS, Segarra RA, Booth MC. An HlyB-type function is required for expression of the *Enterococcus faecalis* hemolysin/bacteriocin. *Infect Immun*. 1990; 58:3914–3923. [PubMed: 2123826]
162. Segarra RA, Booth MC, Morales DA, Huycke MM, Gilmore MS. Molecular characterization of the *Enterococcus faecalis* cytolysin activator. *Infect Immun*. 1991; 59:1239–1246. [PubMed: 1900808]
163. Coburn PS, Hancock LE, Booth MC, Gilmore MS. A novel means of self-protection, unrelated to toxin activation, confers immunity to the bactericidal effects of the *Enterococcus faecalis* cytolysin. *Infect Immun*. 1999; 67:3339–3347. [PubMed: 10377111]

164. Gilmore MS, Segarra RA, Booth MC, Bogie CP, Hall LR, Clewell DB. Genetic structure of the *Enterococcus faecalis* plasmid pAD1-encoded cytolytic toxin system and its relationship to lantibiotic determinants. *J Bacteriol.* 1994; 176:7335–7344. [PubMed: 7961506]
165. Booth MC, Bogie CP, Sahl HG, Siezen RJ, Hatter KL, Gilmore MS. Structural analysis and proteolytic activation of *Enterococcus faecalis* cytolysin, a novel lantibiotic. *Mol Microbiol.* 1996; 21:1175–1184. [PubMed: 8898386]
166. Coburn PS, Pillar CM, Jett BD, Haas W, Gilmore MS. *Enterococcus faecalis* senses target cells and in response expresses cytolysin. *Science.* 2004; 306:2270–2272. [PubMed: 15618522]
167. Rumpel S, Razeto A, Pillar CM, Vijayan V, Taylor A, Giller K, Gilmore MS, Becker S, Zweckstetter M. Structure and DNA-binding properties of the cytolysin regulator CylR2 from *Enterococcus faecalis*. *EMBO J.* 2004; 23:3632–3642. [PubMed: 15359276]
168. Rumpel S, Becker S, Zweckstetter M. High-resolution structure determination of the CylR2 homodimer using paramagnetic relaxation enhancement and structure-based prediction of molecular alignment. *J Biomol NMR.* 2008; 40:1–13. [PubMed: 18026911]
169. Patton GC, van der Donk WA. New developments in lantibiotic biosynthesis and mode of action. *Curr Opin Microbiol.* 2005; 8:543–551. [PubMed: 16118063]
170. Banerjee S, Hansen JN. Structure and expression of a gene encoding the precursor of subtilin, a small protein antibiotic. *J Biol Chem.* 1988; 263:9508–9514. [PubMed: 2837490]
171. Schnell N, Entian KD, Schneider U, Gotz F, Zahner H, Kellner R, Jung G. Prepeptide sequence of epidermin, a ribosomally synthesized antibiotic with four sulphide-rings. *Nature.* 1988; 333:276–278. [PubMed: 2835685]
172. Kaletta C, Entian KD. Nisin, a peptide antibiotic: cloning and sequencing of the *nisA* gene and posttranslational processing of its peptide product. *J Bacteriol.* 1989; 171:1597–1601. [PubMed: 2493449]
173. Willey JM, van der Donk WA. Lantibiotics: peptides of diverse structure and function. *Annu Rev Microbiol.* 2007; 61:477–501. [PubMed: 17506681]
174. Stark J. Antibiotic activity of haemolytic enterococci. *Lancet.* 1960; i:733–734. [PubMed: 13833832]
175. Brock T, Peacher B, Pierson D. Survey of the bacteriocines of enterococci. *J Bacteriol.* 1963; 86:702–707. [PubMed: 14066464]
176. Stevens SX, Jensen HG, Jett BD, Gilmore MS. A hemolysin-encoding plasmid contributes to bacterial virulence in experimental *Enterococcus faecalis* endophthalmitis. *Invest Ophthalmol Vis Sci.* 1992; 33:1650–1656. [PubMed: 1559764]
177. Jett BD, Jensen HG, Nordquist RE, Gilmore MS. Contribution of the pAD1-encoded cytolysin to the severity of experimental *Enterococcus faecalis* endophthalmitis. *Infect Immun.* 1992; 60:2445–2452. [PubMed: 1587612]
178. Huycke MM, Joyce WA, Gilmore MS. *Enterococcus faecalis* cytolysin without effect on the intestinal growth of susceptible enterococci in mice. *J Infect Dis.* 1995; 172:273–276. [PubMed: 7797930]
179. Wells CL, Jechorek RP, Erlandsen SL. Evidence for the translocation of *Enterococcus faecalis* across the mouse intestinal tract. *J Infect Dis.* 1990; 162:82–90. [PubMed: 2113080]
180. Gilmore, MS.; Coburn, PS.; Nallapareddy, SR.; Murray, BE. Enterococcal Virulence. In: Gilmore, MS., editor. *The Enterococci Pathogenesis, Molecular Biology, and Antimicrobial Resistance.* American Society for Microbiology; Washington, D.C: 2002.
181. Yamshchikov AV, Schuetz A, Lyon GM. *Rhodococcus equi* infection. *Lancet Infect Dis.* 2010; 10:350–359. [PubMed: 20417417]
182. Tkachuk-Saad O, Prescott J. *Rhodococcus equi* plasmids: isolation and partial characterization. *J Clin Microbiol.* 1991; 29:2696–2700. [PubMed: 1757535]
183. Takai S, Imai Y, Fukunaga N, Uchida Y, Kamisawa K, Sasaki Y, Tsubaki S, Sekizaki T. Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. *J Infect Dis.* 1995; 172:1306–1311. [PubMed: 7594668]
184. Giguere S, Hondalus MK, Yager JA, Darrah P, Mosser DM, Prescott JF. Role of the 85-kilobase plasmid and plasmid-encoded virulence-associated protein A in intracellular survival and virulence of *Rhodococcus equi*. *Infect Immun.* 1999; 67:3548–3557. [PubMed: 10377138]

185. Takai S, Sekizaki T, Ozawa T, Sugawara T, Watanabe Y, Tsubaki S. Association between a large plasmid and 15- to 17-kilodalton antigens in virulent *Rhodococcus equi*. *Infect Immun*. 1991; 59:4056–4060. [PubMed: 1937765]
186. Yager JA, Prescott CA, Kramar DP, Hannah H, Balson GA, Croy BA. The effect of experimental infection with *Rhodococcus equi* on immunodeficient mice. *Vet Microbiol*. 1991; 28:363–376. [PubMed: 1949550]
187. Takai S, Koike K, Ohbushi S, Izumi C, Tsubaki S. Identification of 15- to 17-kilodalton antigens associated with virulent *Rhodococcus equi*. *J Clin Microbiol*. 1991; 29:439–443. [PubMed: 2037660]
188. Takai S, Watanabe Y, Ikeda T, Ozawa T, Matsukura S, Tamada Y, Tsubaki S, Sekizaki T. Virulence-associated plasmids in *Rhodococcus equi*. *J Clin Microbiol*. 1993; 31:1726–1729. [PubMed: 8349748]
189. Tan C, Prescott JF, Patterson MC, Nicholson VM. Molecular characterization of a lipid-modified virulence-associated protein of *Rhodococcus equi* and its potential in protective immunity. *Can J Vet Res*. 1995; 59:51–59. [PubMed: 7704843]
190. Takai S, Anzai T, Fujita Y, Akita O, Shoda M, Tsubaki S, Wada R. Pathogenicity of *Rhodococcus equi* expressing a virulence-associated 20 kDa protein (VapB) in foals. *Vet Microbiol*. 2000; 76:71–80. [PubMed: 10925043]
191. Jain S, Bloom BR, Hondalus MK. Deletion of vapA encoding Virulence Associated Protein A attenuates the intracellular actinomycete *Rhodococcus equi*. *Mol Microbiol*. 2003; 50:115–128. [PubMed: 14507368]
192. Takai S, Iie M, Watanabe Y, Tsubaki S, Sekizaki T. Virulence-associated 15- to 17-kilodalton antigens in *Rhodococcus equi*: temperature-dependent expression and location of the antigens. *Infect Immun*. 1992; 60:2995–2997. [PubMed: 1612765]
193. Takai S, Fukunaga N, Kamisawa K, Imai Y, Sasaki Y, Tsubaki S. Expression of virulence-associated antigens of *Rhodococcus equi* is regulated by temperature and pH. *Microbiol Immunol*. 1996; 40:591–594. [PubMed: 8887354]
194. Dorman CJ, Porter ME. The *Shigella* virulence gene regulatory cascade: a paradigm of bacterial gene control mechanisms. *Mol Microbiol*. 1998; 29:677–684. [PubMed: 9723908]
195. Lindler LE, Plano GV, Burland V, Mayhew GF, Blattner FR. Complete DNA sequence and detailed analysis of the *Yersinia pestis* KIM5 plasmid encoding murine toxin and capsular antigen. *Infect Immun*. 1998; 66:5731–5742. [PubMed: 9826348]
196. Perry RD, Straley SC, Fetherston JD, Rose DJ, Gregor J, Blattner FR. DNA sequencing and analysis of the low-Ca²⁺-response plasmid pCD1 of *Yersinia pestis* KIM5. *Infect Immun*. 1998; 66:4611–4623. [PubMed: 9746557]
197. Byrne BA, Prescott JF, Palmer GH, Takai S, Nicholson VM, Alperin DC, Hines SA. Virulence plasmid of *Rhodococcus equi* contains inducible gene family encoding secreted proteins. *Infect Immun*. 2001; 69:650–656. [PubMed: 11159951]
198. Letek M, Ocampo-Sosa AA, Sanders M, Fogarty U, Buckley T, Leadon DP, Gonzalez P, Scotti M, Meijer WG, Parkhill J, Bentley S, Vazquez-Boland JA. Evolution of the *Rhodococcus equi* vap pathogenicity island seen through comparison of host-associated vapA and vapB virulence plasmids. *J Bacteriol*. 2008; 190:5797–5805. [PubMed: 18606735]
199. Ribeiro MG, Takai S, de Vargas AC, Mattos-Guaraldi AL, Ferreira Camello TC, Ohno R, Okano H, Silva AV. Short report: Identification of virulence-associated plasmids in *Rhodococcus equi* in humans with and without acquired immunodeficiency syndrome in Brazil. *Am J Trop Med Hyg*. 2011; 85:510–513. [PubMed: 21896813]
200. Takai S, Hines SA, Sekizaki T, Nicholson VM, Alperin DA, Osaki M, Takamatsu D, Nakamura M, Suzuki K, Ogino N, Kakuda T, Dan H, Prescott JF. DNA sequence and comparison of virulence plasmids from *Rhodococcus equi* ATCC 33701 and 103. *Infect Immun*. 2000; 68:6840–6847. [PubMed: 11083803]
201. Vendrell D, Balcazar JL, Ruiz-Zarzuela I, de Blas I, Girones O, Muzquiz JL. *Lactococcus garvieae* in fish: a review. *Comp Immunol Microbiol Infect Dis*. 2006; 29:177–198. [PubMed: 16935332]

202. Chan JF, Woo PC, Teng JL, Lau SK, Leung SS, Tam FC, Yuen KY. Primary infective spondylodiscitis caused by *Lactococcus garvieae* and a review of human *L. garvieae* infections. *Infection*. 2011; 39:259–264. [PubMed: 21424437]
203. Wang CY, Shie HS, Chen SC, Huang JP, Hsieh IC, Wen MS, Lin FC, Wu D. *Lactococcus garvieae* infections in humans: possible association with aquaculture outbreaks. *Int J Clin Pract*. 2007; 61:68–73. [PubMed: 16704679]
204. Aguado-Urda M, Gibello A, Blanco MM, Lopez-Campos GH, Cutuli MT, Fernandez-Garayzabal JF. Characterization of plasmids in a human clinical strain of *Lactococcus garvieae*. *PLoS One*. 2012; 7:e40119. [PubMed: 22768237]
205. National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1992–June 2001, Issued August 2001. *Am J Infect Control*. 2001; 29:404–421. [PubMed: 11743489]
206. Doolittle WF, Sapienza C. Selfish genes, the phenotype paradigm and genome evolution. *Nature*. 1980; 284:601–603. [PubMed: 6245369]
207. Huycke MM, Spiegel CA, Gilmore MS. Bacteremia caused by hemolytic, high-level gentamicin-resistant *Enterococcus faecalis*. *Antimicrob Agents Chemother*. 1991; 35:1626–1634. [PubMed: 1929336]
208. Booth MC, Pence LM, Mahasreshti P, Callegan MC, Gilmore MS. Clonal associations among *Staphylococcus aureus* isolates from various sites of infection. *Infect Immun*. 2001; 69:345–352. [PubMed: 11119523]
209. Hisatsune J, Hirakawa H, Yamaguchi T, Fudaba Y, Oshima K, Hattori M, Kato F, Kayama S, Sugai M. Emergence of *Staphylococcus aureus* carrying multiple drug resistance genes on a plasmid encoding exfoliative toxin B. *Antimicrob Agents Chemother*. 2013; 57:6131–6140. [PubMed: 24080652]

FIGURE 1.

Plasmid map of pIB485, encoding staphylococcal enterotoxins SED (*sed*) and SEJ (*selj*). Toxin genes are colored red. Genes encoding resistance to cadmium sulfate (*cad*) as well as resistance to beta-lactams (*bla*) are colored dark blue.

FIGURE 2.

(A) Plasmid map of a representative staphylococcal enterotoxin B (ETB)-expressing plasmid, TY825 pETB. The outer circle shows genes that are transcribed clockwise, and genes in the inner circle are transcribed counterclockwise. Genes in red are pathogenic factors; genes in green encode antibiotic resistances; genes in blue are involved in DNA replication, recombination and repair; genes in light blue are transcriptional regulators; genes in purple are transposases; genes in yellow are involved in conjugal transfer; genes in orange encode the BacR1/C55 lantibiotic operon; and genes in grey are conserved ORFs. (B) Structural comparison of TY4 pETB and TY825 pETB plasmids of *S. aureus*. Shading indicates homologous regions. Figure is adapted from (209).

FIGURE 3. Schematic showing the principal events during pheromone-responsive plasmid transfer between *E. faecalis* cells.

FIGURE 4.

Pheromone-response regulation and virulence factors encoded on plasmid pAD1 (A) Genes encoded by pAD1 that are involved in plasmid transfer and pheromone sensing. Blue genes are involved in replication and maintenance, red genes are negative regulators of pheromone response, and green genes are positive regulators of pheromone response. (B) Detail of the post-segregation killing (PSK) *par* locus of pAD1, which encodes the Fst toxin. (C) Detailed schematic of individual genes within the cytolysin operon.

UNINDUCED: **INDUCED:** **FIGURE 5.**

Regulation of pheromone sensing and plasmid transfer by the enterococcal Tra regulon. Genes encoding surface exclusion protein (*sea*) and aggregation substance (*asaI*) are shown in dark blue. Positive regulators are shown in green, and negative regulators are shown in red. Straight arrows below the genes indicate transcripts detected in the uninduced and induced states, and arrow thickness indicates relative transcript abundance. Figure is adapted from (87) and (117).

FIGURE 6.

Schematic of *E. faecalis* cytolysin expression, post-translational modification, processing and export. (1) CylL_L and CylL_S precursor peptides are synthesized, (2) and are intracellularly modified by CylM to create CylL_L* and CylL_S*. (3) CylL_L* and CylL_S* are secreted and further modified by CylB, resulting in CylL_L' and CylL_S', (4) which are cleaved extracellularly by CylA to form the active cytolysin components CylL_L'' and CylL_S''. (5) CylL_L'' and CylL_S'' are capable of forming aggregates, and are prevented from lysing cytolysin-expressing cells via CylI (6).

FIGURE 7.

Plasmid map of the virulence plasmid p33701 of *R. equi*. Genes believed to be involved in plasmid maintenance and conjugation are shown in red, genes encoding Vaps are shown in blue, and putative genes within the proposed pathogenicity island are depicted in yellow. Figure is adapted from (200).