

Virulence of Enterococci

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INTRODUCTION

Enterococci are a frequent cause of a wide variety of infections in humans (54, 66, 167, 186). These organisms most commonly infect the urinary tract (59, 65, 72, 92, 99, 144, 165), bloodstream (22, 65, 90, 93, 152, 154, 189, 194, 227), endocardium (62), abdomen (9, 32, 52, 83, 90, 152, 204), biliary tract (134, 152), burn wounds (130, 152), and indwelling foreign devices (such as intravascular catheters [90, 152, 188]). Although enterococci can infect the central nervous system (12), lung (17, 21, 90, 152, 186), soft tissues (109), paranasal sinuses (53, 152), ear (186), eye (211), and periodontal tissue (86), these infections occur less frequently. *Enterococcus faecalis* causes 80 to 90% of human enterococcal infections, while *E. faecium* accounts for a majority of the remainder (162, 167, 192). Other enterococcal species, including *E. avium* (181), *E. casseliflavus* (183), *E. durans*, *E. gallinarum*, *E. hirae*, *E. malodoratus*, *E. mundtii* (131), *E. raffinosus* (38), and *E. solitarius*, are infrequent causes of human infection (67, 192).

In the 1970s and 1980s enterococci became firmly established as major nosocomial pathogens. They are now the

fourth leading cause of hospital-acquired infection and the third leading cause of bacteremia in the United States (65). Case/fatality ratios for enterococcal bacteremia range from 12 to 68%, with death due to enterococcal sepsis in 4 to 50% of these cases (22, 32, 90, 93, 130, 140, 152, 154, 189, 204, 228, 244). In one large study of bloodstream infections, enterococci were the only gram-positive pathogens independently associated with a high risk of death (227). Similarly, in a retrospective cohort study, the mortality rate attributable to enterococcal bacteremia was 31%, with a risk ratio of 4.75 compared with matched controls (140).

Enterococci are normal human commensals adapted to the nutrient-enriched, oxygen-depleted, ecologically complex environments of the oral cavity, gastrointestinal tract, and vaginal vault. As the predominant gram-positive coccus in stool, with concentrations ranging from 10⁵ to 10⁷ CFU/g of feces, enterococci still account for less than 0.01% of normal bowel flora. The bulk of organisms in stool and at other sites of colonization are various obligate anaerobes (224).

For enterococci to act as pathogens they must first adhere to host tissues. During the process of tissue invasion, enterococci encounter an environment vastly different than those at sites of colonization, with higher redox potentials, limited essential nutrients, phagocytic leukocytes, and other host defenses. Infecting enterococci likely express genes favoring growth under these alternate environmental conditions. As has be-

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TABLE 1. Definite and potential virulence factors for enterococci

Factor	Species in which found to date	Observed activities and model systems used ^a	Reference(s)
Cytolysin	<i>E. faecalis</i> , <i>E. faecium</i> 228 ^b	Lytic towards gram-positive bacteria and selected eukaryotic cells; decreased LD ₅₀ ^c and time to death in murine peritoneal infection; destruction of retinal tissue in rabbit endophthalmitis; in combination with aggregation substance, increased mortality in rabbit endocarditis	40, 119, 125, 126
Aggregation substance	<i>E. faecalis</i> , <i>E. faecium</i> 228 ^d	Facilitates binding of donor to recipient cells in pheromone mating response; augmented adherence to cultured renal tubular cells through Arg-Gly-Asp motifs; increased vegetation weight in rabbit endocarditis; in combination with cytolysin, increased mortality in rabbit endocarditis; invasion of enterocytes enhanced	40, 79, 137, 177
Pheromones	<i>E. faecalis</i>	Chemoattractant for neutrophils in vitro	64, 193
Lipoteichoic acid	All enterococci ^e	Stimulation of cytokine production in cultured human monocytes; binding ligand for aggregation substance in pheromone mating response	18, 61, 221
Protease (gelatinase)	<i>E. faecalis</i>	Zinc-endopeptidase; ND ^f	86, 153, 212
Hyaluronidase	<i>E. faecalis</i>	Mucopolysaccharidase; ND	191
AS-48	<i>E. faecalis</i>	Bacteriocin with activity against gram-positive and gram-negative bacteria; ND	81

^a Activities listed include those associated with a possible pathogenic phenomenon and do not necessarily include other known biological activities.

^b Transferable beta-hemolysin active against horse erythrocytes; similarity to prototype pAD1-encoded cytolysin *cyl* not defined (37).

^c LD₅₀, 50% lethal dose (quantity of organisms lethal for 50% of inoculated animals).

^d Presumptive producer of aggregation substance; EDTA-sensitive clumping activity suggestive of pheromone responsiveness; specific clump-inducing pheromone and aggregation substance not determined (37).

^e Most, if not all, gram-positive organisms express lipoteichoic acids as cell wall components.

^f ND, not determined. Activity may be known; however, the factor has not yet been tested in an in vivo and/or in vitro infection model.

come increasingly apparent in recent years, enterococci express factors that permit adherence to host cells and extracellular matrix, facilitate tissue invasion, effect immunomodulation, and cause toxin-mediated damage.

This review discusses enterococcal traits with the potential to contribute to virulence. Increases in enterococcal infections, especially in the hospital, associated with the emergence and proliferation of multi-drug-resistant strains require a careful reexamination of mechanisms that enterococci use to initiate or exacerbate infection. Descriptions of enterococcal infections, their management, and resistance to antibiotics are mentioned here only when relevant to virulence. For information on these topics, readers are instead referred to several recent reviews (5, 62, 63, 103, 146, 152, 162, 167, 182). After a brief overview of selected historical perspectives, this review discusses the current understanding of enterococcal virulence relating to (i) adherence to host tissues, (ii) invasion and abscess formation, (iii) factors potentially relevant to modulation of host inflammatory responses, and (iv) potentially toxic secreted products. A summary of several of these factors can be found in Table 1.

HISTORICAL PERSPECTIVE

The first examination of enterococcal virulence was reported in 1899 (151), the same year the organism was discovered (216). MacCallum and Hastings (151) described a fatal case of endocarditis from the Osler service at The Johns Hopkins Hospital caused by an organism that they termed *Micrococcus zymogenes*. The bacterium expressed cytolytic (or hemolytic) and protease (or gelatinase) activities (151) and likely repre-

sented *E. faecalis* (24). White mice injected intraperitoneally with this new organism mixed with bouillon "... died in the majority of experiments after periods varying from seven hours to three or four days, with evidence of general infection. Autopsy showed always a much swollen spleen and sometimes reddened lymph glands..." (151). Koch's postulates were fully satisfied when endocarditis was reproduced by using the organism in a canine model (151).

The first systematic study of the properties of an enterococcal virulence factor was the study of cytolysin or hemolysin by Todd in 1934 (218). He observed that although some strains of *E. faecalis* produced discernible zones of hemolysis on blood agar plates, "... this group failed to produce haemolytic filtrates when grown in Hewitt's broth" (218). Although he called these strains "pseudo-hemolytic," cytolytic activity was detected by using a "horse flesh infusion" medium (218). We find this infusion, modified by substitution of ground beef for less readily obtainable horse flesh, to be superior to commercially available media for production of cytolysin in liquid culture, although only modest cytolytic activity is produced (83a). The basis for production in some media and not others is unclear and is the subject of ongoing studies on cytolysin expression.

Cytolysin production by *E. faecalis* is recognized by the development of clearing around colonies on certain blood agar media. This *E. faecalis* phenotype, however, frequently has been overlooked in clinical microbiology laboratories because sheep erythrocytes, the target cells commonly used in blood agar plates, are refractory to cytolysin-mediated lysis (11, 136). Erythrocytes from rabbits, humans, horses, and cows are readily lysed by the *E. faecalis* cytolysin (11, 136) and should be used instead to identify this phenotype. The more general term cytolysin is preferred over the historical term hemolysin be-

cause, as discussed below, the target cell range is broad and includes eukaryotic and prokaryotic cells.

Cytolysin production by *E. faecalis* was formerly a criterion for classification as *Streptococcus zymogenes* (24) or, later, *Streptococcus faecalis* var. *zymogenes* (25). The finding that cytolysin was transmissible by plasmids (57, 123), and therefore was a variable trait, coupled with data provided by numerical taxonomic studies placing all *E. faecalis* variants into one cluster (26, 128) caused the *zymogenes* designation to be abandoned (166).

Other secreted products long associated with enterococci, but less well studied, are hyaluronidase (191) and protease (or gelatinase) (3, 151, 202). In 1955, Schultz-Hautd and Scherp postulated that the initial lesion in periodontal disease may be disruption of intercellular cementing substances of the epithelium as a result of mucopolysaccharidase (or hyaluronidase) activity (199). This effect was predominantly ascribed to strains of *Streptococcus mitis* and *Streptococcus salivarius*. Hyaluronidase-producing enterococci were subsequently described by Rosan and Williams in studies of microorganisms causing periodontal disease (191). The contribution of this enzyme to periodontal disease has not been further investigated, even though enterococci are commonly isolated from periodontal infections (75, 132, 174) and were shown in 1955 to readily induce dental caries formation in monoassociated rats infected with a nonproteolytic strain (180).

E. faecalis strains with protease activity were once referred to as *E. faecalis* var. *liquefaciens* (202). Strains with this designation have been used in animal models of enterococcal infection (94, 105, 151), but, as described below, few studies have specifically tested the contribution of protease to this process.

The inability of enterococci to cause chronic or severe infections in laboratory animals after inoculation into subcutaneous tissue or the peritoneal cavity has been used to support arguments for the relative nonvirulence of enterococci. Hite et al. (105), however, reported in 1949 that enterococci caused necrotizing abscess formation in the abdominal walls of mice when mixed with nonsporulating anaerobic bacteria. These investigators went on to note that enterococci mixed with heat-killed anaerobes continued to promote abscess formation, although heat-treated enterococci did not augment the virulence of live anaerobes (105). These remarkable observations have been confirmed by others (30, 157, 159, 178), as discussed below, but the mechanism by which this microbial synergism occurred remains unexplained.

Although urinary tract infections are commonly caused by enterococci, few studies have explored the predisposition of enterococci for this site. Guze et al. (94) were the first to carefully characterize hematogenous infection of the kidney by enterococci. In a rat model, using an unmanipulated urinary tract, these workers consistently produced chronic, progressive enterococcal pyelonephritis through a single intravenous inoculation (94). Histologically, infection at 2 days appeared as focal medullary abscesses. Over 1 week, extensive progression of focal radial cellular infiltrates to the renal cortex occurred (94, 148). Bacteruria invariably developed in 2 to 3 days but only after microabscesses appeared in the renal parenchyma.

ENTEROCOCCAL GENETIC EXCHANGE

Plasmid- and transposon-encoded genes, besides those necessary for replication and transfer, typically confer traits that provide survival advantages to organisms in unusual environments (58). Such traits include antibiotic or heavy metal resistance, bacteriocin activity, metabolin of unusual substrates, and virulence factors. Many putative enterococcal

virulence factors reside on conjugative plasmids. The ease with which these determinants spread horizontally between strains in a natural environment, such as the gastrointestinal tract, likely determines the rate at which adaptive and pathogenic traits are disseminated to potentially less virulent endogenous flora. As such, naturally occurring mechanisms of gene exchange are fundamentally an expression of virulence.

Enterococci possess potent and unique abilities to exchange genetic material among themselves and with other genera (42, 195). At least three conjugative systems exist by which enterococci naturally transfer genetic elements. First, narrow-host-range, pheromone-responsive plasmids unique to enterococci have been described (43). These plasmids transfer at high frequencies on solid surfaces, in broth, and in vivo (43, 112). Second, many plasmids with a broad host range (e.g., pAM β 1) readily transfer at low frequency among enterococci, *Streptococcus* spp., *Staphylococcus aureus*, *Lactobacillus* spp., *Bacillus subtilis*, and other species (42, 195). Coexisting pheromone-responsive plasmids can greatly increase the transfer frequency of these plasmids (43). Transfer requires contact between donor and recipient cells on a solid surface and can occur in vivo (164). Finally, conjugative gene exchange occurs through highly promiscuous transposons found in gram-positive and gram-negative bacteria (200). One transposon, Tn1545, has been shown to transfer at low frequency from *E. faecalis* to *Listeria monocytogenes* in the intestinal tracts of gnotobiotic mice (51). Tn916 is the most thoroughly characterized conjugative transposon and has been used extensively in molecular genetic studies (76, 200).

The only other known mechanism of natural gene exchange for enterococci is through infection with bacteriophages (35, 173). Enterococcal bacteriophages have been isolated from stool (190) and have a narrow host range rendering them of potential use for strain typing (138). The ability of enterococcal bacteriophages to spread virulence traits or antibiotic resistance determinants is largely unknown.

ADHERENCE TO HOST TISSUES

Bacterial adherence to host tissues is a crucial first step in the infection process (8). For gastrointestinal commensals such as enterococci, adhesins that promote binding to eukaryotic receptors on mucosal surfaces would be expected to play a critical role in maintenance of colonization. Without specific means of attachment, enterococci would likely be eliminated by bulk flow of luminal contents through normal intestinal motility. Adherence through surface-exposed adhesins to epithelial cells, endothelial cells, leukocytes, or extracellular matrix is generally a first step in infection. Adhesin systems have been described for several pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Yersinia pseudotuberculosis*, *Bordetella pertussis*, *Streptococcus pyogenes*, and *Streptococcus sanguis* (20, 102, 106, 127, 141). These adhesins have been shown to play diverse roles as effector molecules leading to phagocytosis, inciting or reducing local inflammatory responses, or acting as toxins. Only recently have enterococcal adhesins been investigated.

Exogenous Acquisition of Enterococci

For many years infection-derived enterococci were believed to arise endogenously from a patient's own flora (133). Recent clinical studies, however, have demonstrated that enterococci and enterococcal plasmids transfer between patients (23, 39, 111, 113, 149, 168, 170, 187, 235, 239, 243, 244). Clones of

enterococci have also been reported to cause outbreaks of infection and become endemic in hospitals for months to years (23, 39, 111, 113, 149, 168, 170, 187, 235, 239, 243, 244). The development of simple and precise methods to identify enterococcal strains (98, 169, 245) along with emergence of increasingly antibiotic-resistant strains have, in part, been a catalyst for these studies. One theme noted in many studies has been use of parenteral antibiotics without significant antienterococcal activity in patients preceding the development of an enterococcal infection.

These observations suggest that select exogenous enterococcal strains have heightened capabilities to colonize, overgrow, invade host tissues, and persist compared with endogenous enterococcal flora. Examples of factors conferring such traits might include *E. faecalis* cytolysin or AS-48 peptide. Both are potent bacteriocins (80, 122) that may confer selective advantages for growth on exogenous strains compared with endogenous strains without these phenotypes. In hospitalized patients receiving antibiotics that reduce the resistance of the gastrointestinal tract to microbial colonization (224), endogenous enterococcal flora may be supplanted through selective action of such bacteriocin-producing hospital strains. Alternatively, endogenous flora could acquire these phenotypes through plasmid transfer, as has been demonstrated to occur in the gastrointestinal tracts of hamsters (112). Studies to test these suppositions are in progress. Other enterococcal determinants potentially important to colonization or overgrowth are discussed below.

Aggregation Substance

Aggregation substance is a surface-bound protein encoded by pheromone-responsive plasmids of *E. faecalis* and expressed in response to pheromone induction (55). Aggregation substance converts the surface of the donor bacterium into one adherent to potential recipient cells, causing aggregation or clumping and facilitating transfer of plasmids (55). Transposon insertional mutagenesis has localized the aggregation substance gene on pAD1 to a position 9.4 kb from the origin (Fig. 1a) (60). Nucleotide sequence determination for this gene, *asa1*, revealed two Arg-Gly-Asp motifs, similar to those initially reported for fibronectin (241) and capable of binding eukaryotic cell receptors termed integrins (78).

Aggregation substance appears to be anchored in the bacterial membrane by its C terminus in a manner common to cell wall-associated proteins of gram-positive bacteria (171). In electron micrographs, aggregation substance is a hair-like structure incorporated into old cell wall (225). Genes encoding aggregation substance from a variety of cytolysin-specifying and non-cytolysin-specifying, pheromone-responsive plasmids are highly conserved (79, 104). The deposition pattern of aggregation substance encoded by the pheromone-responsive plasmid pCF10, when cloned and constitutively expressed from the construction pINY1801, is shown at high resolution in Fig. 2 (177). In addition to facilitating exchange of plasmids carrying virulence traits and antibiotic resistance genes, aggregation substance may augment enterococcal adherence to intestinal and renal epithelial cells (137) and cardiac vegetations (40). These subjects are further discussed below in sections on translocation, urinary tract infection, and endocarditis, respectively.

Surface Carbohydrates

Evidence indicating the existence of carbohydrate adhesins for enterococci was first reported by Guzmán et al. (95, 96). This group observed that *E. faecalis* strains isolated from

urinary tract infections adhered efficiently to urinary tract epithelial cells in vitro (95). In contrast, *E. faecalis* strains isolated from the blood of patients with endocarditis adhered efficiently to the Girardi Heart human cell line (95). When urinary tract isolates were grown in the presence of serum, adherence to the heart cell line was enhanced. In vitro competitive inhibition assays using D-mannose, D-glucose, L-fucose, and D-galactose implicated enterococcal surface carbohydrates in binding bacteria to cells in culture (96). Carbohydrate-mediated adherence of *E. faecalis* to different substrates was described as complex and mediated by several adhesins. Indeed, some adhesins were present in all strains and under all growth conditions, while others were expressed only after growth in human serum. Guzmán et al. proposed lipoteichoic acid as a likely candidate for anchoring these carbohydrate moieties to the cell, but the identity of these adhesins has yet to be determined.

Other Adherence Mechanisms

In contrast to detailed characterizations of aggregation substance, limited information is available on other factors promoting adhesion of enterococci to host tissues. Shorrock and Lambert investigated the binding of fibronectin and albumin by *E. faecalis* (205). They found that fibronectin bound *E. faecalis* with higher affinity than albumin did. Treatment of *E. faecalis* with protease or periodate reduced binding, suggesting a surface receptor comprising protein and/or carbohydrate components. Lipoteichoic acid did not interfere with binding, implying that this molecule was not an adhesin for fibronectin as it is for *S. pyogenes* (206). The relevance of fibronectin binding to *E. faecalis* is discussed further below in the section on endocarditis.

INVASION OF HOST TISSUES

Bacteremia and Translocation

Enterococci are currently the third leading cause of nosocomial bacteremia in the United States (65). While many cases are due to identifiable sources, such as intravenous lines, abscesses, and urinary tract infection, a large percentage remain obscure in origin (90, 140) and presumably originate from the intestinal tract (1, 234). According to the translocation model, intestinal epithelial cells or intraepithelial leukocytes phagocytose adherent luminal bacteria. The bacteria exit the apical side of epithelial cells or migrate in phagocytes to mesenteric lymph nodes, proliferate, and hematogenously spread to distant sites. Animal studies and clinical observations are consistent with facultative anaerobic intestinal organisms causing systemic infection via this route (1, 224, 234). Some parenteral antibiotics with little antienterococcal activity, such as cephalosporins, are known to cause a predisposition to enterococcal superinfection (129, 161, 242) and are consistently associated with development of enterococcal bacteremia (6, 90, 93, 113, 175, 243, 244).

Wells et al. (230), expanding on studies linking antibiotic administration to intestinal overgrowth of enterococci (229, 231, 233), described a murine model of enterococcal translocation across an intact intestinal epithelium. In mice orally inoculated with the murine-derived *E. faecalis* strain M20 and given broad-spectrum antibiotics favoring overgrowth of enterococci, translocation of enterococci across a histologically normal intestinal epithelium occurred. Disseminated *E. faecalis* infection resulted, involving the mesenteric lymph nodes,

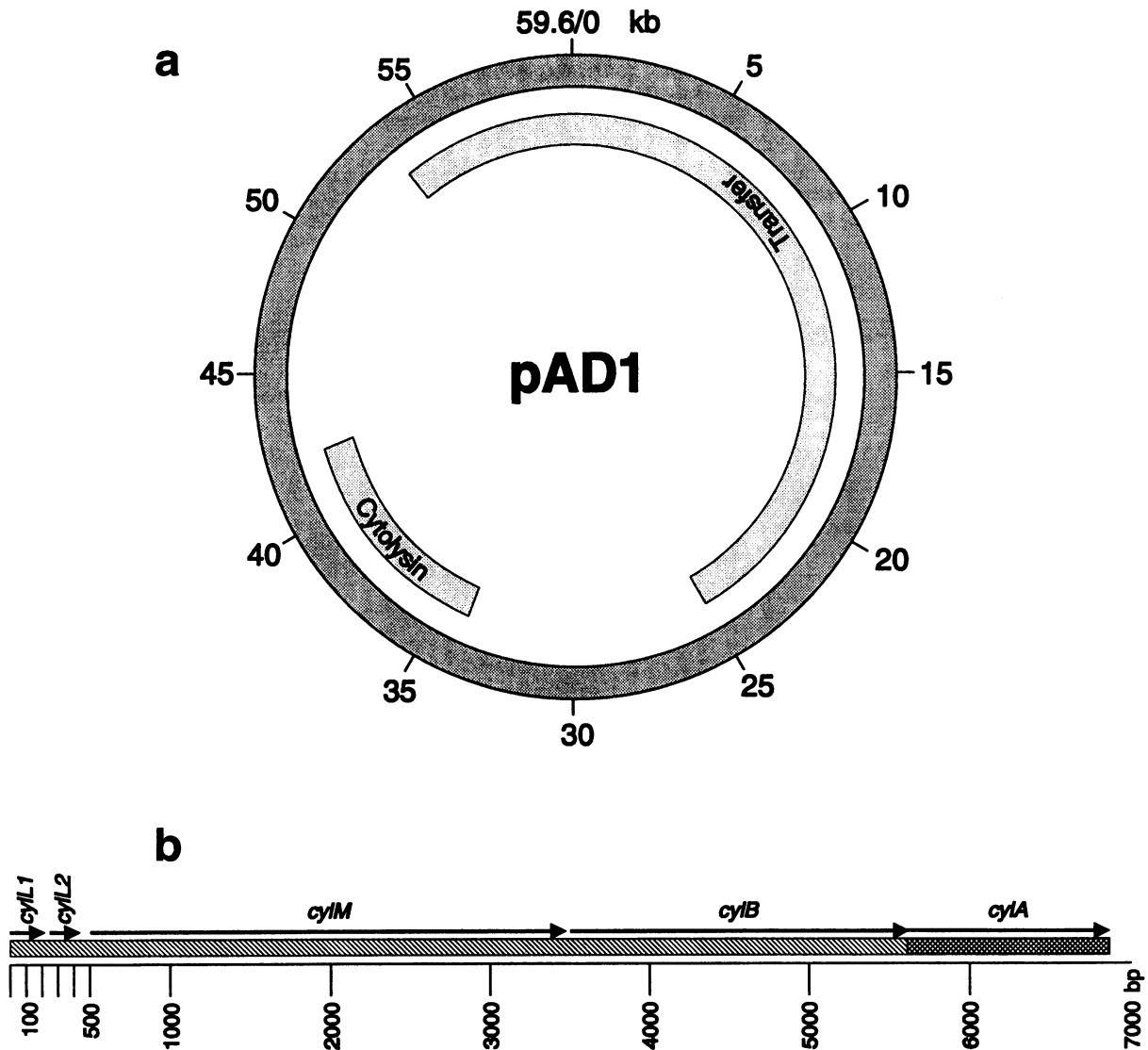


FIG. 1. (a) Physical map of the cytolysin plasmid pAD1. The regions specifying functions related to transfer and cytolysin expression are indicated (45). The aggregation substance gene is located at approximately 9.4 kb (45). (b) Organization of genes encoded by the cytolysin operon. Reading frames *cylL1*, *cylL2*, *cylM*, and *cylB* relate to expression, maturation, and externalization of the lysin precursor (85, 117). *cylA* encodes the cytolysin activator (201). The *cylL1* reading frame would correspond approximately to 42 kb on the pAD1 physical map (45).

liver, and spleen. Thirteen percent of mice died within 3 weeks, but it was unclear whether death was due to overwhelming enterococcal infection or dissemination of other intestinal pathogens. Nevertheless, the model showed *E. faecalis*, under appropriate conditions of intestinal overgrowth, translocating across an intact intestinal epithelium to cause systemic infection. For monoassociated mice, these same investigators suggested that the preferred site of translocation for *E. faecalis* was the cecum or colon (229). Finally, in a study of women undergoing surgical resection of cervical or uterine tumors, 46% of cultures of lymph nodes contained *Enterococcus* spp. (232), suggesting that enterococci also translocate across the vaginal epithelium.

In a collaborative study on the mechanism of enterococcal translocation across intestinal mucosa, Wells and colleagues investigated the role of aggregation substance encoded by the pheromone-responsive plasmid pCF10, termed Asc10. In

HT-29 cells, which in the absence of glucose express terminal enterocyte morphology (246), aggregation substance enhanced uptake of *E. faecalis* (176a). *E. faecalis* INY1801 expressing aggregation substance was equally as invasive for HT-29 as *L. monocytogenes* and *Salmonella typhimurium*. The *E. faecalis* strain M20 used in translocation studies (229, 230), however, was taken up less efficiently by these cells than isogenic strains of INY1801 not expressing aggregation substance. These results suggest that adhesins other than aggregation substance are equally or more important to bacterial-epithelial adherence prior to translocation.

Endocarditis

The avidity of a microorganism for binding to endocardial tissue matrix components or cells is critical to the capacity to cause endocarditis. This concept is consistent with animal

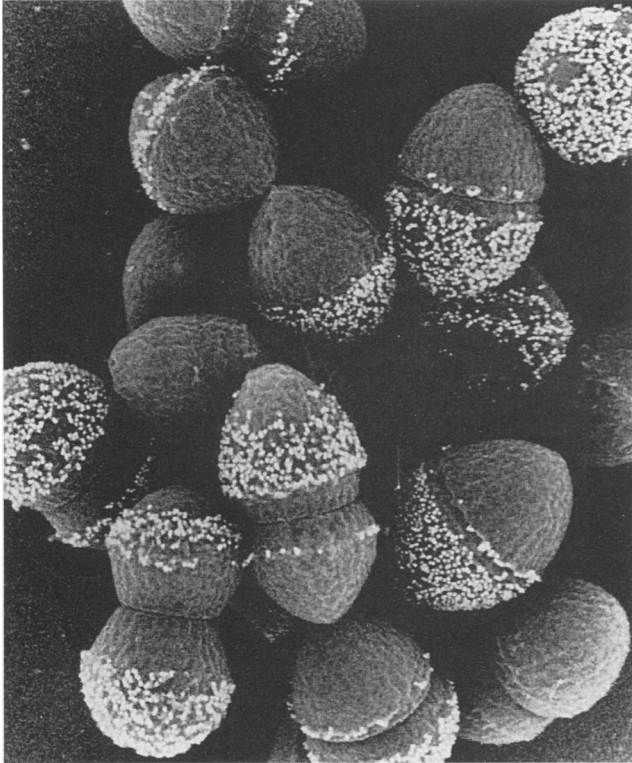


FIG. 2. Immunogold-labeled aggregation substance. Anti-Asc10 monoclonal antibody was used to label aggregation substance expressed on the surface of OG1RF(pINY1801) cells. Antibody specifically bound to aggregation substance was in turn labeled with 12-nm colloidal gold-tagged secondary antibody. From reference 177 with permission of the publisher.

models of catheter-induced endocarditis (7). Vegetations on heart valves in experimental endocarditis are composed primarily of fibrin, platelets, and fibronectin (196). Bacterial or yeast adherence to fibronectin in particular correlates well (but not perfectly) with the propensity of microorganisms to cause endocarditis (139, 196). Animal models have been used extensively to define therapies for enterococcal endocarditis (34, 36, 69, 107, 121, 135, 213, 215, 217, 226, 240), but these models have been used less frequently to investigate enterococcal determinants important to this infection process.

Several investigators have compared the abilities of microorganisms to adhere to endocardial vegetations. Crawford and Russell (48) examined streptococci from patients with subacute bacterial endocarditis and found that *E. faecalis* strains were less adherent than strains of *S. aureus* and *Streptococcus pyogenes* but more adherent than strains of *S. mutans*, *S. milleri*, *S. sanguis*, *S. mitior*, or *S. salivarius*. Although no correlation was found between adherence of strains to fibrin-platelet clots and their ability to cause endocarditis, the authors hypothesized that specific adhesion mechanisms may increase the risk for endocarditis but may not be the most important trait for expression of pathogenicity (48). In contrast, Scheld et al. (196) found that *E. faecalis* bound fibronectin better than gram-negative bacilli but not as well as *S. aureus* or pathogenic *Candida* species. In a rabbit model of catheter-induced endocarditis, the log₁₀ 50% infective dose for *E. faecalis* was only 1.4 times higher than that for *S. aureus* (196).

The seminal investigation of enterococcal microbial determinants in a model of catheter-induced endocarditis was

conducted by Chow et al. (40). Three isogenic *E. faecalis* strains which expressed cytolysin and aggregation substance, cytolysin alone, or aggregation substance alone were compared with a plasmid-free strain for the ability to cause endocarditis. These researchers determined for rabbits with endocarditis the masses and bacterial counts of cardiac vegetations and mortality at 3 days. Rabbits infected with strains expressing aggregation substance exhibited vegetations of increased weight regardless of cytolysin phenotype (40). Although larger vegetations were noted for rabbits infected with noncytolytic *E. faecalis* strains expressing aggregation substance, these strains were no more virulent than the plasmid-free strain with respect to mortality (40). The mortality for rabbits infected with strains producing only aggregation substance or cytolysin was significantly lower than that for animals infected with the strain expressing aggregation substance and cytolysin (7 versus 55%). The 50% infective doses for these strains were not determined, so it is not known whether aggregation substance augments infectivity of *E. faecalis* in this model. Further research will determine how frequently cytolysin-producing and aggregation substance-encoding *E. faecalis* strains cause endocarditis in humans and whether a higher morbidity or mortality is associated with this phenotype.

Abscess and Soft Tissue Infection

The ability of enterococci to infect in pure culture soft tissues or the peritoneum of animals (or humans) is limited (30, 105, 178). Hite et al. (105), however, showed nearly 50 years ago that enterococci could produce severe soft tissue infection when mixed with otherwise avirulent anaerobic microorganisms. These observations fit clinical experience, since pure enterococcal infections are rare at these sites. The concept of microbial synergy is also supported by antimicrobial regimens that lack activity against enterococci but are effective in treating mixed enterococcal infections of soft tissue and peritoneum (10, 237).

Using animal models, Onderdonk et al. (178) and others (30, 157, 159) have confirmed Hite's findings. Onderdonk et al. (178) used a rat model of intraabdominal sepsis and determined that abscesses consistently formed only when combinations of an anaerobe and a facultative microorganism, such as *E. coli* or an enterococcus, were used. In the study of Matlow et al. (159), intraperitoneal inoculation of rats with *E. faecalis* in combination with *E. coli*, *Bacteroides fragilis*, and *Clostridium perfringens* was more often associated with death or large abscess formation than similar inocula without *E. faecalis*. Curiously, these investigators were able to recover *E. faecalis* from 33% of abscesses when *E. faecalis* had not been in the original inoculation. Similarly, Martens et al. (157) observed that nearly one-third of bacteria recovered from experimental pelvic abscesses in female rats had not been part of the original inoculum. Both groups speculated that this phenomenon resulted from translocation of organisms across the bowel wall.

Although microbial synergy between enterococci and anaerobes is well established (30, 105, 157, 159, 178), the mechanism has not been aggressively studied. Possibly encapsulation of anaerobic organisms plays a role (31, 179). Whether other metabolic, toxin-mediated, or immunomodulatory factors contribute to the interplay between anaerobes, enterococci, and host immune cells remains open to further study.

Urinary Tract Infection

The bladder, prostate, and kidney are commonly infected by enterococci, especially in patients with structural abnormalities of the urinary tract or indwelling catheters (72, 144, 165).

Infection likely occurs through organisms ascending the urethra and ureters. In an attempt to mimic this process, Desnottes et al. (49) developed a model of pyelonephritis in male rabbits. Following temporary ligation of a ureter, these investigators injected protease-producing *E. faecalis* into the renal pelvis. All rabbits developed chronic pyelonephritis, with a mortality of approximately 20%. When cystitis was produced by inoculation of organisms into the bladder through a transurethral catheter, chronic pyelonephritis never developed. This model has yet to be used in a study of enterococcal determinants potentially important to urinary tract infection.

Kreft et al. (137) demonstrated a potential role for aggregation substance in mediating adherence of enterococci to renal epithelial cells. These workers demonstrated that isogenic variants of *E. faecalis* OG1X harboring pAD1 bound cultured pig renal tubular cells at modestly higher levels than aggregation substance-deficient mutants. Moreover, this binding was partially inhibited by the tetrapeptide Arg-Gly-Asp-Ser (137). Although an as-yet-unidentified serum component was capable of inducing aggregation substance (137), questions remain as to whether aggregation substance is expressed by enterococci in the urinary tract or whether the gene occurs more frequently in strains isolated from infected urine compared with commensals.

The hematogenous model of enterococcal pyelonephritis developed by Guze et al. (94) has been used to examine the relative virulence levels of a limited number of enterococcal strains with various phenotypes (163). Cytolysin- and protease-producing *E. faecalis* strains were found to cause pyelonephritis no more severe than do strains lacking these phenotypes. *E. durans* and *E. faecium* strains infected kidneys equally as well as *E. faecalis* but at significantly lower concentrations of organisms. The relevance of the rat model to human infection is unclear. Large intravenous inocula are required to infect rats. Pathologic correlates with human pyelonephritis are lacking. Guze et al. (94) speculated that growth persisted in the renal medulla, although initially occurring at multiple sites (such as liver and spleen), because enterococci tolerate the high tonicity found at this site. Microbial or host determinants important to this, the oldest but best-defined model of urinary tract infection, remain to be determined.

MODULATION OF HOST IMMUNITY

For pathogens breaching mucosal or skin barriers and adhering to host tissues or cells, infection can develop only if other defenses are neutralized, avoided, or restricted. Professional phagocytes such as neutrophils, monocytes, and macrophages provide nonspecific, but powerful, host defenses against pathogens of all types. Neutrophils, in particular, migrate efficiently to sites of infection in response to chemotactic signals, use complement and antibody for pathogen recognition, and kill ingested organisms by oxidative and nonoxidative mechanisms (143). Gram-positive pathogens have a diverse array of virulence factors that favor their survival against these host defenses. Survival mechanisms include antiphagocytic polysaccharide capsules for *Streptococcus pneumoniae* (71), antiphagocytic M protein for *S. pyogenes* (73), leucocidin for *S. aureus* (214), hemolysin for *L. monocytogenes* (185), and catalase and superoxide dismutase for *S. aureus* (155) and *Nocardia asteroides* (15).

Lipoteichoic Acids

Membrane-associated lipoteichoic acids common among prokaryotic organisms are amphipathic polymers composed of a hydrophilic polyglycerolphosphate backbone linked via an ester bond to a hydrophobic glycolipid tail. For enterococci these surface molecules (219) have been shown to be identical to the group D antigen (236). Within a single organism, lipoteichoic acids exist as a microheterogeneous species with the glycerophosphate chains varying from 9 to 40 residues in length (145). Several biological characteristics of enterococcal lipoteichoic acids have been investigated. Beachey et al. (13) noted that enterococcal lipoteichoic acid reversibly bound human erythrocytes as well as lipoteichoic acid from *S. pyogenes*. The acyl moiety of lipoteichoic acid was essential for binding (13). Lipoteichoic acid is continually released from *S. pyogenes* independent of stage of growth cycle (2). Whether enterococci also release lipoteichoic acid is not known. These facts may be relevant to a local inflammatory process, because lipoteichoic acid bound by eukaryotic cells retains antigenic specificity. As a result, these cells can suffer complement-mediated lysis when exposed to plasma (110). Conceivably, tissue damage at sites of infection could arise from activation of complement by host cell membrane-associated bacterial lipoteichoic acid (110).

Bhakdi and coworkers tested the ability of lipoteichoic acids from clinically important gram-positive organisms to stimulate production of interleukin-1 β , interleukin-6, and tumor necrosis factor alpha from cultured human monocytes (18). Interestingly, they observed that lipoteichoic acids from *S. aureus* and *S. pneumoniae* failed to induce monokine production, while lipoteichoic acids from several enterococcal species at concentrations ranging from 0.5 to 5.0 μ g/ml induced release of all three monokines. The levels of monokines stimulated by enterococcal lipoteichoic acid were similar to those observed after exposure to gram-negative lipopolysaccharides. Similarly, Tsutsui et al. (221) found enterococcal lipoteichoic acid to be a potent inducer of tumor necrosis factor and interferon.

Finally, Ehrenfeld et al. (61) reported that purified lipoteichoic acid from *E. faecalis* inhibited pheromone-induced aggregation of bacterial cells. They suggested that lipoteichoic acid acted as the binding substance recognized by aggregation substance on donor cells. Analysis of genes affecting binding substance has proven complex and is in progress (16, 220). Together these studies suggest that enterococcal lipoteichoic acid may serve as a virulence factor by modulating inflammatory responses and through facilitation of plasmid transfer.

Complement and Neutrophils

Only recently have interactions between complement, human neutrophils, and enterococci been examined (4, 100, 176). Two groups investigating *E. faecalis* and *E. faecium* reported opsonization of both by the alternative complement pathway (4, 100). Harvey et al. (100) found enhanced neutrophil killing of enterococci in the presence of complement and specific rabbit antienterococcal immune globulin. Arduino et al. (4), however, reported no augmentation of neutrophil killing by antienterococcal hyperimmune rabbit serum. Rather, they suggested that neutrophil killing depended primarily on complement activation and not specific immune globulin (4). Strains capable of expressing protease, cytolysin, or aggregation substance proved no more resistant to phagocytosis than strains lacking these phenotypes (4). Whether these strains actually expressed protease, cytolysin, or aggregation sub-

stance under the *in vitro* assay conditions employed was not demonstrated.

The ease with which enterococci are opsonized is consistent with their apparent lack of capsular polysaccharide. These studies also suggest that enterococci lack significant antiphagocytic determinants. However, pathogens phagocytosed by neutrophils rarely survive exposure to hypohalous acids resulting from a respiratory burst. Macrophages are less efficient in killing ingested pathogens than neutrophils, in part because they lack myeloperoxidase and cannot produce hypohalous acids from reactive oxygen species. Several gram-positive organisms, such as *S. aureus* and *N. asteroides*, can catalyze superoxide and hydrogen peroxide found in phagolysosomes to improve their chances of survival (14, 155). Although enterococci are catalase negative, they express a flavin containing NADH peroxidase to degrade hydrogen peroxide (184). Enterococci also possess an oxygen-inducible superoxide dismutase to catalyze conversion of superoxide to hydrogen peroxide (27, 91). Whether these enzymes enhance survival of enterococci after phagocytosis by macrophages awaits investigation.

Pheromones

Pheromones are small peptides seven to eight amino acids in length secreted by *E. faecalis* that promote conjugative transfer of plasmid DNA between strains (43, 115). These peptides are chromosomally encoded and are referred to as pheromones because they elicit a specific mating response from plasmid-carrying donor cells. Typically, multiple pheromones are secreted simultaneously by a given *E. faecalis* strain. In addition to pheromones, each pheromone-responsive plasmid encodes a secreted peptide that acts as a competitive inhibitor of its corresponding pheromone. Recent reports suggest that some, but not all, pheromones and their peptide inhibitors possess the potential to serve additional roles as chemoattractants for neutrophils, cause granule enzyme secretion, and induce a respiratory burst (64). This last effect may explain the augmentation of neutrophil superoxide production observed by Novak et al. (176) when *E. faecalis* strains containing a conjugative, cytolytic plasmid were phagocytosed. Although pheromones and their inhibitors are nonformylated oligopeptides, they competitively inhibit formyl-Met-Leu-Phe binding to the neutrophil formyl peptide receptor and appear to act as a ligand for this surface receptor (193).

Pheromones cAM373 and cPD1 were reported by Sanomiya et al. (193) to be chemoattractants at nanomolar concentrations. Ember and Hugli (64), however, noted activities 2 to 3 orders of magnitude lower for the same pheromones when slightly different assay conditions were used. In either case, these pheromone concentrations are 2 to 3 orders of magnitude greater than those found in enterococcal culture supernatants (45). Although *E. faecalis* strains typically secrete multiple pheromones (45), and chemotactic effects of pheromones appear to be additive at low concentrations (64), the question of whether these peptides or their inhibitors significantly modulate inflammatory responses *in vivo* remains unanswered. Indeed, formylmethionyl peptides derived from amino-terminal regions of newly synthesized proteins in prokaryotic organisms also serve as chemoattractants for neutrophils (156, 160). Conversely, cell wall components can be antagonistic to leukocyte migration (172) and inducers of inflammation (33). Whether any of these molecules, or complement components such as C5a which would be expected at sites of infection, are more or less important as immunomodulators *in vivo* is in need of further investigation.

SECRETED PRODUCTS

Cytolysin

Generally, the cytolytic phenotype is specified by highly conserved, transmissible plasmids (57, 120, 142). Initially, cytolytic plasmids were found to belong to the same IncHly incompatibility group (46). However, cytolytic plasmids from other incompatibility groups have been reported (116). Moreover, cytolytic genes occasionally occur as chromosomal elements (116). The most extensively characterized cytolytic plasmid is pAD1 (43).

In 1949 Sherwood et al. (203) reported on five of eight beta-hemolytic group D streptococci that inhibited growth of other bacteria. These observations were extended by Stark (209) in 1960, when each of 16 beta-hemolytic *E. faecalis* strains was found to inhibit growth of most gram-positive, but not gram-negative, bacteria. Brock et al. (29) subsequently examined nearly 100 enterococcal isolates and found that over 50% expressed a bacteriocin activity. Cytolytic *E. faecalis* strains expressed a bacteriocin with broad activity against gram-positive, but not gram-negative, bacteria (29). These investigators also demonstrated that cytolytic and bacteriocin activities were simultaneously lost by some *E. faecalis* strains after exposure to UV irradiation and that these activities were simultaneously regained by reversion (28). This coincident behavior provided strong evidence for a single enterococcal product being responsible for cytolytic and bacteriocin activities (28).

Several studies by Granato and Jackson in the late 1960s and early 1970s provided convincing data that the *E. faecalis* cytolytic plasmid was at least bicomponent in nature (87–89). These investigators termed the two operationally defined components L for lysin, and A for activator, on the basis of the kinetics of interaction (87). Transposon insertional mutation localized the cytolytic determinant on pAD1 to 8 kb of DNA (Fig. 1a) (44, 60, 117). These studies permitted direct cloning of restriction fragments related to cytolytic expression and ultimately reassembly of a functional cytolytic expression unit in *E. coli* (117).

Nucleotide sequence determination for the *E. faecalis* cytolytic operon has revealed a complex determinant encoding five gene products (Fig. 1b) (84, 85, 201). Several lines of evidence suggest that the *E. faecalis* cytolytic operon represents a new branch of the lantibiotic family, a group of small secreted proteins with bactericidal activity against gram-positive pathogens such as staphylococci, streptococci, and propionibacteria (198). Reading frames *cylL1*, *cylL2*, *cylM*, and *cylB* are relevant to expression of component L (84, 85, 117), whereas *cylA* is the only reading frame necessary for expression of component A (201). Analysis of *cylA* revealed an inferred extracellular protein with similarity to the serine protease subtilisin BPN' (201). These findings were supported by physical and biochemical evidence and suggested that CylA activates cytolytic precursors extracellularly by protein cleavage (201).

Expression of component L has proven to be considerably more complex than that of CylA. It involves at least two cytolytic precursor peptides and two auxiliary factors (85). Nucleotide sequence determination has revealed two small open reading frames, termed *cylL1* and *cylL2*, at the extreme 5' end of the cytolytic operon (85, 117). From nucleotide sequence analysis, both CylL1 and CylL2 are inferred to be small, relatively cysteine-rich polypeptides. The amino-terminal halves are relatively hydrophilic and are followed by distinctly hydrophobic carboxyl termini (85). A current model of the cytolytic operon suggests that these peptides are the ribosomally synthesized precursors of cytolytic (Fig. 3). Site-specific

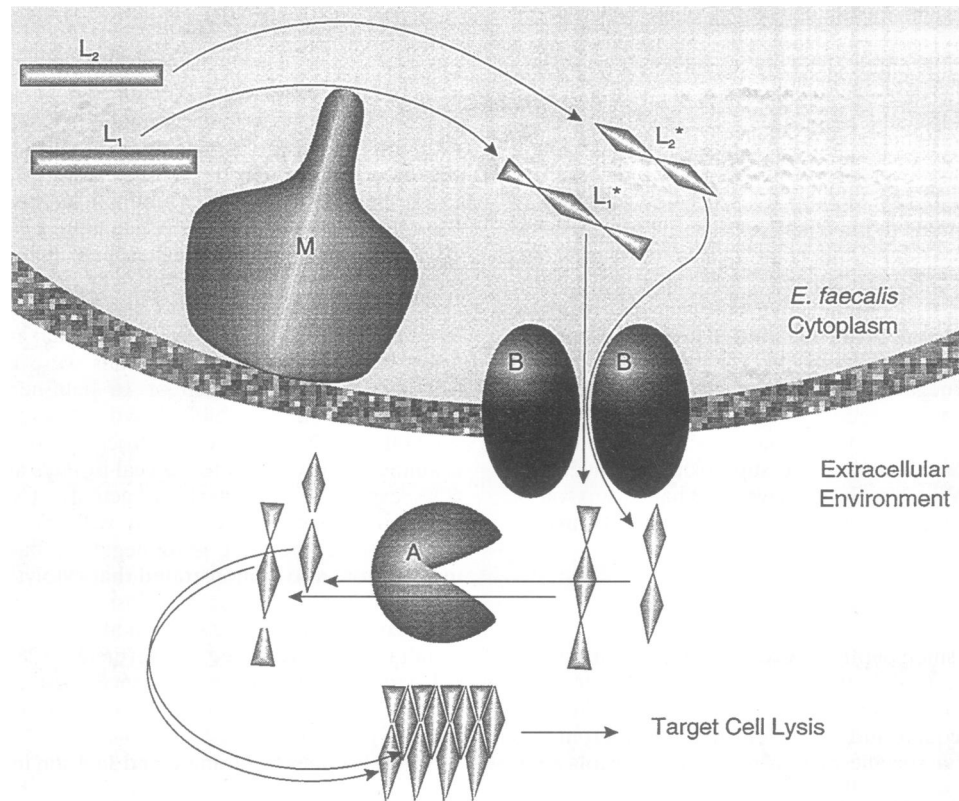


FIG. 3. Model of cytolysin expression, maturation, secretion, and activation. Cytolysin components CylL1 (L_1) and CylL2 (L_2) appear to be modified intracellularly by the CylM (M) gene product. The posttranslationally modified products are transported out of the cell by CylB (B). Once outside the cell, the cytolysin precursor components are activated by CylA (A) and effect target cell lysis by a process that presumably involves interaction of CylL1 and CylL2, since neither is detectably active individually.

mutagenesis in each reading frame demonstrated that CylL1 and CylL2 were both required for expression of cytolysin (85).

A large reading frame encoding a 993-amino-acid polypeptide and termed *cylM* was found adjacent to and immediately 3' to the *cylL1* and *cylL2* reading frames (Fig. 1b) (85). The carboxy-terminal half of CylM has amino acid sequence similarity with analogs occurring in operons related to expression of nisin, epidermin, and subtilin (85). Each of these cell membrane-active peptides is a lantibiotic (198). These peptides are unusual in that posttranslational modifications of serines, threonines, and cysteines form thioether-bridged lanthionines (198, 210). CylM appears to be an intracellular protein that participates in the posttranslational modifications of CylL1 and CylL2, which may include introduction of lanthionine or methylanthionine cross-links as occurs in maturation of other lantibiotics (41, 75, 197, 198). Site-specific mutations in *cylM* block expression of functional CylL1 and CylL2, indicating that both depend on the *cylM* gene product for maturation (85). Studies to analyze the structures of and interaction between activated CylL1 and CylL2 are ongoing.

Immediately 3' to *cylM* is an open reading frame termed *cylB* (Fig. 1b). *cylB* was the first gene encoding an ATP-binding cassette transporter reported for a gram-positive bacterium (84). ATP-binding cassette transporters are transmembrane systems that facilitate translocation of molecules with a high degree of substrate specificity and contain highly conserved ATP-binding motifs (70). CylB is most closely related to ATP-binding cassette transporters identified in nisin and subtilin operons (70). Functional CylB is necessary for external-

ization of both CylL1 and CylL2. However, transposon insertional mutations predicted to truncate CylB near the center of the protein retain an ability to externalize functional CylL2 but not CylL1. The larger CylL1 appears to depend upon the C-terminal ATP-binding domain of CylB for externalization (85). Expression of CylA is not affected by mutations in *cylB* (84).

A model of *E. faecalis* cytolysin activation based on these observations is shown in Fig. 3. This model also incorporates observations on how cytolysin-producing *E. faecalis* cells resist self-lysis. Since CylA is a serine protease, immunity appears to result in part from additional proteolytic cleavage of CylL1 and CylL2 close to the cell, beyond that required for activation. Transformation of cytolysin-susceptible *E. faecalis* cells with DNA containing the *cylA* reading frame renders them cytolysin resistant (201). Moreover, addition of culture fluid containing excessive CylA to a cytolysin assay results in reduction of cytolysin activity (87, 117). Thus, CylA appears to play a dual role in activating cytolysin and simultaneously conferring immunity to it. There is some evidence suggesting that a portion of CylA produced by wild-type cytolytic *E. faecalis* remains associated with the cell surface (201). This association may ensure a protective level of CylA in the microenvironment of cytolysin-producing cells. The structural basis for CylA association with the cell wall remains to be established.

The proposed model for cytolysin activation is limited in several respects. It fails to address additional phenotypic traits that may positively or negatively affect cytolysin activity. For example, superoxide production by *E. faecalis* also contributes

to erythrocyte lysis (27, 68). What interaction, if any, reactive oxygen intermediates have with cytolysin products is unknown. The model also fails to describe how cytolysin expression may be regulated, a prospect suggested by early observations on the production of cytolysin under certain growth conditions and not others (218), or effects resulting from transposon insertions near the 3' end of *cylA* (118). Finally, the model fails to explain how conjugal transfer of plasmids from donors expressing cytolysin to cytolysin-susceptible recipients occurs.

Fewer than 20% of humans are normally colonized with cytolytic enterococci (120, 186, 238), although a well-designed survey of enterococcal phenotypes in the stools of adults and children has yet to be done. Several (111, 113, 120, 147), but not all (97, 186), clinical studies screening for cytolysin on appropriate blood agar have reported an increased frequency of cytolytic strains causing infection compared with strains found as part of the normal flora or among other controls. In a study of patients in Japan, Ike et al. (120) found that 60% of clinical *E. faecalis* isolates were cytolytic compared with 17% of strains from uninfected sources. Similar trends were observed in a study of *E. faecalis* bloodstream isolates in the United States (113). Of 77 strains collected over 21 months, 52% were cytolytic. Many of these isolates were antibiotic resistant, as in other studies (99, 120), and were found to be clonally related (99, 111). In one study, cytolytic strains were determined by regression analysis to be associated with a fivefold-increased risk for death within 3 weeks of bacteremia compared with patients with bacteremia caused by noncytolytic strains (113).

Animal models have been used to demonstrate and quantify the contribution of cytolysin to enterococcal virulence. With mice, Ike and Clewell (119) observed a direct, dose-dependent correlation between cytolysin expression and the toxicity of *E. faecalis* after intraperitoneal injection. In this study, the 50% lethal dose was greater than 3×10^9 CFU for an *E. faecalis* strain harboring a plasmid with blocked expression of cytolysin. After 7 days, no mouse injected with this strain died. In contrast, a lower 50% lethal dose of 2.6×10^8 CFU was found for an isogenic strain expressing wild-type levels of cytolysin. All mice injected with $\geq 10^9$ CFU of this strain died within 4 to 5 h. An even slightly lower 50% lethal dose of 1.6×10^8 CFU was observed for a third strain harboring an isogenic plasmid overexpressing cytolysin. All mice injected with $\geq 3 \times 10^8$ CFU of this strain died within 1 to 2 h (119).

In another comparison of isogenic strains attenuated only in cytolysin expression, Jett et al. (126, 211) demonstrated that the *E. faecalis* cytolysin significantly worsened the severity of endophthalmitis in a rabbit model. These investigators infected eyes by intravitreal injection of as few as 100 CFU of *E. faecalis*. Strains tested harbored Tn917 insertional mutations in pAD1 that blocked expression either of CylA or of CylL1 and CylL2 or, as a control, harbored a pAD1::Tn917 derivative that confers wild-type cytolysin expression. Visual loss, as determined by electroretinography and histopathology, was more severe in eyes infected with cytolytic strains. At 24 h, toxin-mediated damage to all retinal layers, including photoreceptors, was observed only for the cytolytic strain (126, 211). Intravitreal administration of ampicillin, gentamicin, and dexamethasone 24 h following infection was effective in salvaging vision in eyes infected with noncytolytic strains (125). Similar treatment of endophthalmitis with the cytolytic strain had no demonstrable effect on the rapid course of visual loss (125). These observations suggested that improvement in visual outcome for patients with endophthalmitis due to cytolytic *E. faecalis* will be realized only when therapies directly target and abrogate the effects of cytolysin.

Finally, since *E. faecalis* represents approximately 8% of

gingival crevice flora (208), Jett and Gilmore (124) determined whether the common oral streptococci were susceptible to cytolysin. In fact, growth of oral streptococci was uniformly inhibited by cytolysin. These results suggest that colonization or infection of gingival tissue by cytolytic *E. faecalis* might inhibit local gram-positive ecologies and precipitate, worsen, or ameliorate periodontal infections.

Protease (Gelatinase)

Purification of a 28- to 32-kDa metalloproteinase from *E. faecalis* was first described 30 years ago (19). In 1989 Mäkinen et al. (153) published a description of the substrate specificity of protease produced by *E. faecalis* OG1-10, a human oral isolate. The enzyme was an extracellular zinc-endopeptidase (metalloendopeptidase II; microbial proteinase; EC 3.4.24.4) capable of hydrolyzing gelatin, collagen, casein, hemoglobin, and other small biologically active peptides. Su et al. (12) subsequently reported the sequence of the protease gene, *gelE*, which encoded a prozymogen with a mature molecular weight of 34,582. Protease production was easily detected by these investigators using semisolid media supplemented with 3% gelatin or 1.5% skim milk (47, 212).

A potential contribution of enterococcal protease to virulence was first suggested in 1975 by Gold et al. (86), who found that a gelatin-liquefying human oral *E. faecalis* isolate, 2SaR (later designated OG1 [56]), induced caries formation in germfree rats, while nonproteolytic strains did not. In addition, others have linked protease production to human infection. Kühnen et al. (138) reported protease-producing *E. faecalis* to be common among enterococci (63.7%) isolated from surgical and neurosurgical intensive care units in Germany. Coque et al. (47) analyzed 95 enterococcal isolates from patients with endocarditis and other nosocomial infections and found that 54% produced protease. In this same study, only 12 and 14% of enterococcal isolates from uninfected hospitalized patients and healthy volunteers, respectively, produced protease.

Although epidemiologic studies can only suggest associations between protease production and infection, the sequence analysis of *gelE* showed tantalizing amino acid similarity to the 33-kDa zinc-metalloproteinase (elastase) of *Pseudomonas aeruginosa* (77, 212). This enzyme is considered a virulence factor in severe *Pseudomonas* infections, especially for patients with cystic fibrosis (50, 223). A recent review of bacterial zinc-metalloproteases describes the medical importance of these common enzymes (101). Cloning of *gelE* and its introduction and expression in a protease-negative enterococcal strain (212) should permit direct testing of its contribution to infection.

Hyaluronidase

Studies of hyaluronidase in other microorganisms have provided an indirect basis for speculating that this enzyme contributes to enterococcal virulence. Detection of hyaluronidase production by microorganisms is accomplished by inoculation of semisolid media containing hyaluronic acid (114, 207). Unsworth (222) observed hyaluronidase-producing *Streptococcus milleri* strains from abscesses more frequently (83%) than strains that were part of the normal flora (25%). Pneumococcal hyaluronidase appeared to play a role in one animal model of middle ear inflammation (150). In addition, it has been described as a spreading factor for *Ancylostoma duodenale* (hookworm) in cutaneous larva migrans (108) and as important for the dissemination of *Treponema pallidum* (74). These precedents suggest that enterococcal hyaluronidase

could play a role in invasive disease. No studies, however, address this issue for enterococci.

AS-48

AS-48 is a 7.4-kDa peptide produced by *E. faecalis* that inhibits and lyses a wide spectrum of gram-negative and gram-positive bacteria, including enterococci (80). This basic peptide is lytic via the generation of pores in cytoplasmic membranes of target cells that lead to depolarization (81). It also appears to induce lysis of selected enterococci through activation of an autolysin (82). As with the cytolysin operon, AS-48 has been found to be encoded by a transmissible plasmid (158). The significance of this bacteriocin remains uncertain, however, since the prevalence of AS-48-producing strains among human commensal and infection isolates has yet to be defined. No activity of AS-48 against eukaryotic cell membranes has been reported.

CONCLUSIONS AND FUTURE PROSPECTS

Enterococci are commensal organisms well suited to survival in intestinal and vaginal tracts and the oral cavity. However, as for most bacteria described as causing human disease, enterococci also possess properties that can be ascribed roles in pathogenesis. The natural ability of enterococci to readily acquire, accumulate, and share extrachromosomal elements encoding virulence traits or antibiotic resistance genes lends advantages to their survival under unusual environmental stresses and in part explains their increasing importance as nosocomial pathogens. Aggregation substance, surface carbohydrates, or fibronectin-binding moieties may facilitate adherence to host tissues. *E. faecalis* appears to have the capacity to translocate across intact intestinal mucosa in models of antibiotic induced superinfection (230). Extracellular toxins such as cytolysin can induce tissue damage as shown in the endophthalmitis model (124, 211), increase mortality in combination with aggregation substance in an endocarditis model (40), and cause systemic toxicity in a murine peritonitis model (119). Finally, lipoteichoic acid, superoxide production, or pheromones and corresponding peptide inhibitors each may modulate local inflammatory reactions.

The recent interest in enterococcal virulence and host-parasite interaction has been spurred on, in part, by concerns that increasing antibiotic resistance may soon render conventional chemotherapy inadequate for serious enterococcal infections. As our understanding of enterococcal pathogenic mechanisms evolves, so too must our ability to prevent and limit the consequences of these infections and to counter the spread of antibiotic resistance. Much work has been done, but the challenge remains formidable.

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