

ENTEROCOCCAL VIRULENCE - PATHOGENICITY ISLAND OF *E. FAECALIS*

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1. ABSTRACT

Enterococcal species, though most commonly regarded as members of the microbial flora of the intestinal tract, have recently emerged as human pathogens of significant concern. The rapid spread of antibiotic resistance among enterococci, which has resulted in strains now being routinely isolated that are resistant to all bactericidal regimens, has prompted considerable interest in investigating the pathogenesis of enterococcal infection, with a view toward deriving new, information-based treatment strategies. This review summarizes major findings on the pathogenesis of enterococcal infection, fits them into a model for the dual lifestyle of enterococci as commensal and pathogen, and integrates into that model a recently discovered pathogenicity island of *Enterococcus faecalis*.

2. INTRODUCTION

Enterococcal species are exceedingly hardy, and as facultative anaerobes, are well adapted to survive within many complex niches within the human host. Although the ability of enterococci to cause human disease was documented a century ago (1), it was not until the emergence of multiple drug-resistant strains over the past three decades (2-4) that the question of virulence received much attention.

2.1. Commensal/pathogen

Although the most abundant gram-positive coccus in the intestine, enterococci constitute <1% of the microflora of the large bowel of most human adults, a percentage that varies with both age and diet (5). The abundance of enterococci generally decreases from birth to adulthood (5,6). *E. faecalis* and *Enterococcus faecium* are the species most commonly associated with colonization of

the human GI tract, and have been found to occur in 82% and 36% of healthy volunteers respectively (7).

It is now becoming recognized that enterococci are important causes of nosocomial – and to a lesser extent community acquired – infection, and that the pathogenic potential of enterococci is not unique to the antibiotic era. MacCallum and Hastings (1) first cultured an enterococcal isolate from the cardiac vegetations of a patient that died from community acquired endocarditis. This isolate was found to be lethal upon intraperitoneal challenge in mice, and was also capable of causing experimental endocarditis in dogs (1), satisfying Koch's postulates. In addition to endocarditis, enterococci have been long established as causes of urinary tract infection (8,9), peritonitis (9), and bacteremia (9). Although enterococci were known to cause serious infection in the pre-antibiotic era, the rapid spread of multiple antibiotic resistance among strains of enterococci has rendered many of these infections refractory to treatment, bringing the issue of enterococcal infection into sharp focus as a leading public health concern.

2.2. Enterococcal pathogenesis

Currently, enterococci rank as the third leading cause of nosocomial infection. They are the leading cause of surgical site infection (17.1%), are second to staphylococci as the cause of nosocomial bacteremia (11.5%), and are the third leading cause of hospital acquired urinary tract infections (14.3%) (10). The majority of enterococcal infections are caused by either *E. faecalis* or *E. faecium* (11). To a significantly lesser extent, infections are caused by other species of enterococci such as *E. durans*, *E. avium*, *E. gallinarum*, or *E. casseliflavus* (11). While *E. faecium* constitutes the majority of vancomycin

and ampicillin resistant isolates of enterococci, *E. faecalis* accounts for the majority (65-80%) of nosocomial infections caused by enterococci (11-13). That the majority of enterococcal infections are caused by the species *E. faecalis* may reflect either a greater level of bacterial virulence, or the increased prevalence of *faecalis* over *faecium* in intimate association with the host, or both.

A number of findings support the theory that selection for antibiotic resistance and virulence traits occurred independently of one another. With the exception of a few examples where tetracycline resistance and vancomycin resistance have been identified on pheromone-responsive transmissible plasmids (14,15), which by their nature, encode the surface adhesin aggregation substance, antibiotic resistance and virulence traits usually are not physically linked. For example in strain DS16, a clinical isolate resistant to tetracycline and MLS antibiotics (16), the virulence plasmid pAD1 on which both aggregation substance and the cytotoxin operon reside is devoid of resistance determinants. Similarly, the recent determination of the nucleotide sequence of the genome of the first vancomycin resistant enterococcus in the US (17), *E. faecalis* strain V583, revealed that the vancomycin resistance element was located on the genome diametrically opposite from a recently described pathogenicity island (18,19). Moreover, the vancomycin resistance gene cluster represented a localized peak in genome G+C content, whereas the pathogenicity island represented a distinct trough (17). This lack of physical linkage in clinical isolates possessing both virulence and antibiotic resistance traits argues strongly for independent selection for the two types of traits.

Initially it was believed that enterococci native to the patients' intestinal flora were the cause of nosocomial infection (20). However, it has become clear that for multiple antibiotic resistant infections, the situation is more complex. Many instances of clonal infection have been observed among patients in the same hospital ward, as well as among patients in geographically separated institutions (21-26). In contrast, enterococci from the flora of healthy patients are rarely clonal and rarely antibiotic resistant (27,28; M.S. Gilmore, unpublished data). Moreover, point prevalence surveys show that within hours to days of admission, the GI tracts of patients become colonized with multiple antibiotic resistant strains (29). Therefore, an initial step in the pathogenesis of multiple antibiotic resistant enterococcal infection appears to be the asymptomatic colonization of the GI tract of patients following hospital admission.

The first step in the pathogenesis of enterococcal infection appears to be persistence on inanimate surfaces, followed by colonization of the GI tract and amplification of resistant enterococcal numbers (30). Initial colonization of the GI tract appears to result from the acquisition of small numbers of organisms from inanimate surfaces within the hospital, including thermometer handles, bed rails, health care providers, and other sources (31-34). This observation raises two important questions that have not been completely answered: 1) Do multiple antibiotic resistant enterococcal strains exhibit enhanced resistance to

desiccation or enhanced survival on inanimate surfaces? and 2) Do multiple antibiotic resistant enterococci have the capability to displace indigenous, antibiotic sensitive commensal strains or do they colonize other microecologies within the GI tract in a non-competitive fashion as has been suggested (18)? However/wherever pathogenic enterococci establish themselves within the complex microbial ecology of the GI tract of hospital patients, it is likely that antibiotic therapy commonly employed clinically, but largely ineffective against enterococci would also play a role in this stage of pathogenesis, either by the disruption of the intestinal microflora or some other as of yet undetermined mechanism.

From the staging grounds of the patient GI tract, the next step in the pathogenesis of enterococcal infection depends somewhat on the nature of the infection. Bacteremia that does not appear to result from a frank breach of the GI tract, may involve enhanced enterococcal transcytosis into the bloodstream (35), a process discussed in further detail below. Urinary tract infection in patients with indwelling devices, or infection of sutured surgical wounds, appear to result from external contamination, potentially by organisms that have amplified in the GI tract and become intimately associated with the patient upon hospitalization. Colonization of these abiotic devices may involve biofilm formation, resulting in an infection nidus that resists immune clearance and seeds the bloodstream from the exogenous route. Following establishment of a nidus, either within the GI tract at a point leading to enhanced transcytosis, at a post-surgical wound site, or on an abiotic surface, enterococci may then disseminate. Traits that enhance survival to host clearance mechanisms, such as factors that prevent opsonization, impede phagocytosis, or limit phagocytic killing, facilitate systemic spread of the organism.

The final step in the pathogenesis of enterococcal infection is the damage that results to vital tissues as the result of the presence of enterococci. Factors that would enhance the fourth step in the pathogenesis of enterococcal infection include those that induce overt tissue damage, or factors that enhance bystander damage resulting from inflammation.

Variable enterococcal traits with the potential to enhance the ability of the organism to cause disease, as well as intrinsic attributes that enable even commensal strains to colonize inappropriate sites in an immune compromised patient, have been described (12,30,36,37). Although few of these have been shown rigorously to contribute to the pathogenesis of enterococcal infection, a basis of information now exists on which a testable model of enterococcal pathogenesis can be proposed. A schematic of enterococcal pathogenesis as outlined above is shown in Figure 1.

2.3. Model and traits known or suspected to contribute to enterococcal pathogenesis

Traits that may enhance the ability of enterococci to achieve the first step in the proposed model, namely

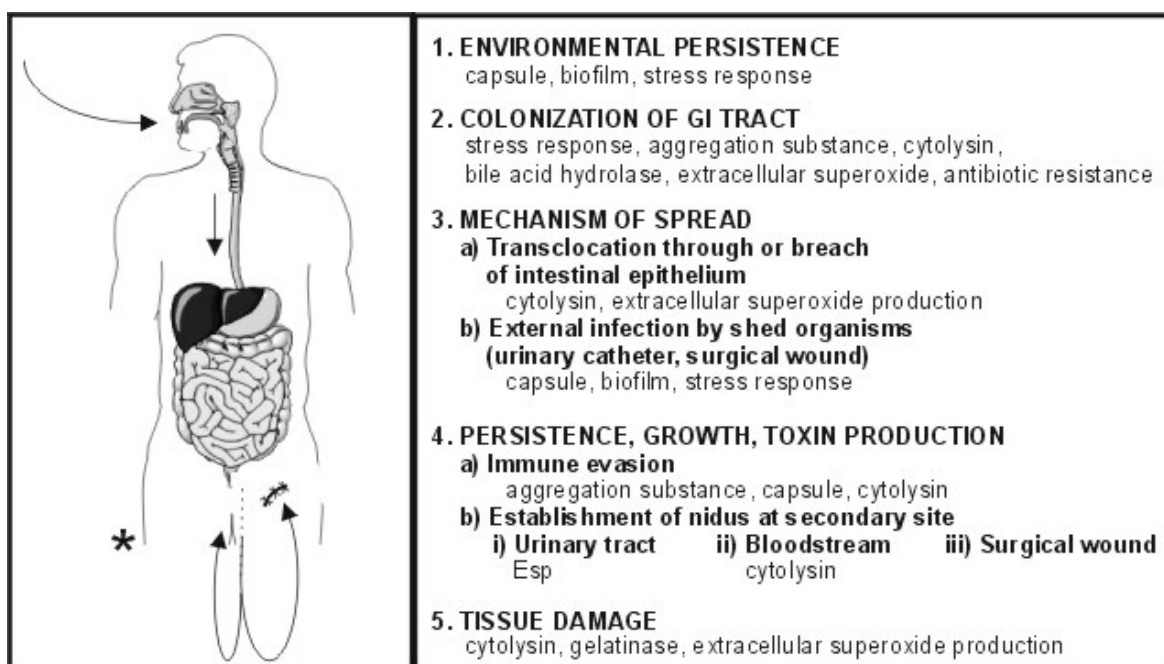


Figure 1. Pathogenesis of disseminated enterococcal infection. A stepwise model of enterococcal pathogenesis and potentially relevant factors of *E. faecalis*. *LifeART image copyright (2003) Lippincott Williams & Wilkins. All rights reserved.

persistence in the hospital environment, include those that confer the ability to survive environmental stress. Enterococci are capable of resisting a variety of environmental stresses including heat, acid, oxidation, hyperosmolarity, and UV irradiation (38-41). They also tolerate ethanol, detergents, and prolonged dessication (41). Biofilm formation and capsular polysaccharide expression may contribute to survival of enterococci outside of the host, although this has not been addressed experimentally.

Some details of the stress response of *E. faecalis* are beginning to be illuminated through proteomic analysis (39). Proteins involved in the response to a variety of environmental stresses, designated general stress proteins, include homologs to heat shock proteins DnaK and GroEL (42), Gsp66 and Gsp67 respectively (39). *E. faecalis* also produces a number of proteins involved in the response to oxidative stress (43) including Npr, an NADH peroxidase (41), Gsp65, a general stress protein homologous to organic hydroperoxidase resistance proteins (44), and an oxygen inducible catalase (41,43). A number of genes involved in alternative metabolic pathways used for growth under nutritional limitation also have been identified (39). Glsl4, a carbamate kinase of the arginine deiminase system, allows for production of ATP from byproducts of arginine metabolism and may also be involved in acid tolerance (39,45). Glsl23, a triose phosphate isomerase, was also found to be induced under starvation conditions (39). CsrA may be involved in peptide repair following cadmium stress (39), and Glsl24 has been implicated in the glucose starvation response as well as resistance to bile salts (39,46). The ability of the organism to respond to rapidly changing environments and stresses likely contributes to the survival of enterococci through all stages of infection.

Traits that may contribute to the second step in the pathogenesis model, those that enhance colonization of the patient's GI tract as a staging ground for disseminated infection, can be divided into four groups: 1) Factors that overcome secreted, non-specific defenses, such as bile, 2) Factors that promote colonization in the presence of overt selection, such as antibiotic resistance determinants, 3) Factors that enhance or alter the ability of enterococci to attach to surfaces within the GI tract, such as adhesins, and 4) Factors that enable invading enterococci to displace resident enterococci or other species contributing to colonization resistance, including bacteriocins and other secreted antimicrobial products. Genomic analysis of *E. faecalis* has uncovered a putative bile acid hydrolase (*cbh*), whose role in pathogenesis has yet to be determined (19). This bile acid hydrolase could aid the organism in occupying a niche within the GI tract closer to the bile duct, where organisms lacking this determinant would not be able to colonize (18,47). The ability of enterococci to degrade intestinal mucin (41,48) also may allow them to occupy deeper layers of the mucosa, perhaps providing a source of carbohydrates.

Pathogenic enterococci are often multi-drug resistant, and thus carry a variety of genetic determinants providing them a potential growth advantage over other susceptible enteric bacteria (49). Antibiotic resistance is not only important as an impediment to treatment, but under selective conditions provides enterococci with a powerful colonization trait, as evidenced by the increased colonization rates of patients undergoing antibiotic therapy by antibiotic resistant enterococci (4,50-52). Enterococci also secrete endogenous bacteriocins, including the cytolysin (53). Enterococci are also capable of producing

other noxious metabolites, including large amounts of extracellular superoxide (54). It is conceivable that the production of extracellular superoxide may be toxic to neighboring anaerobic bacterial species, and thus may aid in the maintenance or expansion of enterococci within the GI tract (55).

Once a pathogenic enterococcus enters a new niche, adherence and growth are required. Aggregation substance, a surface protein involved in the transfer of pheromone-responsive plasmids and clumping, has been associated with the ability of *E. faecalis* to adhere to, and become internalized by intestinal epithelial cells *in vitro* (56,57), and that this effect is mediated by the functional domain of aggregation substance (58). Esp, an enterococcal surface protein common to clinical isolates of both *E. faecalis* and *E. faecium* (59-61), has been observed to promote colonization of the bladder (62). Esp also has been shown to enhance the ability of *E. faecalis* to form a biofilm (63; N. Shankar, personal communication), although recent reports show that *E. faecalis* can form biofilms in the absence of Esp (64), illustrating that biofilm formation is most likely a multifactorial process. The role of biofilms themselves in the colonization of the GI tract or pathogenesis of enterococcal infection is not well understood.

Traits that contribute to the third step, the establishment of infection at a distant site, fall into three categories: Those that promote transcytosis, those that promote survival en route, and those that promote colonization of an extraintestinal site of infection. In order for enterococci to disseminate from the intestine to the bloodstream, bacteria must be able to breach the intestinal epithelium in some manner. In addition to frank penetration of the colon, transcytosis from the lumen of the intestinal tract into the bloodstream may occur. Although the ability of *E. faecalis* to traverse the intestinal epithelium within mice has been reported based on microscopic evidence (35), the factors involved in this process remain to be completely determined. Alternatively, the organism may gain direct access to the bloodstream via cytotoxicity to the intestinal epithelium. The aforementioned production of extracellular superoxide or cytolysin may play a role in damaging nearby intestinal epithelium, allowing adjacent enterococci to escape into the bloodstream. Aggregation substance, has been observed to facilitate internalization of *E. faecalis* by intestinal epithelium (56,57). However, the fate of these bacteria, once internalized, is unknown.

Finally, bacteria including enterococci can be introduced directly into the urinary tract, or can colonize surgical wounds and the bloodstream by colonizing catheters, sutures, or other indwelling devices. A recent study showed that *E. faecalis* bloodstream isolates from catheterized patients exhibited a stronger propensity to form biofilms when compared to isolates from non-catheterized patients or isolates of unclear clinical significance (65). To what extent biofilm formation plays a role in the pathogenesis of enterococci via the exogenous route, i.e. catheterization, is unknown but of great interest to researchers.

Regardless of the route of dissemination, in order to proliferate and cause disease, enterococci must have mechanisms for thwarting the immune response of the host. Aggregation substance has been shown to be involved in the survival of enterococci within activated PMNs (66). The expression of capsular polysaccharide from the *cps* gene has been shown to contribute to host immune evasion (67). Cytolysin expression has been correlated to an increased appearance of *E. faecalis* in the blood by an unknown mechanism in a murine model of bacteremia (12). Enhanced proliferation in the bloodstream observed with cytolytic strains may reflect the ability of these strains to lyse red blood cells and acquire heme, which then results in the synthesis of cytochromes allowing for aerobic respiration and enhanced growth (68,69). There exists the possibility that cytolysin may also have an antiphagocytic role at this stage of infection (70). However, expression of cytolysin did not enhance the ability of *E. faecalis* to survive within macrophages under conditions tested *in vitro* (71).

Various additional factors of enterococci have been implicated in establishing disseminated infections. In infectious endocarditis, cellular carbohydrate was proposed to be involved in adherence to heart cells (72), and aggregation substance was found to influence the weight of vegetations on cardiac tissue (73). The presence of a recently characterized surface protein, Esp, was found to be enriched in pathogenic isolates of *E. faecalis*, particularly those associated with endocarditis (62). Esp also has been identified within *E. faecium* where it is found commonly within clinical isolates irrespective of vancomycin resistance (59,74). Esp enhances colonization of the bladder, perhaps through mediating attachment to uroepithelial cells or promoting biofilm formation at this site (62, 64). Its homology to a staphylococcal protein associated with nosocomial infections in patients with indwelling devices (75) and contribution to biofilm formation (64), suggests that Esp may play a similar role in the establishment of nosocomial enterococcal infections. Esp varies from strain to strain in the numbers of 3 different types of long (~80) amino acid repeats within its structure (61). A small portion of one of the repeat regions is identical to the Rib and C alpha proteins of *S. agalactiae* (76), proteins that have been shown to vary in length on the cell surface under immune pressure (77). The variation observed in repeat number for Esp suggests that it may have a role in immune evasion (36,76). The role of Ace, a surface protein involved in the adherence of heat-stressed *E. faecalis* to collagen type IV and extracellular matrix (78,79), in infection is not well established.

Upon establishing a nidus of infection, enterococci are capable of producing a variety of factors that have been shown or hypothesized to contribute to destruction of host tissue. Cytolysin was initially shown to be toxic for mice in intraperitoneal infection (80). It subsequently was found to be cytotoxic for cells of the rabbit retina (81) and to contribute to toxicity in a rabbit model of endocarditis (73,80). Similarly, the cytolysin was found to be toxic for *C. elegans* (82). Gelatinase (GelE), a zinc-dependent metalloprotease was hypothesized to

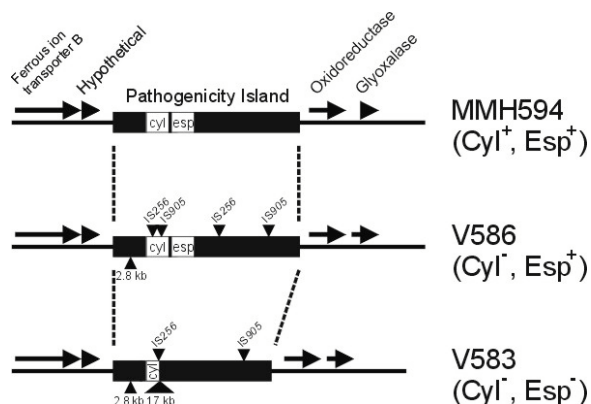


Figure 2. Known variants of *E. faecalis* pathogenicity island. Schematic of variations of pathogenicity island within *E. faecalis*. Entire island present in MMH594 positive for both cytolysin and Esp. IS elements present within island in V583 resulting in the disruption of CylB of cytolysin operon resulting in Cyl⁻, Esp⁺ phenotype. 17 kb deletion within V586 resulting in the loss of the 3' end of the cytolysin operon and the loss of *esp* resulting in Cyl⁻, Esp⁻ phenotype. Adapted with permission from Shankar *et al.* (19) and Nature (<http://www.nature.com>).

contribute to bacterial virulence (83), a prediction that has proven true in several models. Gelatinase is regulated in conjunction with a serine protease (SprE) by the *fsr* two component regulatory system (84). Together, these Fsr-regulated factors have been shown to contribute to the virulence of *E. faecalis* in murine peritonitis and rabbit endophthalmitis (84-86), and is toxic for *C. elegans* (82,86).

3. PATHOGENICITY ISLAND

Pathogenicity islands have been described in a number of Gram-negative species such as *E. coli*, *Shigella flexneri*, *Salmonella enterica*, and *Yersinia spp.* (87-93). These pathogenicity islands typically carry genes associated with virulence, such as toxins, adhesins, other factors that promote colonization, and mechanisms for the delivery of bacterial products directly into host cells. These islands are most often large (20 kb to 200 kb and up) and often differ in G+C content from the remainder of the genome, suggesting that these elements are often acquired via horizontal transfer. Pathogenicity islands often also contain genes encoding recombination functions (integrases, transposases, insertion elements), which likely are involved in the initial integration event, and may contribute to the continuing evolution of these elements. The presence of pathogenicity islands creates a particularly virulent subpopulation within a species such as *E. coli* (87,94), which without the island has an important role as a member of the commensal flora.

3.1. Pathogenicity island of *E. faecalis*

A pathogenicity island recently has been identified within the genome of infection-derived clinical isolates of *E. faecalis* from diverse geographical sources (19). Like *E. coli*, enterococci exist largely as commensal organisms, with some strains exhibiting enhanced virulence.

The pathogenicity island recently described for enterococci encodes most of the known variable traits of *E. faecalis* associated with virulence, including cytolysin, aggregation substance, and Esp, is large in size (approximately 150 kb), and caused terminal duplications at the site of insertion (19), fitting well the paradigm established for pathogenicity islands of *E. coli* and other gram negative organisms (87,88,95).

3.1.1. Identification and characterization

Genomic comparisons of MMH594, the prototype of a strain that infected multiple patients in a hospital ward (96) and OG1, a non-infection derived oral isolate of *E. faecalis* negative for the expression of both Esp and cytolysin (97), revealed the presence of a 153,571 bp insert flanked by 10 bp direct repeats within MMH594 (19). This element was determined also to be present in V583, the first vancomycin-resistant enterococcus isolated in the US (98) and the subject of the *E. faecalis* genome initiative (17). Minor differences between the pathogenicity islands of V583, another vancomycin resistant isolate from the same patient, V586, and MMH594, were observed in that the latter strain possesses a 2.8 kb insertion with the features of a group II intron (19,99). Additional variations between V583 and V586 were identified in the region of the island that encodes Esp, which was noted to be part of a 17 kb deletion in strain V583 (Figure 2) (19).

Analysis of the *E. faecalis* pathogenicity island of strain MMH594 reveals 129 ORFs (Figure 3). The mean G + C content for this region was noted to be 32.2%, which varies from the 37.38% G + C for the genome as a whole (17,100). Based on sequence comparison, it appears as if the 5' portion of the island was derived from a partial integration of a conjugative plasmid into the chromosome in that it bears elements of pheromone-responsive plasmids pAM373, pCF10, and pAD1 (17,19,101). Spread across the pathogenicity island also are ORFs with homology to transposases and insertion elements from species such as *E. faecium* (EF0034, EF0041), *Staphylococcus aureus* (EF0035, EF0057, EF0111), *Listeria innocua* (EF0039), *Staphylococcus epidermidis* (EF0061, EF0062), *Listeria monocytogenes* (EF0075), *Streptococcus pneumoniae* (EF0123), and *E. faecalis* (EF0115) (19). The presence of these elements within the pathogenicity island suggests that portions of the island evolved from the accretion of mobile elements primarily from other low G+C Gram-positive organisms. The exact origin of this island, and the means and selection pressures that affected its evolution are the subjects of ongoing studies.

3.1.2. Association with virulence

3.1.2.1. Established virulence factors on island

Within the 129 ORFs detected on the pathogenicity island of *E. faecalis* strain MMH594 are ORFs encoding cytolysin, aggregation substance, and Esp, all of which have been associated with the pathogenesis of enterococcal infection at a number of different stages. In addition to the details discussed above, a variety of recent reviews and chapters regarding the genetics of these factors, their regulation, and their role in disease are available (30,36,102,103), and the reader is directed to these sources for further information.

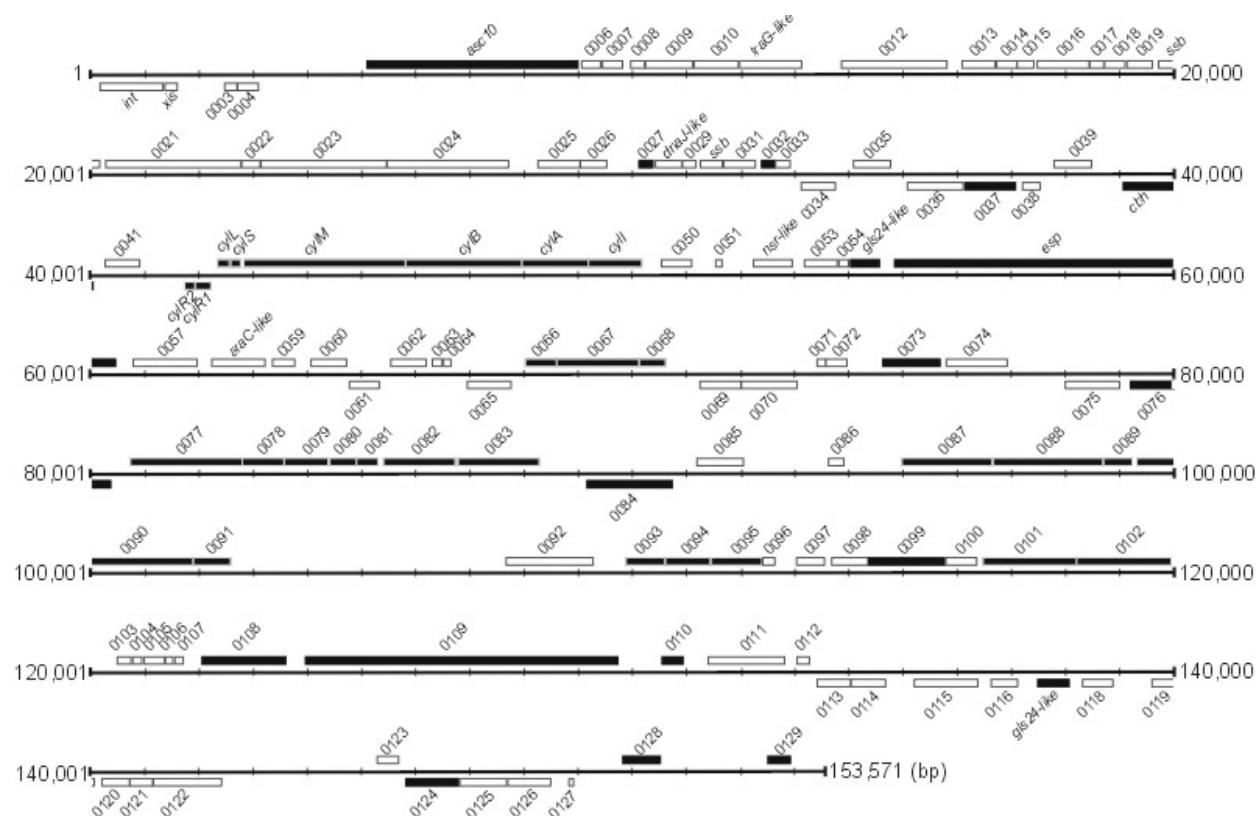


Figure 3. ORFs within pathogenicity island of MMH594. 129 ORFs of pathogenicity island. ORFs of known virulence factors and of genes potentially involved in the pathogenesis of *E. faecalis* are blackened. Adapted with permission from Shankar *et al.* (19) and Nature (<http://www.nature.com>).

3.1.2.2. Additional factors of possible relevance to pathogenesis

In addition to the traits such as Esp, aggregation substance and cytolysin that play well documented roles in the pathogenesis of enterococcal infection, a number of genes within the pathogenicity island of *E. faecalis* may also serve to enhance the ability of enterococci to colonize new niches leading to infection. Such traits include a putative bile acid hydrolase encoded by *cbh*. Enteric bacteria such as *Bacterioides* and lactobacilli commonly express conjugated bile acid hydrolase as one way to defend against the toxicity of bile salts (47), which in addition to their role in the digestion of food are also capable of disrupting bacterial membranes (47,104). While a number of species of enterococci, primarily *E. faecalis* (105), have been found to produce bile acid hydrolases, the contribution of bile acid hydrolases to the pathogenesis of enterococcal infection has not yet been determined. However, *cbh* displays a high degree of similarity to a bile acid hydrolase of *L. monocytogenes*, *bsh* (19). Bsh was found to be unique to pathogenic *Listeria*, was regulated by the global regulator of virulence PrfA, increased the intestinal survival of *L. monocytogenes*, and contributed to lethality in a murine intravenous infection model (47).

Other traits included within the pathogenicity island include a Gls24-like protein, whose homolog Gls24 has been identified as a general stress response protein

whose expression in *E. faecalis* is induced upon nutrient starvation, and exposure to heavy metals and bile salts (39). Strains deficient in Gls24 expression displayed reduced growth rates and variations in cellular morphology when grown under starvation conditions in the presence of bile salts (106). Upon induction, Gls24 was found to result in an increase in the expression of a number of genes, some of which are involved in pyruvate metabolism (106).

The pathogenicity island also contains a number of other genes that may contribute to the survival of *E. faecalis* when nutrients are limited. These ORFs include genes that encode members of phosphotransferase systems (PTS), and various metabolic enzymes used in alternative metabolic pathways including the metabolism of xylose (EF0082, EF0083).

A homolog of KdpD (EF0090) of *L. innocua*, a histidine kinase, and of a neighboring DNA-binding response regulator (EF0091) may be involved in the ability of *E. faecalis* to respond to variations in osmolarity. This two-component regulatory system is known to be involved in the osmoregulation observed in *E. coli* and the sensing of K^+ levels (107). Immediately upstream of the putative regulatory ORFs are three ORFs which encode a potassium ABC transporter (EF0087, EF0088, EF0089), another common element of the osmoregulatory unit characterized in *E. coli* (107).

The pathogenicity island also appears to encode a surface protein, with the closest known homolog being the Dps-family surface protein of *L. rhamnosus* (EF0119), potentially having a role in protection against oxidative stress. A Dps protein on the surface of *Agrobacterium tumefaciens* is involved in the sequestration and reduction of hydroxyl radicals through Fenton chemistry in the extracellular environment, thus protecting the organism from oxidative damage (108). A homolog of PsaA, a protein on the surface of *S. pneumoniae* which has also been suggested to have a role in the defense against oxidative stress (109), can also be found within the *E. faecalis* pathogenicity island (EF0095). PsaA of *S. pneumoniae* is also involved in the adherence of the organism to nasopharyngeal human epithelial cells (110). The involvement of these *E. faecalis* pathogenicity island homologs in adherence or protection against oxidative stress has yet to be assessed.

The island also contains genes that may have a role in repair of damaged cellular components as a result of exposure to harsh environmental conditions. A homolog to DNA-J (EF0028), a chaperone whose primary role in conjunction with DNA-K is the stabilization of proteins under heat stress (42), was identified within the island. In addition, an ORF encoding a putative DNA-damage inducible protein (EF0032) was identified, which may have some role in DNA-damage repair.

There exist a number of ORFs within the pathogenicity island that may also be involved in the establishment of pathogenic *E. faecalis* within the GI tract. The existence of a putative cell-wall associated protein (EF0109) and its function has yet to be investigated. In addition, the aforementioned putative bile acid hydrolase, *cbh* (EF0040), may confer an advantage to colonization in regions of the intestine closer to the bile duct.

As mentioned above, it is possible that pathogenic enterococci must either recognize a new microniche within the GI tract or displace and fill a previously occupied niche. Due to the dense colonization of this site with natural gut flora, remaining niches may be especially inhospitable to colonization. Genes within the pathogenicity island which allow *E. faecalis* to use means of alternative metabolism that may give *E. faecalis* an advantage within nutritionally limited environments of the GI tract are numerous and include the aforementioned phosphotransferase systems and xylose metabolism enzymes, and homologs of an N-acetylmannosamine-6-phosphate epimerase (EF0066) that functions in carbohydrate metabolism (111), a glycosyl hydrolase (EF0077), a ketopantoate reductase involved in coenzyme A metabolism (112), an ornithine cyclodeaminase (EF0124), and a polysaccharide deacetylase (EF0108). As is the case with UPEC, there exist putative ORFs (EF0095, EF0096) that may be involved in providing a means with which to acquire iron and other metals that may be scarce in the intestinal microenvironment.

4. CONCLUSIONS AND PERSPECTIVES

The emergence of enterococci as a leading cause of nosocomial infection has resulted in the recognition of

the pathogenic potential of organisms whose association within the human host primarily was thought to be one of benign commensalism. Through recent evolution, traits enabling enterococci to maintain itself within the harsh environment of the hospital, to establish itself within the patient population, and to act as an etiologic agent of disease within that environment have taken hold among clinical isolates. Although it is clear that the selective pressure applied by antibiotic therapy has resulted in the spread of antibiotic resistance determinants, traits that benefit enterococci within a hospital setting, the selection of virulence traits appears to have occurred independently. Whereas vancomycin resistance is common in *E. faecium* clinical isolates, *E. faecalis*, which rarely exhibits vancomycin resistance, still comprises the majority of enterococcal clinical isolates (12,36). Clinical isolates of *E. faecalis* are enriched for many of the traits that have been shown to affect virulence. Although less is known about virulence factors of *E. faecium*, recent reports have also shown an increase in clinical isolate of *E. faecium* in the presence of determinants suspected to be involved in pathogenesis (including a homolog to *E. faecalis* Esp and a hyaluronidase [113,114]).

Although an understanding of the role of select traits (e.g. cytolysin, Esp, aggregation substance, gelatinase, etc.) that enhance enterococcal virulence has been developed, little is known about the possible contribution to virulence made by many of the genes contained within the pathogenicity island. There are also few clues to its origin. Much remains to be discovered about the relationship between enterococci and man, and the selection forces that drive the appearance of virulence traits within these organisms.

5. ACKNOWLEDGEMENTS

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