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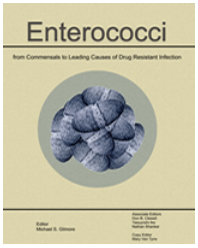

Extrachromosomal and Mobile Elements in Enterococci: Transmission, Maintenance, and Epidemiology

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Introduction

Extrachromosomal elements are ubiquitous in the prokaryotic world and play important roles in the adaptation and survival of cell populations, especially in changing environments. Plasmids are readily found in enterococci, and it is not unusual for clinical and commensal strains (e.g. *Enterococcus faecalis* and *Enterococcus faecium*) to harbor a number of such elements. Indeed, plasmid-free isolates are only infrequently identified. Enterococcal plasmids commonly encode: i) resistance to one or more antibiotics; ii) elevated resistance to ultraviolet light; iii) virulence factors, such as cytolysin and aggregation substance; and iv) bacteriocins. In addition, intercellular transmissibility is frequently a plasmid-determined trait. As in many bacterial species, plasmids generally range in size from 3–4 kb to well over 100 kb and may be present at relatively low copy number (1–2 copies) or up to 20 or more per cell. Table 1 presents a list of enterococcal plasmids recently compiled by one of the authors (Teresa M. Coque).

Conjugation is a primary means for intercellular DNA mobility in enterococci—natural transformation has never been reported, and information is only beginning to be reported with regard to transduction involving a bacteriophage (see Enterococcal bacteriophages and genome defense). Some conjugative plasmids transfer efficiently from donor to recipient in broth, whereas others transfer well only on solid surfaces. In the case of *E. faecalis*, peptide sex pheromones secreted by recipient cells induce conjugation-related mating functions, determined by certain plasmids (*e.g.* pAD1, pCF10, and a host of others). Another group of plasmids, such as pMG1 and related elements identified mainly in *E. faecium*, are also able to transfer efficiently in broth, but do not appear to make use of sex pheromones. A group of plasmids exemplified by pAMβ1 do not transfer well in broth, but are able to move if the cells are on a solid surface. Nonconjugative plasmids are also commonly

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present in enterococci, and some are readily mobilized by conjugative elements *in trans* or move *via* co-integration in some cases. Representatives of some of the above-noted elements have been sequenced, and studies relating to their transfer mechanisms have been published. In addition, reports relating to replication and partitioning provide significant information on the ways in which certain transmissible elements are maintained in their host.

Other types of transmissible elements common in enterococci are the so-called conjugative transposons, which are exemplified by the Tn*916* family. Usually found integrated in the chromosome, their movement involves an excision event that results in a non-replicative circular intermediate that is able to transfer conjugatively, followed by insertion into the genome of a recipient cell. Originally identified in *E. faecalis*, these elements, which commonly encode antibiotic resistance traits, have a broad host range and are widespread among numerous bacterial genera. In a similar vein and as found to be the case for many species of bacteria in recent years, enterococci have been shown to carry a plethora of "genomic islands," some of which are mobile and called "integrative conjugative elements" (ICEs). Some of these represent "pathogenicity islands" that confer significant virulence traits and even antibiotic resistance.

Rapidly accumulating genomic sequencing data are facilitating identification of the enterococcal "mobilome," which includes not only transmissible elements, but also insertion sequences, transposons, and integrons that move intracellularly. Studies based on functionality, including replication and maintenance, complement this rapidly expanding picture, and the significant extent to which enterococci have participated in horizontal transfer within the bacterial world is becoming readily apparent. Below we attempt to summarize recent developments in various aspects of mobile genetic elements (MGEs) in enterococci and try to provide a perspective that is relevant to bacterial-human interaction.

Plasmid Transmission

From a general perspective, the pheromone-responding plasmids and the pMG1-related plasmids, which are usually of sizes greater than 60 kb, are able to transfer at frequencies on the order of 10^{-3} per donor (or higher) within a few hours in broth suspensions (Clewell & Dunny, 2002; Clewell & Francia, 2004); they transfer at much higher frequencies—approaching 100%—on solid surfaces (such as nutrient agar). Thus far, their ability to replicate in a particular host appears limited to the genus *Enterococcus*; although in some cases they are able to transfer to (but do not establish autonomously in) other genera (Francia & Clewell, 2002). pAMβ1, and related elements (commonly 27-50 kb) that do not transfer well in broth, move at frequencies on the order of 10^{-4} or more per donor on solid surfaces. Significantly, these elements often exhibit a broad host range that enables them to move into and establish in a variety of Gram-positive species (Clewell & Francia, 2004; Grohmann, Muth, & Espinosa, 2003).

Sex pheromone systems

E. faecalis was the first bacterial species reported to utilize sex pheromones in the transfer of plasmid DNA—well over 30 years ago—when it was found that recipient bacteria secreted short peptides that were able to induce a mating response by donors that carried certain conjugative plasmids (Dunny, Brown, & Clewell, 1978; Dunny, Craig, Carron, & Clewell, 1979) (*E. faecalis* was designated *Streptococcus faecalis* at that time). Studies showed that such plasmids encode a response to a specific peptide, which leads to expression of a number of mating functions, including a protein "aggregation substance" (AS) that appears on the cell surface. AS facilitates mating-pair formation in liquid cultures, a phenomenon that visibly manifests as a "clumping together" of donors and recipients. AS binds to lipoteichoic acid, which is present on both recipients and donors (Ehrenfeld, Kessler, & Clewell, 1986). Exposure of donors to a culture filtrate of recipients for a few hours results in a self-clumping response facilitated by the synthesized AS; indeed, transfer of plasmid DNA even occurs between donors, although at a significantly reduced rate, as compared to transfer that involves plasmid-free recipients

(Clewell & Brown, 1980). The clumping response proved to be useful in quantifying pheromone activity using a microtiter dilution assay (Dunny, Craig, Carron, & Clewell, 1979). AS was found not to be necessary for transfer if matings were conducted on solid surfaces; thus, its primary role in mating would appear to be the initiation of donor-recipient contact upon random collision of cells in liquid suspensions. AS was later found to also be a virulence factor in various pathogenicity models (see Enterococcal Disease, Epidemiology, and Implications for Treatment and Pathogenesis and models of enterococcal infection).

Plasmid-free strains of *E. faecalis* were found to secrete a number of different pheromones, each specific to a different family of conjugative plasmids. Some of the best-studied plasmid representatives are pAD1, pCF10, pPD1, and pAM373, with corresponding sex pheromones referred to as cAD1, cCF10, cPD1, and cAM373, respectively. Such plasmids are common among clinical isolates, and as many as three different pheromone-responding plasmids have been identified in a single isolate (Clewell, Yagi, Ike, Craig, Brown, & An, 1982; Murray, An, & Clewell, 1988). When a given recipient acquires a copy of a pheromone-responding plasmid, the related pheromone is no longer detectable in culture supernatants; however, the transconjugants (now donors) secrete a plasmid-encoded peptide that acts as a competitive inhibitor of the pheromone. Inhibitors encoded by pAD1, pCF10, pPD1, and pAM373 are referred to as iAD1, iCF10, iPD1, and iAM373, respectively. These pheromones and inhibitors have all been characterized and represent hydrophobic, linear, octa- or hepta-peptides, and synthetic forms of the peptides are active at sub-micromolar concentrations (Clewell & Dunny, 2002). Figure 1 shows their amino acid sequences.

The pheromones derive from a segment of the signal sequences of lipoprotein precursors (Clewell, An, Flannagan, Antiporta, & Dunny, 2000) and result from processing by a lipoprotein signal peptidase and a metallopeptidase that is referred to as Eep (An, Sluvaik, & Clewell, 1999). The latter is a member of a large family of intramembrane proteases known as RIP (regulated intramembrane proteolysis), which are found in both prokaryotic and eukaryotic organisms (Brown, Ye, Rawson, & Goldstein, 2000). As for the plasmid-encoded inhibitors, the precursors correspond to a pre-peptide that resembles an unattached signal sequence, with only Eep being required for processing. There is an interesting exception with regard to processing in that the precursors of cAM373 and iAM373 appear to use a mechanism that does not require Eep; and an alternate Eep-like protein that might serve such a function has not been detected in *E. faecalis* genomes, based on amino acid homology with Eep. Chandler and Dunny (Chandler & Dunny, 2008) have reported evidence that the amino acid residues that precede the cAM373 moiety are likely responsible for the insensitivity of the cAM373 precursor to Eep. This finding was based on the construction of a hybrid pre-peptide that contains cCF10 and the upstream region of the cAM373 precursor; while Eep normally processes the precursor of cCF10, it was not active on the hybrid.

There is no current evidence that the lipoprotein moiety of the various pheromone precursors plays a role in the conjugation or related regulatory processes. In the cases of cAD1 and cAM373, mutations affecting only the lipoprotein component do not affect secretion of the pheromone; and these derivatives behave normally as recipients and donors (An & Clewell, 2002; Flannagan & Clewell, 2002). It is noteworthy, however, that a deletion of the *eep* determinant has been observed by Frank *et al.* (Frank, Barnes, Grindle, Manias, Schlievert, & Dunny, 2012) to severely impair pathogenicity of *E. faecalis* in a rabbit endocarditis model in a manner unrelated to plasmid content. Thus, Eep might be involved in the processing necessary for expression of one or more lipoproteins, if not for precursor-linked peptides that contribute to virulence.

Secretion of pheromones is reduced by a plasmid-encoded membrane protein, which is designated TraB in the case of pAD1 or homologue PrgY in the case of pCF10. Mutations in these related determinants result in increased secretion (An & Clewell, 1994; Chandler & Dunny, 2008; Ike & Clewell, 1984; Weaver & Clewell, 1990); TraB/PrgY, as well as Eep, are possibly associated with a general secretion complex of the host [see Rosch & Caparon, 2004]. Recent studies by Chandler and Dunny (Chandler & Dunny, 2008) have provided some insights into the activity of PrgY, as well as its interaction with cCF10. Interestingly, and perhaps related to the

fact that pre-cAM373 does not get processed by Eep, pAM373 does not encode a TraB/PrgY homologue (De Boever, Clewell, & Fraser, 2000).

With regard to the uptake of pheromones, a specific plasmid-encoded surface-bound sensor/receptor protein (TraC encoded by pAD1 and PrgZ encoded by pCF10) specifically binds and "passes" the peptide to a host ABC oligopeptide transporter complex for internalization (Leonard, Podbielski, Hedberg, & Dunny, 1996; Tanimoto, An, & Clewell, 1993). The TraC/PrgZ protein appears to discriminate between the pheromone and the cognate inhibitor peptides, which results in a competitive inhibition that allows selective passage of the pheromone to the more generalized peptide uptake system (Leonard, Podbielski, Hedberg, & Dunny, 1996). However, there is evidence in the pCF10 system that iCF10 may enter the cell and bind competitively with cCF10 to the negatively regulating PrgX protein, which binds to a key promoter involved in regulation of the pheromone response (see below). For a more detailed and illustrative discussions of pheromone biosynthesis, secretion, and uptake, see the references (Chandler & Dunny, 2008; Clewell, 2007).

cAM373 activity produced by different bacterial genera

It has been known for a number of years that sex pheromone cAM373 activity is also produced by the human pathogen *Staphylococcus aureus* and oral commensal *Streptococcus gordonii*, a component of dental plaque (Clewell, An, White, & Gawron-Burke, 1985). That is, culture filtrates of these species induce clumping by *E. faecalis* strains that specifically harbor pAM373. The related peptides are not identical to cAM373 but are similar (Fig. 1); and similar to the case in enterococci, the related precursors correspond to hydrophobic segments of signal sequences of pre-lipoproteins. The corresponding lipoproteins, however, are very different, and while there is no direct evidence that the peptides serve as pheromones within these non-enterococcal species, it has been shown that they are able to induce intergeneric plasmid transfer from *E. faecalis* (Francia & Clewell, 2002; Vickerman, Flannagan, Jesionowski, Brossard, Clewell, & Sedgley, 2010). While pAM373 is unable to replicate in *S. aureus* or *S. gordonii*, a vector that carries the origin of transfer from pAM373 can be readily mobilized by pAM373 and established as a replicating plasmid in a heterologous host.

Genetic analyses conducted in *S. gordonii* showed that an Eep homologue and a signal peptidase II used in processing of lipoprotein precursors are necessary for the production of detectable gordonii-cAM373 (Vickerman, Flannagan, Jesionowski, Brossard, Clewell, & Sedgley, 2010). As noted above, and contrary to the case in *S. gordonii*, the Eep identified in *E. faecalis* is not necessary for processing the enterococcal cAM373 precursor; this probably relates to differences in the amino acid residues upstream in the two precursors (Chandler & Dunny, 2008).

A potentially significant factor in the production of cAM373 activity by non-enterococcal pathogens, such as *S. aureus,* is the possibility of its involvement in the ever-feared acquisition of vancomycin-resistance, which is now frequently carried by *E. faecalis*. In this regard, at least one plasmid, pAM368, which carries a vancomycin-resistance determinant and encodes a cAM373-response, has been identified in an enterococcal isolate (Showsh, De Boever, & Clewell, 2001) and could be "poised" for transfer in a clinical setting. In a similar vein, *S. aureus* strains that harbor plasmids of the pSK41/pGO1 family secrete a cAD1 activity (in addition to cAM373) that is encoded as part of the signal sequence of the plasmid-encoded lipoprotein TraH precursor (Firth, Fink, Johnson, & Skurray, 1994; Firth, Fink, Johnson, & Skurray, 1994), which could facilitate the uptake of vancomycin-resistance determinants from *E. faecalis* through mobilization or transfer by a pAD1-like element (Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000). While there has been evidence of a limited number of clinical cases of *S. aureus* acquiring high-level vancomycin resistance (VanA) from enterococci in recent years, a direct involvement of pheromone-responding plasmids has not yet been demonstrated (Flannagan, et al., 2003; Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008).

It is noteworthy that the pheromone-responding plasmid pCF10 has been reported to mobilize a small vector plasmid that carries an inserted pCF10-*oriT* site into two different genera, *Lactococcus lactis* and *Streptococcus*

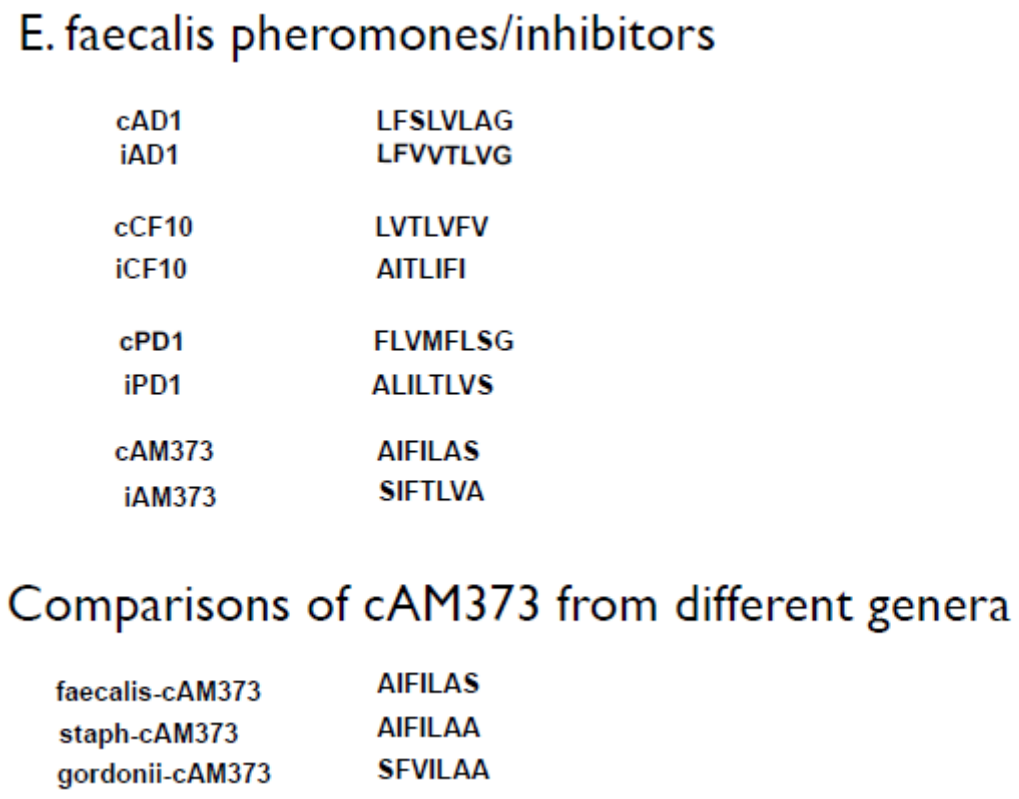

Figure 1. The structure of sex pheromones and cognate inhibitor peptides secreted by *Enterococcus faecalis*, as well as a comparison of the *E. faecalis* cAM373 sequence to those with similar activities secreted by *S. aureus* and *Streptococcus gordonii*.

agalactiae, when matings were conducted on nutrient agar plates (Staddon J. H., Bryan, Manias, Chen, & Dunny, 2006). Since the related recipients are not known to produce cCF10 activity**,** the extent to which the conjugation system was induced is not clear. The fact that pCF10 itself did not establish in these hosts is consistent with the view that while transfer of pheromone-responding plasmids to a variety of other Gram-positive organisms may readily occur, their ability to replicate appears to be limited to enterococci.

A unique regulatory theme involved in the pheromone response

There is a basic similarity in the organization of pheromone-responding plasmids, in that determinants involved in replication and partitioning are located adjacent to a pheromone-response sensing and regulatory region that is, in turn, upstream of an extensive set of structural genes that manifest the overall mating process (see Figure 2). The latter stretch of conjugation genes can involve up to ~30 kb of DNA. Regulation involves both negatively and positively acting features, and structural genes that encode significant surface proteins, such as AS and the product associated with entry/surface exclusion (such as those involved in reduction of uptake of plasmid DNA from other donors), are usually among determinants that are close to the regulatory center. Based on hybridization analyses and sequence comparisons, significant homology exists between most pheromone-responding plasmids, particularly with regard to AS and the determinant for surface exclusion (Clewell & Francia, 2004; Hirt, Wirth, & Muscholl, 1996). An exception is pAM373, which encodes a significantly different AS and no surface exclusion determinant (De Boever, Clewell, & Fraser, 2000). A transfer origin (*oriT*) is generally located well downstream and close to a relaxase determinant. The plasmids pAD1 and pCF10 have been the most heavily studied over the years and have been comprehensively reviewed (Clewell, 2007; Clewell & Dunny, 2002; Dunny G. M., 2007; Dunny & Johnson, 2011). Based on analyses of these systems as well as significant studies of pPD1 (Folli, et al., 2008; Fujimoto, Tomita, Wakamatsu, Tanimoto, & Ike, 1995; Horii,

Nagasawa, & Nakayama, 2002) and more limited analyses of pAM373 (De Boever, Clewell, & Fraser, 2000; Ozawa, De Boever, & Clewell, 2005), a consistent and somewhat unique regulatory theme has become evident.

As illustrated in Fig. 2, a key control feature relates to the expression and interplay (counter transcription) from two opposing promoters P_i and P_n, which ultimately controls transcription termination at a factor independent terminator, t. Because different systems utilize different terminology, the model here is presented in generic terms. P_i governs transcription of the inhibitor peptide precursor determinant, designated as *inh* (*iad* for pAD1 and *icf/prgQ* for pCF10). In the uninduced state, transcription from P_i occurs at a basal level that facilitates production of appropriate amounts of the inhibitor peptide (Inh) with termination occurring at t. The other promoter P_n is located downstream of *inh*, about 150–450 nt from the opposing P_i, depending on the particular plasmid. This promoter relates to the expression of a key regulatory protein Neg (TraA for pAD1, pPD1 and pAM373, and PrgX for pCF10), whose determinant *neg* is located upstream of, and in the opposite orientation to, *inh*. Neg negatively regulates transcription from P_i through its interaction with at least two binding sites. In the presence of a pheromone, however, which binds to the C-terminal region of Neg (Fujimoto & Clewell, 1998; Shi, et al., 2005), the significant up-regulation of transcription from P_i occurs. In at least one system (pAM373), there is evidence that Neg remains associated with the transcription complex during induction (Ozawa, De Boever, & Clewell, 2005). In the uninduced state, transcription from P_n occurs at a high level; however, only a fraction of the transcription extends all the way through *neg*. A significant degree of termination of this transcript occurs relatively early and is apparently related to an as yet unrecognized terminator, which gives rise to a short (102 nt) RNA designated Anti-Inh that can assume an extensive secondary (cloverleaf) structure (Shokeen, Johnson, Greenfield, Manias, Dunny, & Weaver, 2010). An important function of Anti-Inh is its ability to enhance termination of the P_i transcript at t (Bae, Kozlowicz, & Dunny, 2004; Johnson, et al., 2010; Tomita & Clewell, 2000) through regional complementarity and consequent conformational alteration of the *inh* transcript.

During pheromone induction, transcription from P_n becomes greatly reduced, while up-regulated transcription from P_i extends beyond t, which ultimately results in the expression of the conjugation system. An important aspect of regulation concerns the need to make termination at t very "tight" when cells are not exposed to exogenous pheromone. This is because once transcription goes beyond t, it not only facilitates expression of down-stream conjugation functions, but it also results in significant up-regulation. For example, in the case of pAD1, the first determinant beyond t encodes a positively acting regulator, TraE1, that up-regulates itself, as well as certain down-stream genes. While Anti-inh contributes to termination at t, there are suggestions that Neg can also bind near t in some systems, which probably further enhances termination in the absence of pheromone (Clewell, 2007; Folli, et al., 2008). Some plasmids (*e.g.* pCF10 and pAD1) even have a second terminator (t2) closely behind t that probably helps decrease transcriptional leakage in the uninduced state.

Adding to the complexity of the overall process, there is also evidence that in the uninduced state, the P_i transcript interacts with the P_n transcript in such a way as to facilitate cleavage by RNAse III (cleaves double stranded RNA), which possibly facilitates some processing, as well as subsequent degradation (Johnson, Haemig, Chatterjee, Wei-Shou, Weaver, & Dunny, 2011). Finally, mathematical analyses of the pCF10 system has supported the view that transcriptional interference between RNA polymerase molecules that initiate at P_i and P_n is also a component of regulation (Chatterjee, et al., 2011).

With regard to the above regulatory pattern, it is important to note a report by Ibrahim *et al.* (Ibrahim, Nicolas, Bessières, Bolotin, Monnet, & Gardan, 2007) who, in a search of the data base for short, non-annotated ORFs within numerous bacterial genomes, were able to identify a new family of coding sequences for hydrophobic peptides (20-23 amino acids) located just upstream of determinants for positive transcriptional regulators of the Rgg family. Rgg was first identified as a regulator of glycosyl transferase synthesis in *S. gordonii* (Sulavik & Clewell, 1996; Sulavik, Tardif, & Clewell, 1992), but homologues have been subsequently identified in numerous low G+C Gram-positive species, and include proteins involved in the regulation of virulence and biofilm

formation in *Streptococcocus pyogenes* (Chang, LaSarre, Jimenez, Aggarwal, & Federle, 2011; Declerck, et al., 2007; Lyon, Gibson, & Caparon, 1998; Rocha-Estrada, Aceves-Diez, Guarneros, & de la Torre, 2010). Rgg relates to what now corresponds to a superfamily of cytoplasmic regulatory proteins that directly bind to peptide ligands (Chang, LaSarre, Jimenez, Aggarwal, & Federle, 2011; Mashburn-Warren, Morrison, & Federle, 2010), many of which relate to signaling cell density (namely, quorum sensing). Additionally, Neg proteins (e.g. PrgX and TraA) are included in this group. Members of this family do not necessarily exhibit significant amino acid homology; however, secondary and tertiary structure prediction algorithms reveal a high degree of similarity [see Chang, LaSarre, Jimenez, Aggarwal, & Federle, 2011]. Notably, high-resolution structures determined for the pCF10 Neg protein PrgX as an apo-protein, and in complex with its cognate pheromone and inhibitor peptides, represented the first successful structure determinations for any receptor for a bacterial intercellular-signaling peptide (Kozlowicz, Dworkin, & Dunny, 2006; Shi, et al., 2005). Thus, the Neg-Inh peptide aspect of the pheromone response expression system described above bears a resemblance to what may be a widespread pattern of intercellular peptide communication control in Gram-positive bacteria.

Non-pheromone-responding plasmids that transfer efficiently in broth. The pMG1 family.

In 1998, Ike *et al.* (Ike, Tanimoto, Tomita, Takeuchi, & Fujimoto, 1998) reported the identification of a 65 kb plasmid, pMG1, that confers resistance to gentamicin (a Tn*4001*-like transposon) in *E. faecium* that would transfer relatively well to other *E. faecium* strains in broth, as well as to *E. faecalis* and *E. hirae*. Transfer frequencies were on the order of 10^{-4} per donor in 3–4 hour matings. Studies showed that a sex pheromone was not involved.

Donor-recipient aggregates were not generally visible to the naked eye, in contrast to systems that involved a pheromone response, although microscopic examination revealed the formation of aggregates (Ike, Tanimoto, Tomita, Takeuchi, & Fujimoto, 1998). In addition, unlike the case for pheromone-responding plasmids like pPD1 and pAD1, the aggregates were not dispersed upon EDTA exposure. It was then found that closely related plasmids, including those that carry high-level vancomycin-resistance (VanA) determinants associated with Tn*1546*–like elements, were common among antibiotic-resistant strains of *E. faecium* and *E. avium* (Tomita, Pierson, Lim, Clewell, & Ike, 2002; Tomita, et al., 2003). These plasmids exhibited incompatibility (namely, an inability to co-exist), and members of the group now constitute the pMG1 family. DNA hybridization studies showed no detectable homology with pheromone-responding plasmids or the broad host-range pAMβ1 and pIP501 plasmids (Ike, Tanimoto, Tomita, Takeuchi, & Fujimoto, 1998). It would appear that pMG1 family elements have contributed significantly to the spread of vancomycin- and gentamicin-resistance among enterococci, particularly within *E. faecium*. However, while they exhibit transfer between various species of enterococci, the extent to which they might transfer to different genera is not yet known.

pMG1 and the related pHTβ carrying a vancomycin-resistance transposon (Tn*1546*-like) have been sequenced and found to closely resemble each other (Tanimoto & Ike, 2008; Tomita & Ike, 2005). Conjugation-related determinants identified by BLAST analysis appear to span well over 30 kb, and there are similarities at the amino acid level to proteins encoded by pXO2 (10-50% identity with regard to 22 different proteins), a virulence-related plasmid carrying capsule determinants in *Bacillus anthracis*. In the *E. faecalis* host FA2-2, the presence of pHTβ is associated with a self-aggregation of cells, which are visible when allowed to grow overnight in broth. Genetic analyses suggested that this is related to the aggregation that occurs between donors and recipients during mating. The phenomenon was narrowed down to a region that spans 6 kb (Tomita & Ike, 2005), which contained 5 ORFs (an operon) with one (ORF10) being relatively large (1209 amino acids) and possibly encoding an "aggregation substance." There was no similarity of this protein to the AS of pAD1, pCF10, pPD1, *etc.*; however, it has exhibited amino acid homology with a surface protein encoded by pXO2. Analyses revealed additional regions that are important in the mating process, as well as a key positive regulator, *traB*. Based on BLAST

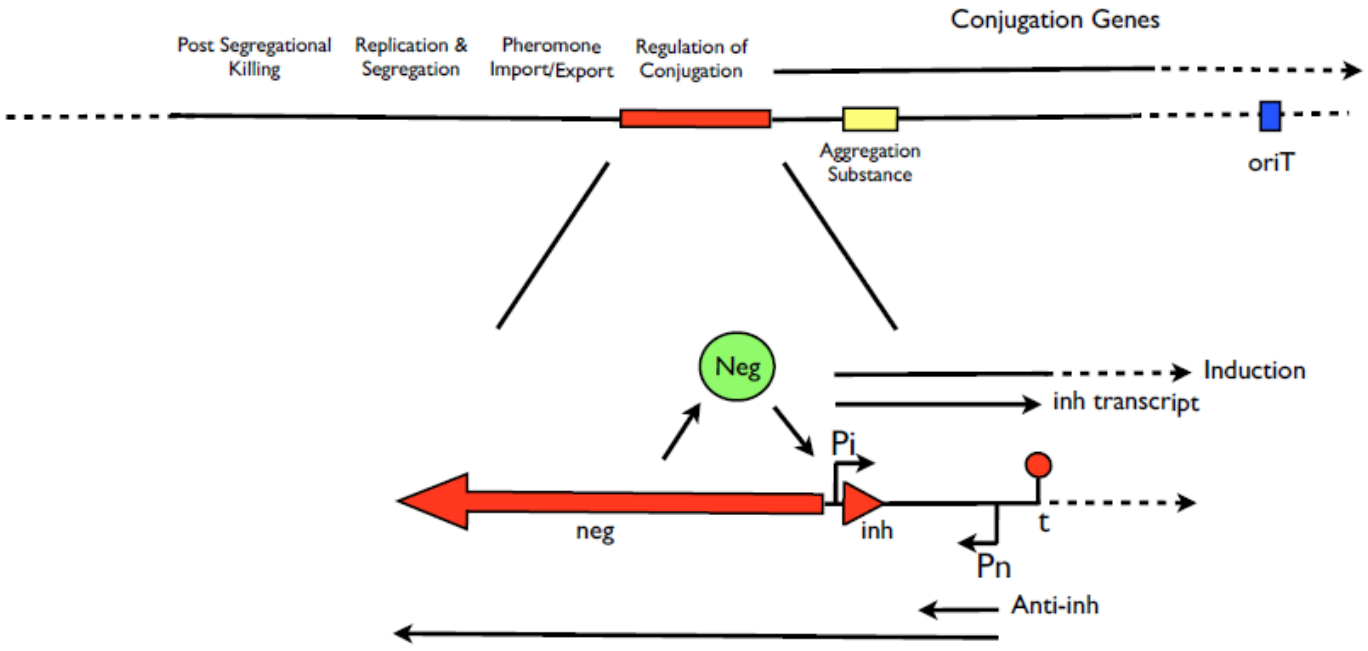

Figure 2. A generic model that focuses on key aspects of regulation of the plasmid-encoded sex pheromone response. The primary promoters are P_i and P_n, which govern expression of oppositely oriented transcripts. Neg (which corresponds to TraA or PrgX, depending on the particular plasmid system) acts on P_i to influence expression of *inh*, whose product corresponds to the inhibitor peptide. In the uninduced state, P_i expresses at a basal level, which allows the expression of Inh. Exposure of cells to sex pheromone results in the internalized peptide binding to Neg and up-regulating expression from P_i. This results in expression beyond the transcription terminator t and into the structural genes related to conjugation. P_n transcription is elevated in the absence of sex pheromone. It gives rise to a short transcript that enhances termination of the P_i transcript at t and a lower amount of an extended product that serves as the *neg* transcript. During induction, transcription from P_n ceases.

analyses, there was evidence for the presence of a coupling protein (namely, a protein utilized in DNA transfer), a VirB11-family sec protein, a transfer origin, and a relaxase (Tomita & Ike, 2005).

Plasmids that transfer well on solid surfaces but not in broth (the Inc18 elements)

Broad host range conjugative enterococcal plasmids, which are transferable mainly on solid surfaces and not responsive to pheromones, and are typically between 26 and 50 kb, are usually members of what has been referred to as the Inc18 family, based on their incompatibility with each other (Horaud, Le Bouguenec, & Pepper, 1985; Jannière, Gruss, & Ehrlich, 1993) (see Table 1). Homologs can be found in a wide variety of bacterial genera [see Clewell & Francia, 2004; Grohmann, Muth, & Espinosa, 2003]. In enterococci, they typically encode resistance to Em (MLS), and often encode resistance to additional antibiotics. Representative enterococcal plasmids of this class are the *E. faecalis* plasmids pAMβ1 (28 kb) and pRE25 (50 kb), and related conjugation determinants closely resemble those of the *S. agalactiae* plasmid pIP501 (30 kb) (Grohmann, Muth, & Espinosa, 2003; Horodniceanu, Bouanchaud, Bieth, & Chabbert, 1976; Schwarz, PerretenV., & Teuber, 2001), whose transfer functions have been analyzed in greater detail. The host-range of the latter includes numerous Gram-positive genera, and appears to extend to the multicellular *Streptomyces lividans,* as well as the Gram-negative *Escherichia coli,* where both replication and conjugation genes appear functional (Kurenbach, Bohn, Prabhu,

Abudukerim, Szewzyk, & Grohmann, 2003). Sequence comparisons show that there is significant similarity with the tra regions of the *S. aureus* plasmids pGO1 and pSK41, as well as the *Lactococcus lactis* plasmid, pMRC01 (Grohmann, Muth, & Espinosa, 2003).

pAMβ1 and pIP501 have been observed to transfer in the environment as well as in animal intestines (Byzov, et al., 1999; Igimi, Ryu, Park, Sasaki, Sasaki, & Kumagai, 1996; McConnell, Mercer, & Tannock, 1991; Morelli, Sarra, & Bottazzi, 1988). In mixed infections in humans, closely related plasmids appear to have been involved in the delivery of a Tn*1546*-like vancomycin-resistance transposon from *E. faecalis* to *S. aureus* (Flannagan, et al., 2003; Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008; Zhu, et al., 2010). It is noteworthy that the above-mentioned pRE25, which was originally identified in raw-fermented sausage, confers resistance to 12 different antibiotics (Schwarz, PerretenV., & Teuber, 2001; Teuber, Schwarz, & Perreten, 2003)—which supports the view that Inc18 elements are likely involved in human acquisition of antibiotic-resistant bacteria from many sources.

Origins of transfer, relaxases and mobilization

As previously mentioned, conjugative plasmids generally have a specific origin of transfer (*oriT*) that corresponds to a site at which a plasmid-encoded relaxase binds, with the potential to introduce a strand-specific nick that initiates, and also terminates, the process of intercellular DNA transfer. A complex that consists of relaxase and accessory proteins associated with *oriT* is referred to as the relaxosome. In the case of enterococci, examples of plasmids where *oriT*s and relaxases have been identified include: i) pheromone-responding plasmids pAD1, pAM373 and pCF10; ii) a member of the pMG1 family; iii) the Inc18-type pIP501; and iv) the mobilizable plasmid pAMα1. A number of pheromone-responding plasmids are able to mobilize otherwise non-conjugative elements (Clewell, Yagi, Ike, Craig, Brown, & An, 1982; Murray, An, & Clewell, 1988; Oliver, Brown, & Clewell, 1977) as well as various cryptic elements (404), and there is strong evidence for the mobilization of non-conjugative elements facilitated by pAMβ1, pIP501, and related plasmids (Burdett, 1980). In addition, it is likely that the relaxase of certain plasmids might act on *oriT* sites that happen to be located on the bacterial chromosome (see the subsequent section on conjugative transposons and integrative conjugative elements). Interestingly, and for reasons not yet known, the pheromone responding plasmid pAD1 strongly inhibits the transfer of pAMβ1 if both are present in the same host (Clewell, Yagi, Ike, Craig, Brown, & An, 1982).

In the case of pAD1, there are two transfer origins, *oriT1* and *oriT2*; the latter of which is utilized about 1000-fold more efficiently (Francia & Clewell, 2002; Francia, et al., 2001). The *oriT1* site is located within the *repA* determinant (which is required for replication; see below) and exhibits strong similarity to the IncP-type of *oriT*s (8). The *oriT2* site closely resembles the single known *oriT* site of pAM373, both of which are located within a ~285 bp segment upstream and are relatively close to a relaxase determinant (designated *traX* for pAD1). When a fragment of DNA that contains the *oriT2* segment of pAD1 is cloned in an otherwise non-mobilizable vector, the chimera can be readily mobilized by the pAD1, but not by the pAM373 system, and vice versa. These *oriT* loci are unique compared to those of other characterized transfer systems. The *oriT nic* sites are located within large inverted repeat structures spanning about 140 bp, and specificity is based primarily on a series of adjacent 5–6 bp direct repeats that are separated by similarly-sized spacer sequences (Figure 3). The inverted repeats of pAD1 and pAM373 exhibit strong homology; however, the adjacent direct repeats are non-homologous, and there is strong evidence that they are involved in the binding of the specific relaxases (Francia & Clewell, 2002). The relaxases differ significantly from others that have been characterized, in that they are lacking the relatively common "3-histidine motif" (Francia, Varsaki, Garcillán-Barcia, Latorre, Drainas, & de la Cruz, 2004; Garcillán-Barcia, Francia, & de la Cruz, 2009). However, highly related pAD1/pAM373 relaxase-like sequences are readily found in databases that contain recently reported enterococcal genomes.

The pheromone-responding pCF10 bears *oriT* and relaxase determinants that are similar to those of a large superfamily of conjugation systems, which includes the staphylococcal pC221 and pC223 (53) and is completely different from those present in pAD1 and pAM373. The relaxase (PcfG) contains the more typical 3-histidine

domain, and studies by Chen *et al.* (53) showed that the interaction of PcfG with *oriT* includes an accessory protein PcfF. Analysis at the protein and DNA levels of the specificity determinants for recognition of *oriT* by the PcfG relaxase and PcfF accessory protein allowed for a detailed model for coordinated DNA binding, unwinding, and site-specific nicking within the pCF10 *oriT* region. The DNA sequence that includes *oriT* (Fig. 3) and *pcfG* is very similar to that of the *Lactococcus lactis* plasmid pRS01. The relaxase determinant of pRS01 contains a group II intron within a segment identical to a region of *pcfG*, and studies showed that the intron could insert in the related pCF10 site through a homing mechanism (339). The data suggest a mechanism whereby group II introns may spread horizontally. A group II intron also has been identified on the pMG1-like plasmid pHTγ, although not within a relaxase determinant (365).

The *oriT* sites of the non–pheromone-responsive pMG1 and the closely related pHTβ are located in a noncoding region that is close, but not immediately adjacent, to a relaxase determinant (351, 362). In this case, the relaxase contains the three domains typical of many relaxases, including the three-histidine motif (135). The *oriT* region contains no significant similarity to those of other known classes of plasmids. It contains three direct repeats and two inverted repeats, one of which probably contains the *nic* site.

The pIP501 conjugation system uses an *oriT* site that resembles that of IncQ plasmids, and a *nic* site that is identical to that of RSF1010. Genetic studies on conjugation were initially conducted by Macrina and coworkers (197, 372), who identified the *oriT* site and showed that it could be specifically nicked by the product of the adjacent relaxase determinant *traA*. Grohmann and colleagues (199, 200) subsequently analyzed the system in some detail using an *E. faecalis* host background. They showed that *traA* is the first of 15 open reading frames that constituted an operon that was expressed at a similar level during various stages of growth (200). The relaxase was found to negatively regulate the operon, and the *oriT* nicking target overlapped the promoter of the operon (200). The relaxase, TraA, contains characteristic motifs and was shown to exist as a dimer in solution. With a full length of 654 amino acids, a 246-amino acid truncated (N-terminal) version, which also exhibits dimer formation, maintained nicking activity on DNA that contained the specific *oriT*. pIP501 and pAMβ1 have highly homologous relaxases (96% identity) and only slightly different *oriT* sequences (Figure 4). (See below for comments on the identification and association of the pIP501 conjugation with a Type IV secretion system.)

The interesting case of pAMα1 and the role of relaxase

E. faecalis strain DS5 harbors a nonconjugative multicopy plasmid pAMα1 (9.75 kb) that encodes resistance to tetracycline. It was originally of interest because of the generation of multiple tandem copies of its resistance determinant *tet*(L), when cells were grown protractedly in the presence of tetracycline (Clewell, Yagi, & Bauer, 1975; Yagi, Clewell, & Bauer, 1976). The phenomenon was shown to involve recombination between two directly repeated homologous, but not identical, ~380 bp recombination sequences, RS1 and RS2, which flanked *tet*(L) (Yagi & Clewell, 1977). It was subsequently reported that pAMα1 corresponded to a structure that resembled a cointegrate of plasmids similar to pBC16 (from *Bacillus cereus*) and pS86 (from *E. faecalis*), and that the two RS sequences probably arose as a result of a site-specific recombination between homologous sites on the original plasmids (Perkins & Youngman, 1983). The pBC16-like element, referred to as pAMα1Δ1, carries the *tet*(L) determinant and appears to be unable to replicate alone in the *E. faecalis* host. The pS86-like element was dubbed pAMα1Δ2. Amplification in pAMα1-containing cells during growth in the presence of tetracycline corresponds to the generation of tandem repeats of the pBC16-like component, separated by RS sequences. The RS sequences were eventually found to represent *oriT* sites. pAMα1 encodes two relaxases (MobB and MobE) that are encoded by the two components of the cointegrate (Francia & Clewell, 2002). Each is able to facilitate mobilization through its interaction with one or both *oriT* sites, when present with a conjugative plasmid, such as pAD1. Both MobB and MobE are members of the pMV158 family of relaxases, and both RS1 and RS2 contain regions that resemble the pMV158 *oriT* sites (Fig. 4).

The pAMα1 amplification was shown to begin with a site-specific recombination between RS1 and RS2 that is catalyzed by either MobB or MobE (Francia & Clewell, 2002). However, after the initial generation of two copies

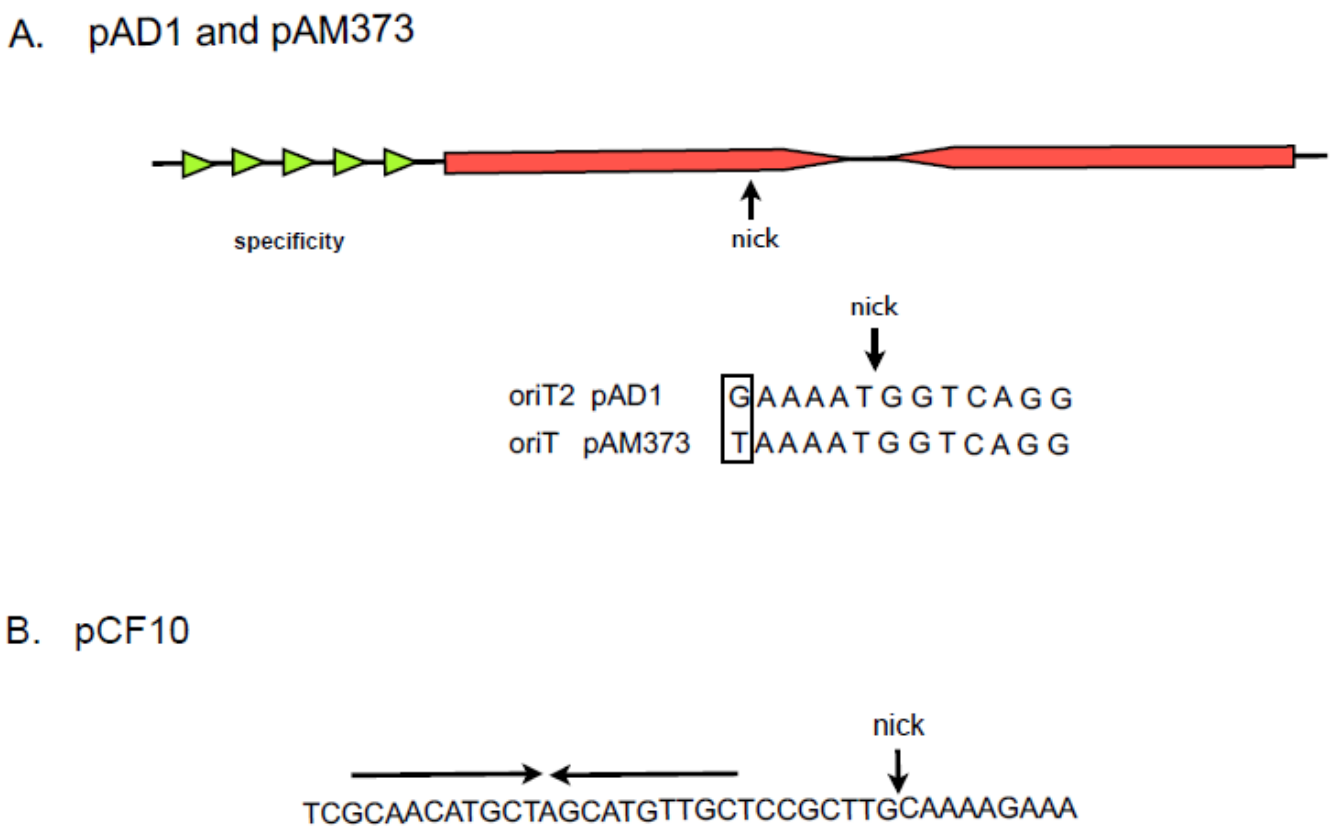

Figure 3. The *oriT* sites of three sex pheromone plasmids with the corresponding nick sites indicated by the arrows. A. The red indicates a large inverted repeat structure that is similar for both pAD1 and pAM373. The green represents short direct repeats that differ for pAD1 and pAM373 and relate to the specificity of binding of the corresponding relaxases. B. The *oriT* for pCF10 is very different from pAD1 and pAM373, as indicated by the nick site located outside of a much shorter and very different inverted repeat.

of the *tet*(L)-containing segment, additional amplification is accomplished by homologous (RecA-dependent) recombination between the larger redundant DNA segments (Yagi & Clewell, 1980). Site-specific recombination activity of relaxases has been previously reported (Broome-Smith, 1980; Llosa, Bolland, Grandoso, & de la Cruz, 1994); the TraX relaxase of pAD1 was found to exhibit such an activity (Francia & Clewell, 2002).

The above system illuminates multiple functions that a site-specific recombination system, which involves a relaxase in this case, can serve: 1) Initiation of plasmid transfer; 2) Facilitation of plasmid cointegration, which could subsequently enable transfer and establishment of a component plasmid that is otherwise not able to replicate in a particular host; and 3) Initiating the amplification of tandem copies of a gene.

On the presence of Type IV secretion systems

The structures involved in DNA transfer constitute a divergent Type IV secretion system (T4SS). A key feature of T4SS is the intercellular transfer of a protein substrate (a DNA-protein in the case of conjugation). What is known about Type IV-mediated DNA transfer comes primarily from studies of plasmids in Gram-negative bacteria (Cascales & Christie, 2003; Zechner, et al., 2000), but it has become evident in the past ten years that Gram-positive systems, including enterococci, make use of analogous systems. This was first recognized based on comparative analysis of DNA sequence data (Grohmann, Muth, & Espinosa, 2003), whereas an understanding of protein organization and function for the Gram-positive systems has emerged more slowly. A

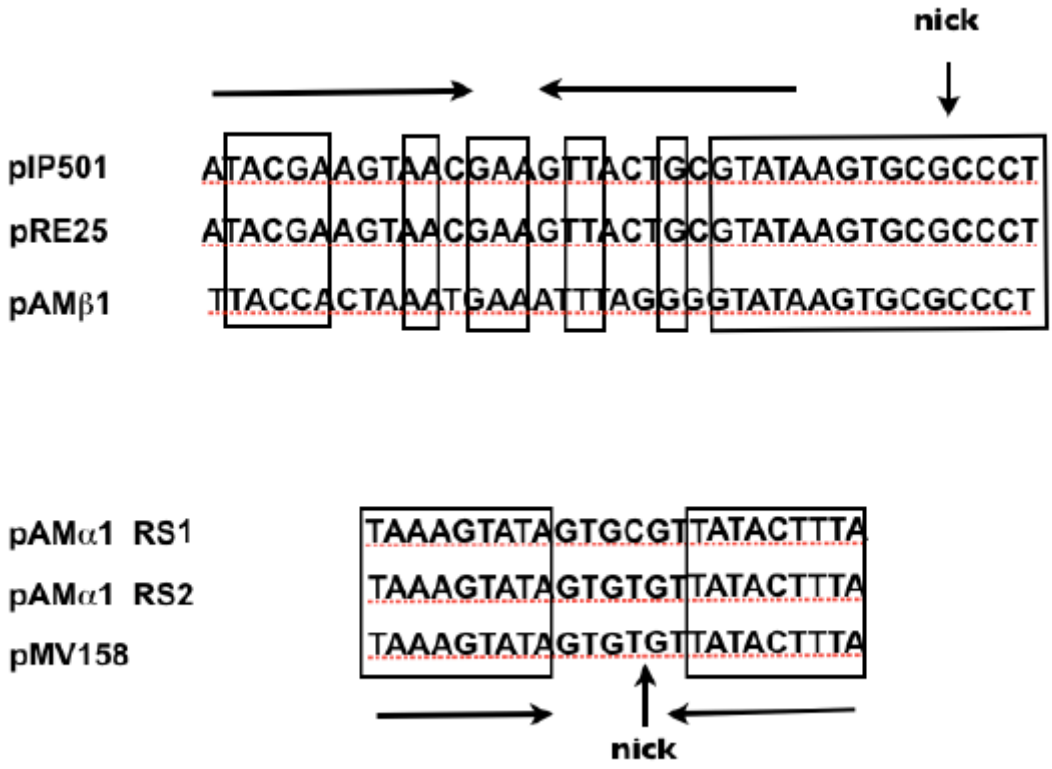

Figure 4. The *oriT* sites of several enterococcal plasmids. The horizontal arrows represent inverted repeats and the vertical arrows indicate the nick sites.

combined genetic (yeast 2-hybrid analysis) and biochemical (pull-down) analysis of the pIP501 conjugation system established that the protein products of the *tra* operon interact to form a T4SS machine (Abajy, et al., 2007). However, the proteins involved in the Gram-positive T4SS are surprisingly divergent from counterparts of Gram-negatives, which is likely the result of major differences in the cell envelopes that are traversed by the DNA.

A highly conserved T4SS component, termed a coupling protein, interfaces with the relaxosome and a complex involved in mating pair formation (MPF) (Cascales & Christie, 2003). In the case of enterococcal plasmids, homologs of coupling proteins include: Orf10 of pIP501 (Abajy, et al., 2007); TraW (Orf53) of pAD1 (Francia & Clewell, 2002); PcfC of pCF10 (53); and Orf22 of pHTβ (a pMG1-type plasmid) (Tomita & Ike, 2008). Studies of pCF10 demonstrated that PcfC is indeed the functional coupling protein that physically links the relaxosome with the T4SS machine and leads to ATP-dependent transfer through the mating channel (Chen, Zhang, Manias, Yeo, Dunny, & Christie, 2008). Common and unique features of T4SSs from Gram-positive and Gram-negative bacteria have recently been detailed (Alvarez-Martinez & Christie, 2009).

Conjugative transposons and integrative conjugative elements

Conjugative transposons are widespread in bacteria and have been found in numerous genera, including in both Gram-negative and Gram-positive bacteria. The prototype, Tn*916*, was first identified in *E. faecalis* DS16, and consists of an 18 kb element that encodes resistance to tetracycline/minocycline [Tet(M)]. It was originally recognized as a transposon because of its ability to insert at multiple sites on the co-resident plasmid pAD1 (Franke & Clewell, 1981; Gawron-Burke & Clewell, 1982). Conjugative transposons have a remarkably broad host range (including Gram-negatives), due to the presence of comparatively few restriction sites, an anti-

restriction system, and proteins that function in multiple host backgrounds. The properties of Tn*916* and its extended family of related elements have been reviewed in detail (Clewell, Flannagan, & Jaworski, 1995; Clewell & Gawron-Burke, 1986; Rice L. B., 1998; Roberts & Mullany, 2010; Roberts & Mullany, 2011; Scott & Churchward, 1995).

Tn*916*-like elements are usually found to be integrated in the bacterial chromosome, although they can occur on low-copy plasmids (Clewell & Francia, 2004). They are characterized by their ability to excise, which generates a circular intermediate that is non-replicative but is able to transfer conjugatively to a recipient by a plasmid-like mechanism. Excision of Tn*916* has been shown to require the activity of a specific integrase (Int_{Tn916}), a member of the lambda family, and excisionase, Xis (Su & Clewell, 1993), and is accomplished by a mechanism that has been well characterized (Hinerfeld & Churchward, 2001; Jia & Churchward, 1999; Rocco & Churchward, 2006). The transposon is generally flanked by 6-bp coupling sequences (usually not identical) that may influence the rate of excision (Caparon & Scott, 1989; Jaworski & Clewell, 1994). Int_{Tn916} brings the ends of the transposon together by binding to direct repeats (DR2) located within each terminus. It then generates a staggered cut, which gives rise to a 6-bp "core" (heteroduplexed if the flanking coupling sequences differ), that enables the ends to join to form the excised circular intermediate.

Intercellular transfer is initiated by a transposon-encoded relaxase (Orf20) that acts at an *oriT* site (adjacent to the relaxase determinant) on the circular intermediate (Jaworski & Clewell, 1995; Rocco & Churchward, 2006). BLAST searching has not resulted in any hits against known relaxases; however, similarity to members of the Rep_trans superfamily of replication initiators has been noted (Garcillán-Barcia, Francia, & de la Cruz, 2009). Int_{Tn916} also binds within *oriT* and facilitates recognition of the specific *nic* site by the relaxase (Rocco & Churchward, 2006). Orf21, encoded on the other side of the *oriT* from the relaxase determinant, is a member of the FtsK/SpoIIIE family, and therefore may correspond to a coupling protein, as such proteins are distantly related [see (228)]. In any case, support for the involvement of a T4SS system in Tn*916* conjugation is also provided by Orfs 14-16, which exhibit similar sequences to products that participate in such a process.

Upon entering the recipient with a single strand transferring and the complementary strand being synthesized in the recipient, a double-stranded circular form then inserts into the genome through a mechanism that resembles the reverse of excision. Insertion of the transposon occurs with relatively low target specificity at sites that are generally AT-rich. Transfer frequencies can range from 10^{-9} or less to as high as 10^{-4} per donor, depending on the particular insertion in the donor. Tn*916* contains 26-bp terminal inverted repeats (identity at 20 of 26 bp), which provide an AT-richness that may facilitate the alignment of intermediates with the AT-rich target sites. Transfer studies in the laboratory are generally conducted on solid surfaces, where frequencies are 1-2 orders of magnitude higher than in broth (Jaworski & Clewell, 1994).

Transfer of Tn*916* often gives rise to multiple insertions; indeed as many as half of the transconjugants in a given mating experiment may exhibit more than one and as many as six insertions in different sites (Gawron-Burke & Clewell, 1982). When more than one copy is present in a donor, the transfer of one may activate in *trans* the transfer of another (Flannagan & Clewell, 1991). The basis of multiple insertions is not known, although at least two possibilities can be considered: 1) during intercellular DNA transfer, replacement replication from the 3′ end within the donor might result in multiple rounds that, in the recipient, give rise to dimeric/oligomeric forms that subsequently resolve with monomers inserting at different sites; and 2) an initial insertion may take time to stabilize, during which it might, after the chromosomal replication fork has passed through it, undergo an intracellular transposition into a new target.

Rice et al. (Rice L. B., Carias, Rudin, Hutton, & Marshall, 2010) have identified a clinical isolate of *E. faecium* (C68) that contains three closely-related functional Tn*916*-like elements, which suggests that the acquisition of multiple copies can occur naturally. In a different clinical isolate of *E. faecium* (D344R), the same group (Rice L. B., Carias, Marshall, Rudin, & Hutton-Thomas, 2005) reported the presence of an 18 kb Tn*916*-like element, together with a heterologous, functional 29 kb Tn*916*-like element (Tn*5386*). In this case, they showed that the

integrase from either could act on both transposons, giving rise to a deletion of the entire 178 kb of DNA between the two elements (Rice L. B., Carias, Hutton-Thomas, & Rudin, 2007)—a segment that contained a determinant, *pbp5*, that is associated with increased resistance to ampicillin. One could envision a case whereby the entire 178 kb segment along with one (or even both) of the flanking transposons could transfer as a unit, which enables the movement of the ampicillin-resistance trait. The reader is referred to a later section of this chapter for a broader and more epidemiologically-related description of conjugative transposons in enterococci.

As noted earlier, analyses of complete genome sequences, which are continuing to be reported at ever-increasing rates, are revealing the great extent to which horizontal gene transfer occurs in the bacterial world. Large segments of DNA that are being identified as components of the bacterial chromosome appear to have been "acquired" from an unrelated source;, and in some cases they appear to be able to conjugate. Such segments, which can correspond to well over 100 kb, are turning out to be ubiquitous and quite diverse (Guglielmini, Quintais, Garcillán-Barcia, de la Cruz, & Rocha, 2011; Wozniak & Waldor, 2010); and they frequently bear multiple determinants for integrases and insertion sequences, as well as genes that resemble those involved in conjugative transfer. Putative *oriT* sites, relaxase determinants and plasmid-like conjugation genes—sometimes even similar to those found in Tn*916*—have also been identified. The presence of such determinants has given rise to the term "integrative conjugative elements" (ICEs), although direct demonstration of such transfer has not always been possible. The additional presence in ICEs of genes that facilitate survival or the ability to take advantage of a new environment is common, with determinants that encode antibiotic resistance and virulence being good examples. Conjugation frequencies can be quite low (such as 10^{-10} to 10^{-9}); and insertion into the recipient genome is often into a specific site, such as a tRNA determinant. Secondary transfer events observed in the laboratory sometimes occur at much higher frequencies, which suggests that the initial transfer event may have been associated with an alteration/rearrangement of the element or help from a resident conjugative plasmid. "Pathogenicity island" (PAI) and "genomic island" are commonly used terms to describe these entities, which may or may not be capable of conjugation without a "helper" system (like a mobilizing plasmid). Many of these "islands" include modules derived from plasmids, transposons and/or bacteriophages. Some excellent reviews of ICEs can be found in the literature (Burrus, Pavlovic, Decaris, & Guédon, 2002; Guglielmini, Quintais, Garcillán-Barcia, de la Cruz, & Rocha, 2011; Osborn & Böltner, 2002; Wozniak & Waldor, 2010).

In enterococci, a PAI was initially identified in E. faecalis strain MMH594; it corresponded to about 150 kb, contained numerous putative virulence determinants and 11 transposases/IS elements, and was flanked by 10-bp direct repeats (Shankar, Baghdayan, & Gilmore, 2002). Similar islands have been identified in E. faecium (Leavis H. L., et al., 2003; Oancea, Klare, Witte, & Werner, 2004). Many strains of enterococci associated with nosocomial infections have since been shown to contain PAIs, although with significant variability; and genomic analyses have suggested the involvement of a modular accretion in their evolution (McBride, et al., 2009; McBride, Fischetti, Moellering, Jr., & Gilmore, 2007). In the case of the MMH594 strain, a ~28 kb segment that contains virulence determinants from within the PAI was shown to excise, circularize, and transfer at a very low frequency (10-10 per donor) to the plasmid-free E. faecalis strain OG1RF; however, secondary transfer occurred at a high frequency (10-1 per donor) and was shown to involve a pheromone-like plasmid into which the segment integrated (Coburn, Baghdayan, Dolan, & Shankar, 2007). The transfer of PAI components between E. faecalis and E. faecium has also been reported (Coburn, Baghdayan, Dolan, & Shankar, 2007; Oancea, Klare, Witte, & Werner, 2004).

Although most reports appear to demonstrate the movement of only internal segments of PAIs, Laverde-Gomez et al. (Laverde-Gomez, et al., 2011) have recently shown that an entire PAI (~200 kb) in *E. faecalis* UW3114 was able to precisely excise, transfer, and integrate site-specifically into the chromosome of *E. faecalis*, as well as *E. faecium*. (It is noteworthy that along with precise excisions, imprecise excisions of internal segments of the PAI were also detected in the donor.) A 66-kb pheromone-responsive plasmid pLG2 (which encoded resistance to erythromycin) was transferred in parallel and was probably involved in promoting transfer of the PAI (Laverde-Gomez, et al., 2011). Manson et al (Manson, Hancock, & Gilmore, 2010) reported that in the case of *E. faecalis*

V583 transfer of PAI sequences was accompanied by a resident plasmid (pTEF1 or pTEF2). They also observed plasmid-promoted transfer of DNA outside the PAI that involved determinants widely scattered around the chromosome, which is a finding that is consistent with earlier reports in enterococci, where pheromone-responding plasmids were found to mobilize certain chromosome-borne mutational markers (Franke & Clewell, 1981; Franke, et al., 1978).

Inasmuch as PAIs in enterococci represent a variety of segments that carry different clusters of genes, including conjugation-related determinants, integrases, transposons, and IS sequences, it is likely that there are multiple ways by which a given "island," part of it, or even segments that include a region outside of the island, could be excised and mobilized. Multiple IS sequences, as well as resident plasmids (for example, strain V583 carries at least ten *IS256* sequences, ten *IS1216*s, and six *ISEf1*s, as well as three plasmids) might easily participate in the incorporation, rearrangement, or deletion of segments of DNA in the ever-changing PAI mosaic.

Plasmid Maintenance

Replication

Bacterial plasmids require three basic components for replication within their host cells: 1) a replication initiator, which serves to recruit the cellular DNA replication machinery; 2) an origin of replication, at which the initiator acts; 3) a negative regulatory circuit that serves to limit the frequency of replication. These three components comprise the minimal replicon and will be the focus of this section of the chapter.

A recent survey of plasmid replicons in enterococci identified eleven classes of replicons, based on sequence homology and phylogeny of the initiator proteins (185). These plasmids can be further grouped into four families, based on sequence homology and the presence of conserved domains. The RepA_N plasmid family encodes initiators with the RepA_N domain in their N-terminus, usually within the first 80 amino acids. This family includes the pheromone responsive conjugative plasmids of *E. faecalis*, as well as the pRUM-related plasmids of *E. faecium* (Weaver, Kwong, Firth, & Francia, 2009).

The Inc18 family (Brantl, Behnke, & Alonso, 1990) includes the well-studied broad host range plasmids pAMβ1, pIP501, and pSM19035, and provides the backbone of many cloning vectors used in the enterococci. The initiators of this plasmid family include the PriCT_1 domain, which is associated with primases, although these initiators have not been shown to have primase activity. The RCR family (Khan, 2005) is a large group of ubiquitous plasmids that replicate through a rolling circle mechanism. Several classes of RCR initiators have been detected in the enterococci. The Rep_3 family of replicons includes several plasmids detected in both *E. faecalis* and *E. faecium*, but these plasmids have not yet been well studied in these species. However, the Rep_3 domain is conserved in the initiators of many well-studied enterobacterial plasmids, and may provide a model for studies of those identified in enterococci. The details of what is known about the enterococcal members of these plasmid families, as well as what we may infer about their function from relatives in other species, will be discussed below.

RepA_N plasmids

The RepA_N plasmid family is broadly distributed in the low G+C Gram-positive bacteria, including both conjugative and non-conjugative plasmids that range in size from the 3.3 kb *Lactobacillus helveticus* plasmid pLJ1 (Takiguchi, Hashiba, Aoyama, & Ishii, 1989) to the 281 kb *E. faecium* plasmid pLG1 (Laverde-Gomez, et al., 2011). Proteins that contain the RepA_N conserved domain have also been associated with streptococcal bacteriophages and ICE elements, although their precise function on these elements is unclear (Weaver, Kwong, Firth, & Francia, 2009). While the replicon family appears to be widespread, individual plasmids have a relatively narrow host range that is frequently restricted to their species of origin. Phylogenetic analysis further suggests that they have not been recently disseminated by horizontal transfer (Weaver, Kwong, Firth, & Francia, 2009).

The RepA_N initiator proteins have a tripartite domain organization (Weaver, Kwong, Firth, & Francia, 2009). The N-terminal domain is the most highly conserved among family members and contains the eponymous conserved domain. The C-terminal domain is relatively poorly conserved across phylogenetic boundaries, but is better conserved among plasmids present in the same genus. This pattern of conservation suggests that the N-terminal domain performs some essential function common to all plasmid family members, such as DNA binding and/or strand separation, while the C-terminal domain performs some host-specific function. In plasmids of Gram-negative bacteria, such host specificity has been associated with interaction with host replication proteins, particularly the loading of the host replicative helicase, DnaB (195). The C-terminal domain of the RepA_N plasmids could perform a similar function.

The central domain of the RepA_N initiator proteins is highly variable and the corresponding genes contain multiple complex nucleotide repeats, which usually consist of short 5-12 nucleotide (nt) inverted repeats that are embedded in longer direct repeats. In some cases, the repeats are translated into amino acid repeats, but in others they are not, which suggests that it is the DNA sequence and not the protein sequence that is important. This region has been shown to function as an origin of replication *in vivo* in several plasmids, including the prototype pheromone-responsive plasmid pAD1 (Francia, Fujimoto, Tille, Weaver, & Clewell, 2004). *In vitro* studies demonstrated that pAD1 RepA_N protein RepA binds to the inverted repeats within the origin region and that the conserved N-terminal domain is sufficient for binding. The C-terminal domain, including two of the repeats, did not bind the origin (Francia, Fujimoto, Tille, Weaver, & Clewell, 2004). Interestingly, in several RepA_N family plasmids, including the pheromone-responsive plasmids pAD1 and pCF10 and the non-conjugative *E. faecium* plasmid pRUM, the inverted repeats converge on a poly-A tract of 3-5 nt. In the *S. aureus* RepA_N plasmid pSK41, RepA has been shown to leave a single A unprotected in binding studies (Kwong, Skurray, & Firth, 2004). This suggests that the structure of the repeats is important for function, but precisely what this function is remains unclear. The origin repeats have been demonstrated to function as incompatibility determinants in pAD1 (Francia, Fujimoto, Tille, Weaver, & Clewell, 2004), the *Bacillus subtilis* plasmid pLS32 (Tanaka & Ogura, 1998) and the *S. aureus* plasmid pSK1 (Kwong, Lim, Lebard, Skurray, & Firth, 2008), which suggests that the high sequence variability within this region may be required to prevent competition between related plasmids in the same host.

The molecular events that allow RepA_N proteins to facilitate replication initiation at their origins of replication are as yet unknown. It has been demonstrated that pAD1 RepA has non-sequence specific single stranded DNA binding activity, in addition to its sequence specific double stranded DNA binding activity (Francia, Fujimoto, Tille, Weaver, & Clewell, 2004). Similar activities have been observed in the unrelated initiator proteins of the Inc18 family plasmids, where single-stranded DNA binding facilitates strand separation (Le Chatelier, Jannière, Ehrlich, & Canceill, 2001). Origin function apparently requires multiple *rep*-associated repeats, since recombination between the two large repeats in pAD1 eliminates origin function (Francia, Fujimoto, Tille, Weaver, & Clewell, 2004). Note that the RepA protein retains initiator function, despite an in-frame 35-codon deletion.

The mechanism of copy number control in the enterococcal RepA_N plasmids is also unknown**.** In pSK41, an antisense RNA has been implicated in regulating translation of the RepA protein (Kwong, Lim, Lebard, Skurray, & Firth, 2008; Kwong, Skurray, & Firth, 2006; Kwong, Skurray, & Firth, 2004) but an examination of the pAD1 sequence in the analogous region showed no evidence of a similar regulatory RNA on this plasmid (Weaver, Kwong, Firth, & Francia, 2009) In pLS32, mutations in some of the repeats increase the copy number of the plasmid. Similar mutations have not yet been made in the enterococcal RepA_N origin regions (Tanaka, Ishida, & Maehara, 2005). Interestingly, there are several potential binding sites for the RepC partition protein, similar to the iterons involved in partition [see the following section and Francia et al. (Francia, Weaver, Goicoechea, Tille, & Clewell, 2007)], in the putative promoter for pAD1 RepA, but it remains unknown whether these binding sites are actually bound by RepC or are important for regulation.

Inc18 plasmids

The term "incompatibility group" was traditionally applied to plasmids whose replication functions were so closely related that they could not be maintained together in the same cell culture, due to competition for limiting replication and/or stability components (Novick, 1987). In keeping with this traditional definition, the Inc18 incompatibility group originally included plasmids pIP501 from *Streptococcus agalactiae*, pSM19035 from *Streptococcus pyogenes*, and pAMβ1 from *E. faecalis* (Brantl, Behnke, & Alonso, 1990). Since that time, the terms Inc18-like or Inc18 family have been used, perhaps inappropriately, to refer to plasmids that encode replication initiators with sequence homology to the initiators of the original three plasmids of the group. While this homology probably indicates a similar replication initiation mechanism, it does not imply that the replicons are closely related enough to be truly considered incompatible. With this caveat in mind, the term Inc18 plasmid family will be used below to refer to plasmids with related initiator proteins, without regard for their actual incompatibility properties.

The Inc18 plasmid family is comprised of relatively low copy number (<10 copies/cell) plasmids that range in size from 25 to 50 kb and frequently carry multiple antibiotic-resistance genes. They have a broad host range and many are self-transmissible. As noted in an earlier section, they appear to be particularly important in the spread of vancomycin resistance both within the enterococci and to other species (Flannagan, et al., 2003; Freitas, et al., 2013; Freitas, et al., 2012; Freitas, et al., 2010; Rosvoll, et al., 2010; Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008). A recent PCR-based screening of enterococcal isolates indicated that Inc18 family plasmids were the most common plasmids in both *E. faecalis* and *E. faecium* (Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010). In addition, their replicons form the backbone of some of the most broadly used cloning vectors in the enterococci.

Another class of plasmids that may be related to the Inc18 family is the pMG1/pHT plasmids. While these plasmids have not been reported to have a broad host range, the only replication initiator protein homolog identified in the pMG1 sequence is approximately 30% identical to Inc18 initiators (Tanimoto & Ike, 2008) and includes the PriCT_1 domain. This protein was shown to be essential in supporting the replication of a mini-plasmid in *E. faecalis*, perhaps due to its function at an essential inverted repeat sequence. Sequence differences or alterations in origin function may limit the host range of these replicons, relative to the better-studied Inc18 replicons.

Studies on the replication of Inc18 family plasmids have focused primarily on the original three members of the group. Most of this work was performed in the model Gram-positive bacterium *B. subtilis*, because of its amenability to genetic manipulation, but the plasmids in enterococci most likely replicate by a similar mechanism. Nomenclature is somewhat confusing, since homologous proteins on the three plasmids have different letter designations, despite the fact that the replicons share an overall >92% DNA sequence homology (Lioy, Pratto, de la Hoz, Ayora, & Alonso, 2010). For simplicity, initiator and copy control proteins for all three plasmids will be designated Rep and Cop, respectively, and the regulatory RNA will be referred to as *ct* for countertranscript. It will be assumed that the molecular mechanisms of replication and copy control described for one plasmid apply to all three.

Replication initiation of Inc18 plasmids requires the Rep protein, a small origin region located immediately downstream of the *rep* gene, a transcription fork that passes through the replication origin, and the host DNA Pol I (Bruand, Le Chatelier, Ehrlich, & Jannière, 1993). The replication primer is provided by a transcript that initiates from the *repE* promoter and passes through the origin region (Bruand & Ehrlich, 1998). Rep has both sequence-specific dsDNA binding and non-specific ssDNA binding capabilities. It uses the former to bind to the origin region, which causes bending and supercoiling-dependent melting, while it uses the latter to extend and stabilize the single-stranded bubble within the origin. The *rep* transcript then passes through the replication bubble and is processed in a poorly defined Rep-dependent manner to provide the primer for DNA Pol I-dependent replication (Le Chatelier, Jannière, Ehrlich, & Canceill, 2001). Replication proceeds for approximately

150 bp until an *ssiA* site for *priA*-dependent priming of lagging strand synthesis is exposed. At this point, DNA Pol I is replaced by DNA Pol III, which directs simultaneous unidirectional replication of both strands away from the *rep* gene.

The Rep proteins are rate-limiting for replication and as a result, their production must be tightly regulated. Regulation of Rep synthesis occurs by the concerted action of two regulators, an antisense RNA, *ct*, and a transcriptional repressor, Cop. Binding of *ct* to the 5′ untranslated region of the *rep* message causes a refolding of the nascent transcript that results in the premature termination of transcription (Brantl, Birch-Mirschfeld, & Behnke, 1993; Heidrich & Brantl, 2007; Le Chatelier, Ehrlich, & Jannière, 1996). Cop acts as a transcriptional repressor at the *rep* promoter by competing with RNA polymerase binding (Brantl, Birch-Mirschfeld, & Behnke, 1993; Le Chatelier, Ehrlich, & Jannière, 1996; Licht, Freede, & Brantl, 2011). In addition, Cop-mediated repression of the *rep* promoter reduces transcriptional interference with the convergent *ct* promoter, which indirectly increases the production of *ct* and tightens the repression of replication (Brantl & Wagner, 1997). When the copy number decreases, decreased Cop levels result in an increase in *rep* transcription and a concommitant decrease in the production of *ct*. This extra layer of control is essential because, unlike the regulatory RNAs of most other plasmids, *ct* is relatively stable and therefore does not directly reflect the plasmid copy number (Brantl & Wagner, 1996). The cooperativity of this system is indicated by the fact that interference with either component alone results in a 10-20 fold increase in copy number, but simultaneously disabling both Cop and *ct* has no further effect (Brantl & Behnke, 1991). Another layer of regulation is provided by the plasmid-encoded ω protein, which represses the synthesis of Cop (de la Hoz, et al., 2000). ω also binds to the promoters for genes involved in active partition and post-segregational killing, and presumably coordinates replication and stable inheritance functions.

Because plasmids are commonly present in multiple copies in bacterial cells and those copies are 100% identical, they are frequently subjected to recombination that results in multimerization. Because copy control systems count numbers of origins rather than the number of plasmid molecules, and plasmids with multiple origins have a replication advantage, systems to resolve multimers are an important adjunct to copy control mechanisms (Summers, Beton, & Withers, 1993). In Inc18 plasmids, this function is accomplished by β recombinase, which acts at a resolution site that is located immediately upstream of its gene (Alonso, Ayora, Canosa, Weise, & Rojo, 1996). In addition to its role in multimer resolution, β also has been implicated in the switch from DNA Pol I to DNA Pol III-directed DNA replication at *ssiA* (Jannière, Bidnenko, McGovern, Ehrlich, & Petit, 1997). Binding of β to its target presents a roadblock to continued DNA Pol I-directed replication, and the resolvase site is appropriately positioned to facilitate primosome assembly at *ssiA*. The product of the *top* gene has also been implicated in the polymerase switch (Bidnenko, Ehrlich, & Jannière, 1998). The Top protein displays significant sequence homology to *E. coli* topoisomerase I, and has been shown to possess DNA relaxation activity, but only on plasmids that use DNA Pol I for initiating replication. Top-mediated relaxation of negative supercoils has been proposed to interfere with the ability of DNA Pol I to separate the DNA strands for replication, thereby causing its arrest.

RCR plasmids

RCR plasmids are a ubiquitous family of plasmids that were originally described in *S. aureus*, are widespread in Gram-positive bacteria, and include representatives in Gram-negative bacteria and Archaea. Replication initiator proteins from three classes of RCR replicons have been detected in enterococci; the pT181-family that contains the Rep_trans conserved domain, the pMV158 family that contains the Rep_2 conserved domain, and the pUB110 family that contains the Rep_1 conserved domain (see the section below on categorizing plasmids). Only two natural isolates have been described in any detail in the enterococci—the *E. faecium* plasmids pRI1 (Rep_trans) (Garcia-Migura, Hasman, & Jensen, 2009) and pJB01 (Rep_2) (Kim, et al., 2006)—but related initiators have been detected in sequencing projects and PCR-based screens (Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010). While Rep_1 initiators have been identified in the enterococci, they are

commonly associated with alternative replicons and may not be functional. Indeed, the pAMα1 Rep_1 initiator is known not to function in *E. faecalis*, and replication of this plasmid depends on the Rep_3 initiator. (See previous section on pAMα1.) Several commonly used enterococcal cloning vectors have been derived from RCR plasmids that were isolated from other organisms. RCR plasmids have been the subject of numerous extensive reviews (del Solar, Giraldo, Ruiz-Echevarría, Espinosa, & Díaz-Orejas, 1998; del Solar, Hernández-Arriaga, Gomis-Rüth, Coll, & Espinosa, 2002; Espinosa, del Solar, Rojo, & Alonso, 1995; Khan, 2005), and the reader is referred to these for more extensive information and detailed citations on this subject.

Rolling circle replication was originally described in single-stranded DNA (ssDNA) coliphages, such as φX174, and the basic mechanisms of ssDNA phage and RCR plasmid replication are similar, except for several modifications to the plasmid systems that serve to regulate replication frequency and limit copy number. Replication in all RCR replicons is initiated by the introduction of a single-strand nick within a specific origin: the double-stranded origin, or *dso*, by the replicon-encoded initiator protein. Nicking is accomplished through a conserved active site tyrosine that is present in all RCR initiators. The plasmid *dso* contains two separate sequence elements, one that is required for sequence-specific binding of the initiator and a second that contains the nick site. The initiator protein consists of two domains, a DNA-binding domain that binds specifically to its cognate origin and a nicking domain that contains the active site tyrosine. Binding of the initiator to supercoiled plasmid DNA causes a conformational change in the *dso* that exposes the nick site as ssDNA. This is essential, since the initiator-nicking domain cannot recognize the nick site in double-stranded DNA (dsDNA). The active site tyrosine becomes covalently attached to the 5′ nucleotide at the nick site and remains attached until the replication cycle is complete. A DNA helicase (PcrA in Gram-positive bacteria) and DNA Pol III are then attracted to the origin and replication is primed by the free 3′-OH at the nick site. After a single round of replication, synthesis through the origin regenerates a copy of the *dso*. A nicking/religation reaction catalyzed by the bound initiator terminates replication, which results in the release of a completely replicated duplex plasmid copy and the displaced ssDNA strand. Replication of the displaced strand is accomplished from an origin distinct from the *dso*, which is referred to as the single-strand origin, or *sso*. These are imperfect palindromes that are recognized by the host RNA polymerase, which synthesizes a short RNA primer. Host DNA Pol I initiates DNA synthesis from the primer and eventually is displaced by DNA Pol III, which completes the synthesis of the complementary strand.

In a bacteriophage, RCR initiators function as monomers and contain two closely-spaced active site tyrosines. Following completion of the first round of replication, a second round of replication can be immediately initiated by using the second tyrosine. By alternating between the two tyrosines, multiple replication rounds can be completed after a single initiation event. Some RCR plasmid initiators also function as monomers, but contain only a single tyrosine. Termination is accomplished by an acidic residue close to the active site tyrosine, thereby preventing reinitiation. In other plasmids, the initiator also contains a single tyrosine, but functions as a dimer. In these plasmids, the noninitiating tyrosine of the dimer could, in principle, terminate and reinitiate replication as in a bacteriophage. However, while this second tyrosine is used for termination, the termination reaction results in the attachment of an oligonucleotide that corresponds to approximately ten nucleotides of newly synthesized DNA beyond the *dso*. Oligonucleotide attachment inactivates the initiator, which prevents reuse and requires *de novo* initiator synthesis prior to a second round of replication.

Initiator synthesis is also regulated in RCR plasmids by a regulatory RNA that is transcribed from the opposite strand at the 5′ end of the initiator gene. This antisense RNA or ctRNA binds to the initiator message within the complementary region, which either causes a refolding of the message that results in premature transcription termination, or interferes directly with ribosome binding. Because the antisense RNA is unstable, its level in the cell always reflects the number of plasmid copies, which makes it an effective control element. RCR plasmids of the pMV158-family (Rep_2) also encode a small transcriptional repressor protein, CopG, that acts synergistically with the antisense RNA to repress initiator synthesis.

Replication at an RCR plasmid *dso* initiates with a nick, which provides the free 3′-OH required for the priming of DNA Pol III. Therefore, RCR plasmids have no need for interaction with host proteins, like DnaA or primase. This fact is probably responsible for the broad host range of RCR replicons, some of which are capable of replicating in both Gram-positive and Gram-negative bacteria. However, inefficient use of *sso*s, either by the host RNA polymerase or DNA Pol I, may result in the accumulation of ssDNA in non-native hosts. Some plasmids have partially solved this problem by evolving *ssos* that are capable of functioning in more than one host. Others may contain two or three *sso*s and presumably use the best one in different hosts.

The production of ssDNA as an intermediate of rolling circle replication imposes certain limits on the biology of RCR plasmids, and on their utility as cloning vectors. Most naturally-occurring RCR plasmids are less than 12 kb in size, presumably because larger sizes would increase the probability of the presence of partially homologous sequences that would lead to recombination and deletion during the ssDNA phase of replication. This propensity for recombination also limits the usefulness of RCR plasmids as cloning vectors. Such vectors are notoriously "structurally" unstable, often deleting all or portions of cloned genes over time. Cloning of large DNA fragments, fragments with repeat sequences, or fragments carrying genes toxic to the host is particularly problematic.

Rep_3 plasmids

Plasmid replication initiator proteins that contain the Rep_3 conserved domain are ubiquitous in bacteria, and include some of the best-studied bacterial plasmids, including the enterobacterial plasmids F, P1, pSC101, and R6K (del Solar, Giraldo, Ruiz-Echevarría, Espinosa, & Díaz-Orejas, 1998). Several plasmids that contain initiators with the Rep_3 domain have been identified in the enterococci, including *E. faecium* plasmids pMBB1 (399), pDT1 (Todokoro, Tomita, Inoue, & Ike, 2006) and pCIZ2 (Criado, et al., 2008), and *E. faecalis* plasmids pS86 (Martínez-Bueno, Valdivia, Gálvez, & Maqueda, 2000), pAMα1 (Francia & Clewell, 2002), and pEF1071 (Balla & Dicks, 2005). Of these plasmids, the pCIZ2 replicon is the best characterized. The minimal replicon consists of the initiator protein gene, *repE*, and two sets of direct repeats, DR1 and DR2, immediately upstream of the *repE* gene. The DR1 repeats are distal to the *repE* promoter and consist of 3.5 copies of a 12 bp sequence. The DR2 repeats are between the DR1 repeats and the *repE* promoter and consist of 4.5 copies of a 22 bp sequence. A fragment that contains both sets of repeats is capable of supporting replication if the *repE* gene is supplied *in trans*, which indicates that the repeats function as an origin of replication. 2D-gel analysis confirmed that the plasmid replicates through a theta mode rather than by rolling circle. Attempted transformation into several species indicated that the plasmid has a relatively narrow host range. Other enterococcal plasmids within this group have a similar organization of iterons upstream of the Rep_3-containing gene.

While the mechanism of copy control is not known for the enterococcal plasmids, it has been intensively studied in their Gram-negative counterparts. In these plasmids, replication initiator monomers represent the active initiator form, while dimers are inhibitory. As plasmid copy number increases, so does initiator concentration leading to increased dimer formation. Initiator dimers cross-link the excess plasmid copies at their initiator repeats, or iterons, "handcuffing" replication and simultaneously inhibiting initiator transcription. As copy number falls, handcuffing is released, initiators are monomerized by chaperones, and plasmid replication can proceed (Chattoraj, 2000). Whether a similar cycle occurs in the Gram-positive representatives remains to be determined.

Several Rep_3 replicons from Gram-positive plasmids have been used for the construction of cloning vectors [see Benachour, Auffray, & Hartke, 2007; Criado, et al., 2008]. Theoretically, these plasmids should make good vectors, because they are generally smaller than the RepA_N plasmids and their theta mode of replication makes them structurally more stable than rolling circle replicons. Further studies on copy control mechanisms should help determine if high copy derivatives can be constructed.

Plasmid partition cassettes: organization and activity

Replication control systems maintain plasmid copy numbers within defined windows that are characteristic for different plasmids, as outlined above. Low copy number plasmids, including those that mediate antibiotic resistance and virulence in enterococci, cannot rely on passive diffusion through the cytoplasm to ensure their stable maintenance. Instead, these plasmids possess accessory mechanisms to promote their persistence in the population. As discussed below, plasmids frequently encode post-segregational cell-killing mechanisms that act specifically in plasmid-free progeny (Hayes & Van Melderen, 2011). Low–copy-number plasmids also specify active partition mechanisms that guarantee their precise distribution during bacterial cytokinesis. Recent studies focused on biochemical, structural and subcellular localization analyses have revealed crucial aspects of the partitioning process, especially in *E. coli* and in certain *Bacillus* spp. In particular, understanding of assembly of the segrosome, the nucleoprotein complex that drives plasmid segregation, and of the action of motor protein components of the complex have provided fascinating glimpses into plasmid trafficking and positioning during partition (Hayes & Barillà, 2006; Schumacher M. A., 2012).

The loci that mediate plasmid segregation are of four currently known types, based on genetic organization and evolutionary relationships of the encoded proteins (Schumacher M. A., 2008). Types I to III have been most well characterized, whereas type IV has been investigated less thoroughly. Types I to III are each comprised of two autoregulated genes and a centromere-like sequence. In each case, one of the genes specifies an NTPase, either an ATPase of the ParA (type I) or actin (type II) superfamilies, or a GTPase related to tubulin (type III). ParA proteins vary in size from ~200 to ~400 amino acids. The larger proteins typically possess a DNA binding domain that is required for transcriptional regulation of the locus and which is absent from smaller homologs. Type II actin-like proteins are similarly evolutionarily diverse (Derman, et al., 2009), whereas type III tubulin-like proteins are apparently confined to *Bacillus thuringiensis* and related bacilli (Larsen, Cusumano, Fujioka, Lim-Fong, Patterson, & Pogliano, 2007).

The second gene in type I to III partition cassettes invariably encodes a centromere-binding factor (CBF). The proteins that accompany large ParA homologs in the type I class are members of the ParB family of dimeric DNA-binding proteins. ParB proteins possess two DNA binding motifs: a helix-turn-helix (HTH) motif and a six-stranded β-sheet coiled-coil that contact distinct repeat motifs in the centromere site (Schumacher & Funnell, 2005). By contrast, the primary sequences of the CBFs that associate with small ParA proteins are more variable (Fothergill, Barillà, & Hayes, 2005). Nevertheless, in the few cases for which tertiary structures of these CBFs have been solved, they have proven to possess a dimeric ribbon-helix-helix (RHH) fold. This fold is characteristic of a family of DNA-binding proteins that are widely disseminated in both bacteria and archaea (Schreiter & Drennan, 2007). Similarly, the only CBF of type II segregation complexes for which structural information is available is a RHH protein. Here, multiple RHH dimers assemble on the cognate centromere to form an extended, higher order nucleoprotein structure (Møller-Jensen, Ringgard, Mercogliano, Gerdes, & Löwe, 2007; Schumacher, et al., 2007). The crystal structure of one type III class CBF has been determined, and reveals a dimeric winged HTH motif. However, instead of canonical HTH-DNA interactions, the protein inserts the N termini of the recognition helices into a single DNA groove and the wings into adjacent grooves (Ni, Xu, Kumaraswami, & Schumacher, 2010). Thus, there is considerable diversity in the modes of centromere recognition by CBFs in the three major classes of partition complexes.

In accord with the variations in CBF sequences and tertiary structures, plasmid centromeres are remarkably heterogeneous (Hayes & Barillà, 2006). The sites typically comprise multiple direct and/or inverted repeat motifs that may be arrayed over ≈100-bp. However, the sequences, lengths, and numbers of repeats differ from centromere to centromere (Wu, Zampini, Bussiek, Hoischen, Diekmann, & Hayes, 2011). Recognition of centromeres by the cognate CBFs is specific, with little cross-talk with non-cognate centromeres (Fothergill, Barillà, & Hayes, 2005). Thus, different plasmids that co-exist in the same cell may ensure their independent segregation by using distinct CBF-centromere interactions.

The centromere is not contacted directly by the partition NTPase that instead is recruited to the mature segrosome by interactions with the CBF. Plasmid pairing through the segrosomes is considered to be an early step in the partitioning process. Subsequently, nucleotide-induced polymerization of the NTPase into filamentous structures directs the attached plasmids to opposite halves of the dividing bacterial cell. However, the mechanisms by which the polymerizing NTPases in type I-III partition complexes achieve partitioning differ. For the type II system, ATP-mediated symmetrical growth of the actin-like filament propels bound plasmids towards opposite cell poles (Campbell & Mullins, 2007). By contrast, the tubulin-like polymers in type III complexes undergo treadmilling, in which protein subunits are added to the plus end and disassembled from the minus end of the filament. The attached CBF-plasmid can be transferred from the minus end to the subunits in the elongating plus end, which induces plasmid movement towards the cell pole (Barillà, Rosenberg, Nobbmann, & Hayes, 2005; Larsen, Cusumano, Fujioka, Lim-Fong, Patterson, & Pogliano, 2007; Ni, Xu, Kumaraswami, & Schumacher, 2010). The ParA protein in type I partition complexes also undergoes nucleotide-mediated polymerization that is modulated by the partner CBF (Barillà, Rosenberg, Nobbmann, & Hayes, 2005). Current models suggest that depolymerization of ParA filaments pulls bound plasmids towards the cell poles (Ringgaard, van Zon, Howard, & Gerdes, 2009), but the molecular mechanism by which plasmid transport is achieved in type I systems remains uncertain.

Enterococcal plasmid segregation cassettes

Despite their prevalence and significance in disseminating antibiotic resistance (see the later section on epidemiology), the molecular mechanisms that underpin segregation of enterococcal plasmids are poorly understood. Only the partition cassettes from the prototypical, pheromone-responsive plasmid pAD1 (see above) and the gentamicin-resistance plasmid pGENT have been described to date. The two modules conform to the type I archetype: both cassettes include genes for similarly sized ParA homologs, but encode distinctive CBFs and centromeric sites (Figure 5) (Derome, et al., 2008; Francia, Weaver, Goicoechea, Tille, & Clewell, 2007). For pAD1, the centromere comprises arrays of 13 and 12 8-bp direct repeats with the consensus 5′-TAGTARRR-3′. Provision of the RepB (ParA) and RepC (CBF) proteins *in trans* promoted enhanced segregational stability of a vector bearing either all 25 repeats or 12 motifs, but not a vector that possessed 3 repeats. Thus, more than three repeats are required for effective segrosome assembly (Francia, Weaver, Goicoechea, Tille, & Clewell, 2007). RepC binds the centromere directly and specifically *in vitro* and recruits the RepB protein, which itself does not contact the site. Interestingly, although other enterococcal plasmids carry *repB* genes, the sequences of the accompanying downstream genes and of the putative centromeres are variable which emphasizes how diversity in the CBF-centromere interaction may confer segregation-specificity in multiplasmid strains (Francia, Weaver, Goicoechea, Tille, & Clewell, 2007). Moreover, although RepB homologs are widely distributed in enterococci and other Gram-positive species, there is no linkage between the homologs and any particular plasmid replicon type, which suggests mosacism in enterococcal plasmids, as has been observed in plasmids of other Gram-positives (Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009).

The pGENT plasmid (≈70-kb) was detected in a clinical isolate of *E. faecium*, and confers high-level resistance to gentamicin and a range of other aminoglycosides (Simjee, Fraise, & Gill, 1999). Screening of a library of pGENT fragments cloned in a segregation probe vector identified *cenE-prgP-prgO* as a maintenance locus (Derome, et al., 2008). The *prgP-prgO* genes were previously noted on other enterococcal plasmids, but were of uncertain function (Hedberg, Leonard, Ruhfel, & Dunny, 1996). PrgP is a ParA homolog, whereas the dimeric PrgO protein binds the *cenE* centromere site. Like the pAD1 centromere, the *cenE* site consists of two clusters of repeats, although in this case, seven 5′-TATA-3′ motifs separated by half-helical turns are located in each array (Fig. 5). The *cenE* site shows a modest intrinsic curvature that may be important in assembling a functional segrosome (Derome, et al., 2008). Recent database searches revealed >100 PrgO homologs, principally in *Enterococcus sp*. but occasionally in other Gram-positives. Although annotation of many of these homologs is incomplete and it is frequently unclear whether they are encoded by chromosomal or plasmid genes, *prgP-prgO* loci may prove to be the most common partition cassettes in enterococci.

The descriptions of the pAD1 and pGENT segregation cassettes represent only the first steps in probing the molecular basis of plasmid segregation in enterococci. For example, it remains unclear whether the RepB and PrgP proteins polymerize in response to nucleotide binding, as established for other ParA superfamily members, and whether the cognate CBFs modulate this process. No tertiary structures of enterococcal segregation proteins, nor of these proteins in complex with centromeric DNA are currently available, which precludes a detailed understanding of the mechanics of segrosome assembly in these important bacteria. Moreover, it is unknown whether enterococcal chromosomal factors contribute to plasmid segregation, whether *repB-repC* and *prgP-prgO* are the only type I partition modules in enterococci, and whether type II and III partition cassettes also are prevalent.

Enterococcal partitioning as an antibacterial drug target

High-throughput screening (HTS) of small molecule libraries is a powerful approach that is geared at identification of lead compounds that can be developed into new drugs. Moreover, recent progress in compound synthesis and screening now make mechanism-directed small molecule strategies a robust and feasible experimental alternative (Fischbach & Walsh, 2009). The bacterial DNA segregation apparatus has significant potential as a novel target for antibacterial agents that are now urgently required, as existing antibiotics inexorably fail in treating infections caused by enterococci and other pathogens. Thus, small-molecule HTS directed at the enterococcal partition machinery may produce new compounds that could be developed into new antibacterial drugs. Protein-protein interactions involved in CBF dimerization, ParA homolog polymerization, or in CBF-ParA associations might be targeted. Small molecules that disrupt the assembly of enterococcal CBFs on their centromeres or on operator sites that reflect their anticipated roles in partition loci transcriptional regulation might be identified. Compounds isolated from a library screen that suppressed the ATPase activity of a mycobacterial ParA protein were recently described (Nisa, et al., 2010). Nucleotide binding or hydrolysis by enterococcal ParA proteins could be targeted analogously. Thus, as our understanding of plasmid segregation in enterococci develops and as potential HTS approaches evolve, the identification of small molecules that intervene in defined steps of the process may open new avenues towards combating enterococcal infections.

Toxin-Antitoxin Systems

Post-segregational killing (PSK) systems were originally defined as loci that stabilize bacterial plasmids by programming the death of any host cell that fails to inherit a plasmid copy at cell division (Gerdes, Rasmussen, & Molin, 1986; Jaffé, Ogura, & Hiraga, 1985; Ogura & Hiraga, 1983). They accomplish this feat by producing a stable toxin and an unstable antitoxin. As long as the plasmid is retained, the antitoxin is continually replenished and the toxin is inhibited. If the plasmid is lost, the antitoxin is degraded and the toxin kills the cell. PSK systems have also been referred to as addiction modules; the host cell is said to become "addicted" to the presence of the plasmid and suffers the ultimate withdrawal symptom (death) upon plasmid loss (Yarmolinsky, 1995).

PSK systems come in three varieties, designated Types I, II, and III, which differ in the composition and mode of action of their antitoxins (Fineran, Blower, Foulds, Humphreys, Lilley, & Salmond, 2009; Hayes, 2003). In Type I and Type III systems, the antitoxin is a regulatory RNA. In Type III systems, the RNA binds directly to the toxin protein and inhibits its activity (Blower, Fineran, Johnson, Toth, Humphreys, & Salmond, 2009; Blower, et al., 2011). No Type III system has yet been described in enterococci. In Type I systems, the RNA antitoxin binds to complementary sequences in the toxin mRNA, inhibiting translation and/or stimulating degradation. The paradigm for Type I systems is the *hok/sok* system of the *E. coli* plasmid R1 (Gerdes, Rasmussen, & Molin, 1986). The *par* determinant of the *E. faecalis* plasmid pAD1 is the only Type I PSK system characterized so far in Gram-positive bacteria (Weaver, Jensen, Colwell, & Sriram, 1996; Weaver & Tritle, 1994). In Type II systems, the antitoxin is a protein that inhibits toxin activity by direct binding to toxin proteins (Jensen & Gerdes, 1995). These antitoxins typically consist of two domains, a disordered toxin-binding domain and a DNA-binding domain. The toxin-binding domain adopts a more orderly structure upon toxin binding, but remains more

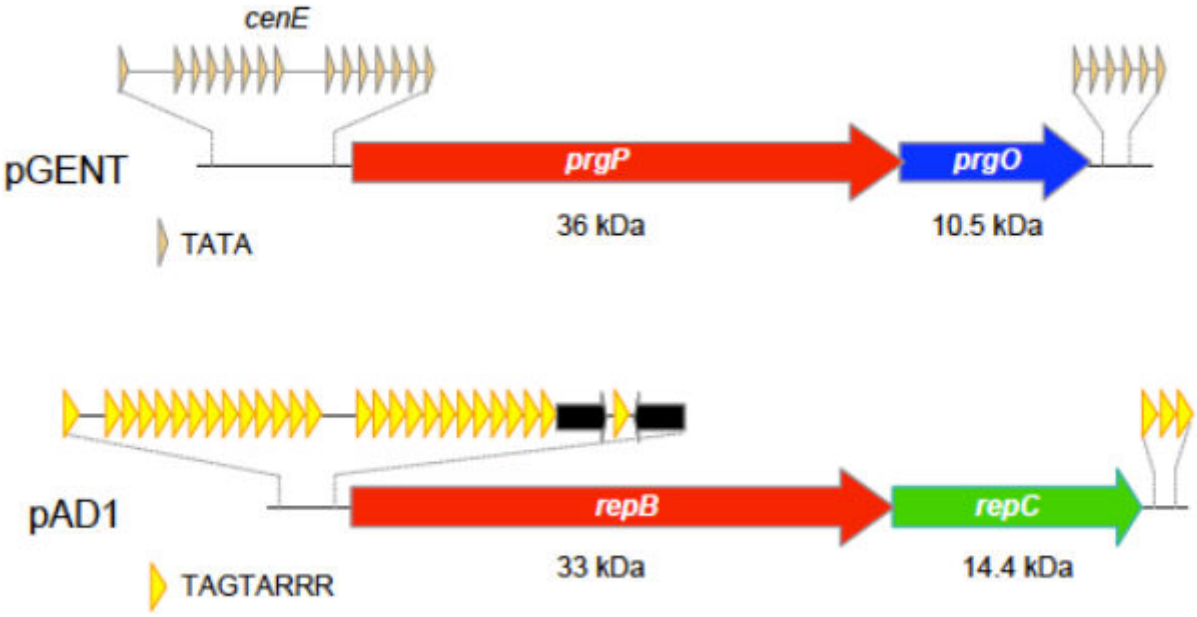

Figure 5. Organization of the pGENT and pAD1 segregation loci (90, 119). ParA homologs are denoted by red arrows and genes for CBFs by blue and green arrows. Molecular masses of protein monomers are indicated. Repeat motifs in the centromere sites located upstream of the genes are shown by arrowheads. Additional repeats of unknown function are situated downstream of the genes.

susceptible to recognition by proteases than the toxin. The DNA binding domain binds to the promoter responsible for transcription of the bicistronic antitoxin-toxin mRNA, and is responsible for autorepression of the system. In most cases, the toxin facilitates promoter binding and expression may be controlled by the ratio of the toxin and antitoxin proteins. The best-studied Type II system of Gram-positive bacteria is the ωεζ system found on Inc18 plasmids (Ceglowski, Boitsov, Karamyan, Chai, & Alonso, 1993; Lioy, Pratto, de la Hoz, Ayora, & Alonso, 2010). A second enterococcal Type II system, Axe-Txe, was identified on the *E. faecium* plasmid pRUM (Grady & Hayes, 2003). Both the ωεζ and the Axe-Txe systems have been shown to be common in enterococci (Moritz & Hergenrother, 2007; Rosvoll, et al., 2010). Other Type II systems related to those described in *E. coli,* including RelBE and MazEF, have been identified on enterococcal plasmids (Moritz & Hergenrother, 2007), and ribonuclease activity has been demonstrated for an enterococcal MazF toxin (Wang & Hergenrother, 2007). The RelBE and MazEF systems are ubiquitous in both Gram-positive and Gram-negative bacteria and have been extensively reviewed elsewhere (Condon, 2006; Gerdes, Christensen, & Løbner-Olesen, 2005; Hayes & Van Melderen, 2011), and as a result, will not be discussed in detail here.

Shortly after their description, homologs of plasmid-encoded PSK systems were identified on bacterial chromosomes (Masuda, Miyakawa, Nishimura, & Ohtsubo, 1993; Poulsen, Larsen, Molin, & Andersson, 1989). Evaluation of accumulating genomic sequences has revealed that most bacterial chromosomes encode multiple homologs of both Type I and Type II PSK systems (Fozo, Makarova, Shabalina, Yutin, Koonin, & Storz, 2010; Leplae, Geeraerts, Hallez, Guglielmini, Drèze, & Van Melderen, 2011). Since these chromosomal homologs clearly are not involved in post-segregational killing, they have been referred to as toxin-antitoxin or TA modules. This designation is now commonly applied to both plasmid and chromosomal systems. The precise role of many of these systems is still obscure, and in some cases, is controversial (Hayes, 2003). These systems have been implicated in stress response, persistence, programmed cell death, biofilm formation, and maintenance of integrated mobile genetic elements. Numerous extensive reviews have been published on TA

systems, of which a few of the most recent are cited here (Fozo, Hemm, & Storz, 2008; Hayes & Van Melderen, 2011; Van Melderen, 2010; Wang & Wood, 2011). This section will focus only on those systems that have been identified in the enterococci.

Type I systems: *par*$_{pAD1}$ and its relatives

The pAD1 *par* determinant was originally identified as a locus required for maximal stability of the plasmid's basic replicon (Weaver, Clewell, & An, 1993). The first indication that *par* might be a PSK/TA module came from the investigation of a serendipitously isolated pAD1 mini-plasmid that triggered host cell death when induced with pheromone (Weaver & Clewell, 1989). Later work showed that this phenomenon resulted from the fortuitous fusion of a pheromone-inducible promoter, P_0 (or the generic P_i described in the section above on conjugation) to RNA I (Weaver, Jensen, Colwell, & Sriram, 1996; Weaver & Tritle, 1994), an mRNA that encodes the 33–amino-acid peptide toxin of the *par* locus Fst. Sequence and RNA analysis identified a short transcript convergently transcribed and partially complementary to RNA I (384), which is designated RNA II. It was later demonstrated that RNA II was capable of counteracting the toxic effects of RNA I both *in cis* and *in trans*, which confirms its role as the antitoxin of the system (Greenfield & Weaver, 2000; Weaver, Jensen, Colwell, & Sriram, 1996). It was further demonstrated that the *par* locus, which is contained on a fragment of 457 bp, stabilized heterologous plasmids at the expense of host cell growth, which confirms its role as a PSK system (Weaver K. E., 1995; Weaver, Jensen, Colwell, & Sriram, 1996; Weaver, Walz, & Heine, 1998).

The genetic organization of *par* and the structure of its transcripts are shown in Figures 6 and 7. The *par* RNAs are convergently transcribed and share a bidirectional intrinsic terminator. The terminator loop provides one region of complementarity at which the two RNAs interact. The RNAs are also transcribed across a pair of direct repeats, DRa and DRb, which provide a second region of complementarity between RNA I and RNA II. Interaction at both the terminator loop and the direct repeats is essential for the proper regulation of RNA I translation, but the function of these interactions differs. The interaction between RNA I and RNA II is initiated at a U-turn motif that is present in the terminator loop of RNA I (Greenfield, Franch, Gerdes, & Weaver, 2001). These motifs, with a consensus sequence of YUNR, have been demonstrated to accelerate the rate of RNA-RNA interaction in a variety of systems (Franch & Gerdes, 2000). Indeed, mutations in the terminator loop reduce the rate of interaction of the two RNAs *in vitro* (Greenfield, Franch, Gerdes, & Weaver, 2001) and abrogate RNA II-mediated protection *in vivo*, which suggests that the rate of interaction is important to translational suppression (Greenfield & Weaver, 2000; Shokeen, Patel, Greenfield, Brinkman, & Weaver, 2008). Following the initial reversible interaction between the terminator loops, binding is rapidly extended to the DRa and DRb repeats that sequester the initiation codon, interfere with ribosomal binding, and inhibit translation of the toxic peptide, Fst (Greenfield & Weaver, 2000; Shokeen, Patel, Greenfield, Brinkman, & Weaver, 2008).

Two RNA I intramolecular structures, 5′-SL and 5′-UH (which are boxed and labeled in Fig. 7), also impact the regulation of *par* function. The 5′-SL is a stem-loop structure that sequesters the ribosome-binding site for the Fst open reading frame, which suppresses translation (Greenfield, Ehli, Kirshenmann, Franch, Gerdes, & Weaver, 2000; Shokeen, Patel, Greenfield, Brinkman, & Weaver, 2008). Translational suppression is not complete, since low levels of translation can be observed *in vitro* and wild-type RNA I is toxic *in vivo* in the absence of RNA II. However, mutations that destabilize the 5′-SL cannot be established in cells that express RNA II (Shokeen, Patel, Greenfield, Brinkman, & Weaver, 2008), in spite of the fact that RNA II is capable of binding to and suppressing translation from such mutants *in vitro* (Greenfield, Ehli, Kirshenmann, Franch, Gerdes, & Weaver, 2000). This discrepancy between *in vivo* and *in vitro* results may relate to the timing of RNA I's interaction with its two competing partners, ribosomes and RNA II. Because the interaction between RNA I and RNA II is initiated at the terminator loop, the ribosome binding site is transcribed and available for ribosome binding before RNA II can initiate binding. The 5′-SL is postulated to temporarily inhibit ribosome binding until the terminator loop can be transcribed. It is also possible that RNA I is processed to remove the 5′-SL *in vivo* before it can be translated, but no such processing product has been observed, despite multiple attempts.

The 5′-UH is an "upstream helix" composed of the extreme 5′ end of the RNA I transcript and a complementary sequence further downstream that folds back to interact with it. This helix sequesters the 5′ nucleotides from digestion by cellular RNases, and is at least partially responsible for the greater stability of RNA I relative to RNA II (Shokeen, Greenfield, Ehli, Rasmussen, Perrault, & Weaver, 2009). Mutations in the 5′-UH result in a >4-fold drop in RNA I half-life from >40 minutes to around 9 minutes; the half-life of free RNA II is approximately 4 minutes. Mutation of the 5′-UH makes RNA I more susceptible to RNases J1 and J2, which have 5′ to 3′-exonuclease activity (Mathy, Bénard, Pellegrini, Daou, Wen, & Condon, 2007; Shokeen, Greenfield, Ehli, Rasmussen, Perrault, & Weaver, 2009). Whether these are the primary RNases responsible for degradation of RNA I is not clear. It is also possible that other features of RNA I, such as its relatively inaccessible 3′ end and its compact structure, may also contribute to its stability.

In order for TA loci to function as plasmid stabilization systems, the antitoxin must be less stable than the toxin. Indeed, *par* RNA II is significantly less stable than RNA I, with a half-life of 4 minutes, while RNA I shows negligible decay even after 40 minutes. Interestingly, in the presence of RNA I, RNA II basal levels increase more than two-fold, and its half-life increases to 16 minutes (Weaver, Ehli, Nelson, & Patel, 2004). Similarly, the basal level and stability of the RNA I 5′-UH mutant was increased more than two-fold in the presence of RNA II (Shokeen, Greenfield, Ehli, Rasmussen, Perrault, & Weaver, 2009). These results suggest that formation of the RNA I-RNA II complex protects both RNAs from degradation by cellular RNases. While most regulatory RNAs appear to destabilize their targets, target stabilization is not without precedent (Opdyke, Kang, & Storz, 2004). Indeed, target destabilization would not be an effective mechanism of regulation for Type I PSK systems, since a pool of toxin mRNA must persist in plasmid-free segregants in order to kill the cells. In the *hok-sok* PSK system, newly transcribed *hok* mRNA adopts a conformation in which both the translation initiation region and *sok* interacting region are sequestered by intramolecular secondary structures, which allows for the accumulation of a pool of translationally inactive *hok* mRNA. Slow processing from the 3′ end of the *hok* message results in a conformational change that exposes both the *sok* and ribosome interacting sequences (Franch, Gultyaev, & Gerdes, 1997). If the plasmid is still present, *sok* RNA binds to *hok*, and the complex is rapidly degraded by RNase III (Gerdes, Nielsen, Thorsted, & Wagner, 1992). If the plasmid is lost, ribosomes translate the *hok* toxin, which kills the cell. In the case of *par*, the 5′-SL appears to prevent ribosome binding to RNA I until RNA II binding can occur. The translationally inactive complex then accumulates as a pool in the cells, with RNA I to RNA II ratios maintained at about 1:1.1 (Weaver, Ehli, Nelson, & Patel, 2004). It is possible that the discontinuous nature of the interacting sites in the RNA I-RNA II complex prevents efficient degradation by RNase III, which requires at least two helical turns of double-stranded RNA for binding and activity (301). The lower stability of RNA II suggests that it is preferentially removed from the complex and is degraded by means that have yet to be described. If plasmid remains in the cell, sufficient RNA II is produced to replace that which has been removed from the complex. If the plasmid is lost, degraded RNA II cannot be replaced, the Fst ribosome binding site becomes accessible—either through the processing of the 5′-SL or by the utilization of a ribosomal standby site (de Smit & van Duin, 2003)—and sufficient Fst is produced to kill the cell.

The toxin of the *par* TA module is a 33–amino-acid peptide, designated Fst for *faecalis* stabilizing toxin. The peptide has a charged N-terminus, a hydrophobic central region with features of a transmembrane domain, and a highly charged C-terminal tail. Amino-acid substitution experiments revealed that the central hydrophobic domain was critical for toxin function (Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009) and that charged amino acids in the N terminus were also important. In contrast, the charged C-terminal tail appears to have little contribution to toxin function; a nonsense mutation that resulted in truncation of the C-terminal eight amino acids retained its toxicity.

Fst is toxic to *E. faecalis* (Patel & Weaver, 2006; Weaver, Weaver, Wells, Waters, Gardner, & Ehli, 2003), *S. aureus* (Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009), and *B. subtilis* (Patel & Weaver, 2006) when over-expressed from the native RNA I transcript. Toxicity can also be observed in *E. coli* if the 5′-SL structure is disrupted (Shokeen, Patel, Greenfield, Brinkman, & Weaver, 2008). In all four species, the primary effect is

condensation of the nucleoid. In *E. coli* and *B. subtilis*, this results in elongation of cells, perhaps because the collapsed nucleoid interferes with formation of the division septum at the cell center through nucleoid occlusion (NO) (Wu & Errington, 2011). In *S. aureus*, the division septum forms and invaginates, but the nucleoid is frequently trapped at the convergence point, and the completion of cell division is inhibited. In this case, NO appears to be ineffective in stopping invagination of the cell wall, or Fst abrogates its function. Since new cell wall growth occurs only at the septum (Pinho & Errington, 2003) in *S. aureus*, the presence of a condensed nucleoid effectively blocks both division and growth. In *E. faecalis*, cells initially elongate, then produce mis-placed division septae, and finally mis-segregate the nucleoids producing cells that contain little or no DNA. Since cell wall growth in chaining ovococci, like enterococci, occurs both longitudinally and septally (Morlot, Zapun, Dideberg, & Vernet, 2003), elongation apparently occurs even though the nucleoid trapped at the division site blocks septation. In at least some cells, the partition apparatus mobilizes the condensed chromosome, but only into one of the daughter cells. Thus, specific differences in the effects of Fst may relate directly to fundamental differences in cell growth and division.

The specific target of Fst is unknown. The putative transmembrane domain and its importance to toxin function suggest that it is membrane-localized. However, exposure to Fst, unlike Hok (Gerdes, Rasmussen, & Molin, 1986), does not result in the leakage of cell contents and the formation of "ghost cells." An increase in cell permeability is observed following Fst over-expression, but only after the appearance of cell growth and division anomalies, which suggests that membrane defects may be a secondary effect (Patel & Weaver, 2006; Weaver, Weaver, Wells, Waters, Gardner, & Ehli, 2003). Nisin and Fst have a synergistic effect, which suggests that they have different, but complementary targets (Weaver, Weaver, Wells, Waters, Gardner, & Ehli, 2003). Nisin is a pore-forming lantibiotic that docks on lipid II and also affects peptidoglycan synthesis (Wiedemann, et al., 2001). Unlike nisin, synthetic Fst has no effect on cell growth when added externally (Weaver, Weaver, Wells, Waters, Gardner, & Ehli, 2003), which suggests either that it is modified in some way within the cell, or that is targets a component present only on the inner surface of the membrane or in the cytoplasm. Recent microarray data indicates that exposure to Fst results in the induction of a variety of energy-dependent membrane transporters; interference with this induction by RNA polymerase mutation or interference of ABC transporter activity with reserpine leads to Fst resistance (Brinkman, Bumgarner, Kittichotirat, Dunman, Kuechenmeister, & Weaver, 2013). It is possible that hyperactivity of energy-utilizing membrane transporters depletes the cells of energy, which thereby leads to the observed toxic effect.

An atomic resolution structure of Fst has been determined in the membrane mimetic dodecylphosphocholine (DPC) by NMR spectroscopy (Göbl, Kosol, Stockner, Rückert, & Zangger, 2010). These results indicated that Fst forms a transmembrane α-helix with the first two and the last seven amino acids protruding. The charged C-terminal seven amino acids are disordered and were predicted to extend from the cytoplasmic side of the membrane. These authors suggested that the primary function of membrane insertion was to facilitate interactions with a specific target, rather than being directed against the membrane itself. They also predicted that the disordered C–terminus might become structured upon recognition of the target, but this conclusion conflicts with mutagenic studies that indicate that the last eight amino acids are not required for toxicity (Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009).

Work by several groups has revealed that Fst belongs to a large family of RNA-regulated peptide toxins (Fozo, Hemm, & Storz, 2008; Fozo, Makarova, Shabalina, Yutin, Koonin, & Storz, 2010; Kwong, Jensen, & Firth, 2010; Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009). These peptides are smaller than 60 amino acids, are hydrophobic, and are predicted to contain an α-helical transmembrane domain. Indeed, many of the smaller peptides may consist solely of the transmembrane helix. Most are toxic when over-expressed in their native hosts (Fozo, Hemm, & Storz, 2008). An exhaustive bioinformatic search across 774 bacterial genomes identified hundreds of these peptides in the γ-proteobacteria and Firmicutes that were divided into eight families (Fozo, Makarova, Shabalina, Yutin, Koonin, & Storz, 2010). Fst is the founding member of the Fst/Ldr family of peptide toxins, which in this analysis consisted of 161 members. In addition, Kwong, *et al*. reported the identification of

more than 200 Fst related peptides in a diversity of Gram-positive bacteria (Kwong, Jensen, & Firth, 2010). While there is likely significant overlap between these two lists, it seems apparent that Fst-related peptides are ubiquitous in Gram-positive bacteria and that the related Ldr peptides are prevalent in the γ-proteobacteria. Although Fst homologs were frequently present on mobile genetic elements, phylogenetic analysis showing coherence between the phylogeny of the peptides and their hosts of origin suggests that their distribution is not due to recent horizontal gene transfer (Fozo, Makarova, Shabalina, Yutin, Koonin, & Storz, 2010). The gene organization of the Fst/Ldr-homologs further distinguishes the two subgroups. Examination of the DNA sequences that surround the Fst-like peptides revealed the existence of all of the elements originally defined in the pAD1 *par* locus, including the convergent promoters for RNA I and RNA II transcripts, a bi-directional intrinsic terminator, the DRa and DRb interacting sequences, and sequences that provide the 5′-SL and 5′-UH of RNA I, which suggests that they may be regulated in a similar manner to *par* (Kwong, Jensen, & Firth, 2010; Weaver, Kwong, Firth, & Francia, 2009). In contrast, the regulatory RNA of the Ldr message, the Rdl-RNA, is transcribed from the opposite strand of the 5′ end of its target in a manner reminiscent of the *hok/sok* system (Gerdes & Wagner, 2007; Kawano, Oshima, Kasai, & Mori, 2002). Nevertheless, overexpression of Ldr in *E. coli* produces nucleoid condensation effects similar to those seen upon over-expression of Fst, which indicates functional as well as sequence homology (Kawano, Oshima, Kasai, & Mori, 2002). Whether this is a case of variation in regulation of an ancestral locus or convergent evolution is unclear.

It is important to note that, while the general features of the Fst-encoding *par* homologs are conserved, their sequences are not, particularly in the regions that are predicted to provide interactions between the Fst message and its regulatory RNA (Kwong, Jensen, & Firth, 2010; Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009). This feature would allow related *par* systems present on different plasmids to operate in the same cell without interfering with one another. Furthermore, a number of *par* homologs are chromosomally encoded. While many of these are associated with integrated MGE or their remnants, some are not. For example, the Fst homolog EF0409 is located between genes that appear to be associated with mannitol transport and metabolism, and is present in all sequenced *E. faecalis*, but not *E. faecium* strains. Recent work in the Weaver laboratory (unpublished) indicates that it neither interferes with nor is essential for pAD1 *par* function.

Chromosomal homologs of both Type I and II TA systems are common in bacteria, and their function is a matter of ongoing debate (Gerdes, Christensen, & Løbner-Olesen, 2005; Hayes F. , 2003; Van Melderen, 2010). Some chromosomal TA systems are clearly associated with integrated MGE, including plasmids, prophage, pathogenicity islands, and ICE elements, and could serve to facilitate retention of these elements in the absence of overt selection for them. Others are not apparently associated with MGE and have been suggested to perform various functions in response to stress, including suppression of translation, formation of a subpopulation of persister cells, and apoptosis. Others have been suggested to be merely selfish elements that ensure nothing but their own maintenance. It seems likely that no single function will apply to all chromosomally encoded TA systems. Five of the *par* homologs not associated with MGE are intimately linked to genes involved in carbohydrate metabolism (Kwong, Jensen, & Firth, 2010; Weaver, Reddy, Brinkman, Patel, Bayles, & Endres, 2009). This includes the *E. faecalis* EF0409 locus described above, the *Staphylococcus saprophyticus* SSP0870 locus situated between genes for 6-phosphogucono-lactonase and an aldehyde dehydrogenase, the *Lactobacillus caseii* LSEI2682 locus situated between genes for a mannose-6-P isomerase and a two-component signal transduction system, a locus in *S. aureus* MRSA252 located between genes that encode a putative ABC transporter and glycerate kinase, and a *Listeria monocytogenes* locus downstream of a gene that encodes a glycosyl hydrolase. The locations of these *par* homologs, along with the association of Fst effects with ABC transporters, are suggestive of a role in fine-tuning carbohydrate metabolism. This possibility is under active investigation.

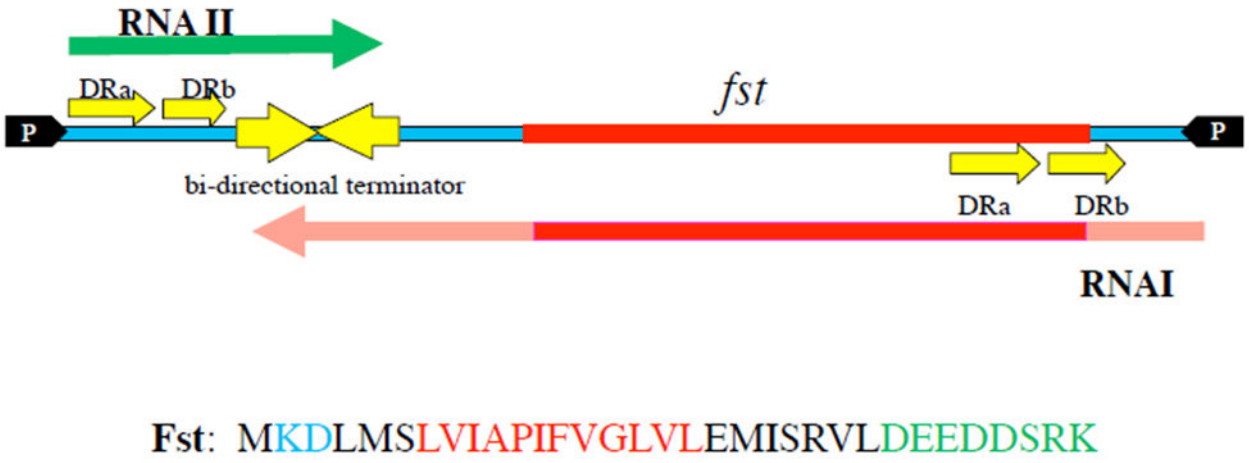

Figure 6. Organization of the pAD1 *par* locus. Converging promoters (black arrowheads labeled P) transcribe the toxin-encoding RNA I (red arrow) and the antitoxin RNA II (green arrow) toward a bi-directional intrinsic transcriptional terminator (converging yellow arrows). The RNAs are transcribed across direct repeats (yellow arrows labeled DRa and DRb) at which interaction occurs, which suppresses translation of the Fst coding sequence (red box on blue line). The protein sequence of the Fst toxin is shown below using standard single-letter amino-acid designations. The essential, conserved hydrophobic domain is shown in red. This forms part of a transmembrane domain in the recently published structure of Fst (142). The two amino acids in blue at the N-terminus must be charged to retain toxin function. The non-essential C-terminal tail is shown in green.

Type II (Proteic) systems

a. ωεζ

The ωεζ PSK system was originally identified and characterized on the *S. pyogenes* plasmid pSM19035 (Ceglowski, Boitsov, Karamyan, Chai, & Alonso, 1993; Lioy, Pratto, de la Hoz, Ayora, & Alonso, 2010). An identical locus is present on the related plasmid, pAMβ1 from *E. faecalis* strain DS5 (Clewell, Yagi, Dunny, & Schultz, 1974), and related loci have been identified on the pheromone plasmid pSL1 from *E. faecalis* (Lim, Tanimoto, Tomita, & Ike, 2006), as well as several vancomycin-resistance plasmids from *E. faecium* (Rosvoll, et al., 2010; Sletvold, Johnsen, Hamre, Simonsen, Sundsfjord, & Nielsen, 2008; Sletvold, Johnsen, Simonsen, Aasnæs, Sundsfjord, & Nielsen, 2007). pSM19035, pAMβ1, and the *E. faecium* plasmids are members of the Inc18 plasmid family (see above).

The ε and ζ proteins are the antitoxin and toxin components, respectively, of the pSM19035 system. As in other TA systems, the ε antitoxin is less stable than the ζ toxin and is specifically targeted by the ATP-dependent protease Lon *in vivo*, although *in vitro* studies have failed to reconstitute degradation of ε from the toxin-antitoxin complex. *In vivo* ε has a half-life of <20 minutes, while ζ has a half-life >60 minutes (Camacho, et al., 2002; Lioy, et al., 2006). The toxin and antitoxin form an extremely stable heterotetramer complex organized as $\zeta\varepsilon_2\zeta$. Structural data and mutagenesis experiments revealed that the ζ toxin contains a P-loop NTPase superfamily domain, and indicated that toxicity is due to an ATP-dependent phosphorylation event. ε antitoxin was inferred to interfere with the binding of ATP at the P-loop of the toxin in the $\zeta\varepsilon_2\zeta$ complex, thereby inhibiting toxin function (Meinhart, Alonso, Sträter, & Saenger, 2003).

Unlike most Type II TA systems, which are autoregulated by their antitoxin components, transcription of the εζ TA pair is regulated by a third component, ω. ω, ε, and ζ are transcribed in order as a tricistronic message from the P_ω promoter, which is repressed by the binding of ω dimers (ω_2) to seven binding sites within the promoter

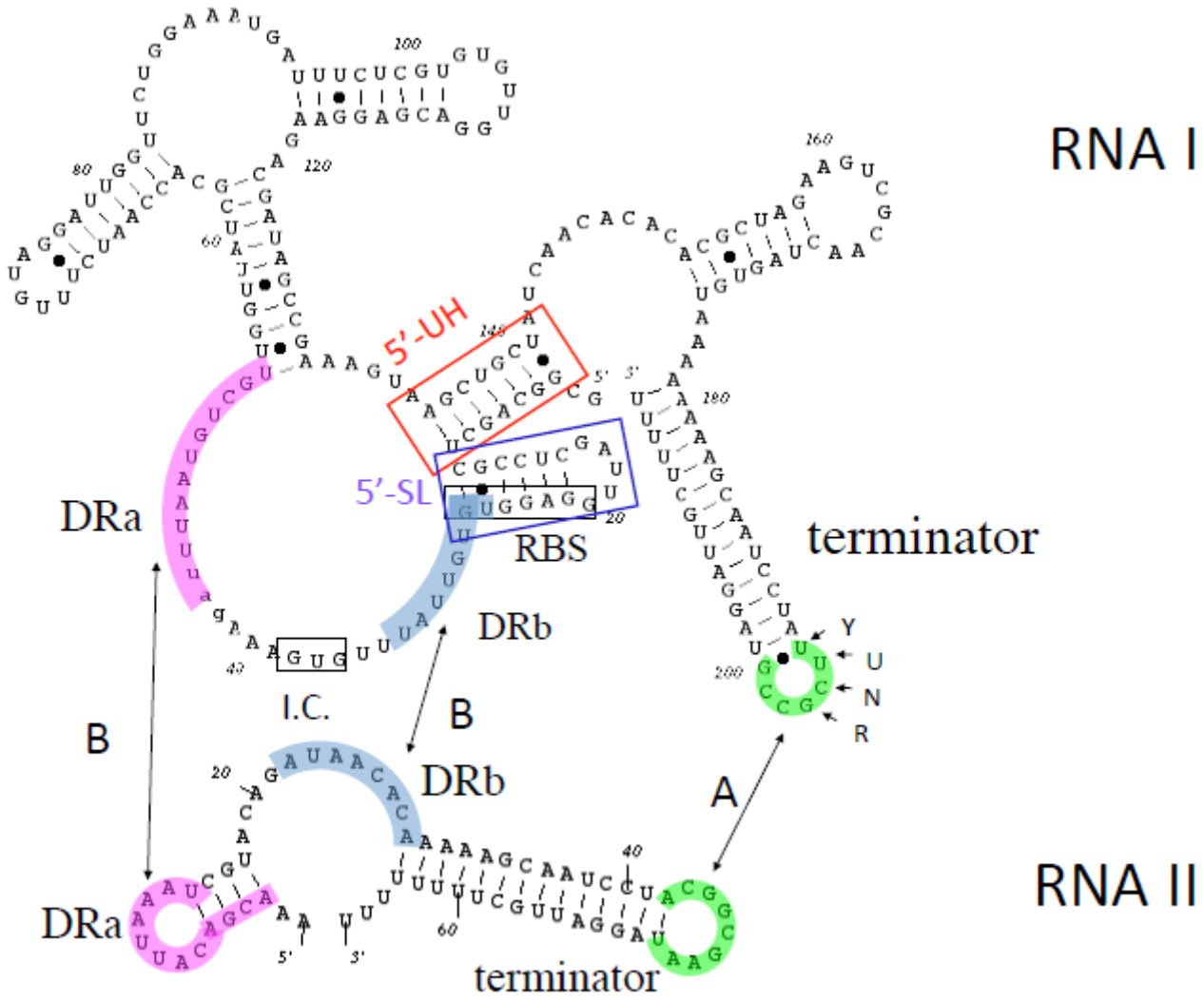

Figure 7. Secondary structure of RNA I and RNA II. The terminator region and the direct repeats (DRa and DRb) are shaded pink and blue, respectively. The two 5′ structures, 5′-SL and 5′-UH, of RNA I are boxed and labeled as are the fst ribosome binding site (SD) and initiation codon (I. C.). The two RNAs have three dispersed complementary segments. Interaction between the two RNAs is initiated at the U-turn motif (labeled YUNR) present in the loop of the terminator of RNA I (green shaded). This interaction is indicated by the arrow labeled A. The interaction then extends to the direct repeat sequences (with interaction indicated by arrows labeled B) and prevents the translation of the toxin, Fst, since the initiation region of the toxin is overlapped by the interacting RNAs.

region. This binding inhibits transcription without inhibiting RNA polymerase promoter binding (de la Hoz, et al., 2000). ε and ζ are also expressed from a weak promoter, $P_ε$, upstream of the ε gene, which provides a low basal level of the two TA components in the presence of $ω_2$-mediated repression. It is hypothesized that, in the event of a downward fluctuation in copy number, $ω_2$ levels drop, which leads to increased production of ε and ζ to a threshold level at which the amount of stable ζ protein exceeds the level of the unstable ε protein, and leads to toxin expression. $ω_2$ acts to repress not only the ωεζ operon, but also the copy control function CopS and the active partition protein δ, thereby coordinating the replication and stability functions.

Work with the pSM19035 system suggested that the effects of ζ toxin are bacteriostatic, inducing a viable but non-culturable (VBNC) state in which most major metabolic pathways are inhibited. The effects of ζ over-expression can be partially reversed by expression of the ε antitoxin, but a proportion of cells (~20%) are killed by lysis (Lioy, et al., 2006). Recently, a chromosomal homolog of ζ toxin, PezT of *Streptococcus pneumoniae*, was

demonstrated to be a kinase that phosphorylates the nucleotide sugar uridine diphosphate-N-acetylglucosamine (UNAG), a key cell wall precursor (Mutschler, Gebhardt, Shoeman, & Meinhart, 2011). This activity inhibits cell wall synthesis by depleting the pool of UNAG required for peptidoglycan synthesis and by competitive inhibition of MurA by the UNAG-3P product. The difference between the predominantly bacteriostatic effect of ζ toxin over-production and the lytic effect of PezT over-production may simply reflect a difference in levels of the two toxins produced. Interestingly, ζ toxin has been shown to inhibit yeast cell growth, as well as bacterial cell growth (Zielenkiewicz, Kowalewska, Kaczor, & Cegłowski, 2009). Since yeast do not produce peptidoglycan, inhibition of these cells must occur through a different mechanism.

As observed in most other TA systems, the toxin proteins of ωεζ-like systems are more highly conserved than the antitoxin proteins, which probably reflects the constraints that are required to maintain biological activity. Even though the PezT toxin shares only 42% homology with ζ, their 3D structures are identical (Khoo, et al., 2007; Meinhart, Alonso, Sträter, & Saenger, 2003). ω, on the other hand is perfectly conserved in plasmid systems, but is absent from the chromosomal systems, which indicates that it performs plasmid-specific functions. In the PezAT chromosomal system, the PezA antitoxin functions as an autorepressor with PezT as a corepressor, as in canonical Type II TA systems (Khoo, et al., 2007), which suggests that the εζ TA pair was brought under ω control to more efficiently coordinate its expression with other plasmid-maintenance functions.

b. Axe-Txe

The Axe-Txe locus was originally described on the multi-resistance plasmid pRUM from a clinical isolate of *E. faecium* (Grady & Hayes, 2003). It was demonstrated to stabilize heterologous plasmids in its native host, as well as in *B. thuringiensis* and *E. coli*, and over-expression of Txe was shown to be toxic in *E. coli*. Toxicity was alleviated by coexpression of Axe, which confirmed that Axe-Txe was a TA locus. An identical locus was later identified on a vancomycin-resistance plasmid related to pRUM, designated pS177, in a second *E. faecium* clinical isolate (Halvorsen, Williams, Bhimani, Billings, & Hergenrother, 2011). The Txe toxin was shown to inhibit translation by cleaving mRNA one base downstream of the AUG initiation codon in *E. coli*. These results are consistent with phylogenetic analyses, which suggests that Txe belongs to the RelE superfamily of RNA interferases, a widespread group of proteins found on both bacterial plasmids and chromosomes that possess ribosome-dependent RNase activity (Condon, 2006; Gerdes, Christensen, & Løbner-Olesen, 2005). RelE toxins are commonly encoded in TA systems with RelB antitoxins. However, the Axe antitoxin is more closely related to Doc, the antitoxin for the unrelated Phd toxin, suggesting that recombinational "mixing and matching" has occurred in the evolution of TA systems (Hayes & Van Melderen, 2011). pS177 also encodes a *relBE* TA system, designated $relBE_{Ef}$. It is not known whether this locus is functional.

Work on a chromosomally-located *relBE* locus in *E. coli* suggests that the RelE "toxin" is not bactericidal (Pedersen, Christensen, & Gerdes, 2002). Cells affected by RelE over-expression can be rescued by subsequent expression of the antitoxin. It has been postulated that the function of chromosomally-encoded RNA interferases is to rapidly and temporarily halt translation during periods of nutritional stress, which puts cells into a kind of stasis from which they can awaken when circumstances become more favorable for their growth. Similarly, the effects of ζ toxin were also shown to be reversible by over-expression of the ε antitoxin (Lioy, et al., 2006). The fact that the activities of some toxins have been shown to be reversible begs the question of whether the function of PSK "toxins" in general is to kill plasmid-free segregants. In this regard, it is important to note that most studies of toxin function have relied on regulated over-production of the toxin, and it is unlikely that toxins ever reach such high levels under natural circumstances. It is possible that the real role of plasmid-encoded PSK systems is simply to delay cell division in the case of downward fluctuations in plasmid copy number in order to allow sufficient time for plasmid replication to restore homeostasis. Once plasmid copy numbers are restored and antitoxin levels are increased, the effects of the toxin could be reversed and the plasmid-containing cell would be free to grow. Alternately, in the case of conjugative plasmids (particularly in chained bacteria like enterococci), the division of plasmid-free segregants could be delayed long enough for cells

to regain a copy of the plasmid by conjugation. Evaluation of these possibilities awaits methods that will allow the investigation of toxin and antitoxin levels expressed from their native loci in single cells.

On the Categorization of Plasmids

Plasmids have been traditionally classified as RCR or theta based on their mode of replication, and Table 1 lists currently known plasmids in enterococci accordingly, with theta replication being the most prevalent. As described in the earlier section on plasmid replication, plasmids can be further subdivided into six families, based on the presence of conserved domains in their replication initiators: Rep_3, Inc18 and RepA_N families of theta-replicating plasmids and Rep_trans, Rep_1 and Rep_2 classes of RCR plasmids. This classification scheme was initially developed by Jensen *et al.* (Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010), and the related multiplex-PCR approach has recently been applied to different collections of enterococcal isolates (Freitas, et al., 2013; Freitas, et al., 2012; Garcia-Migura, Sanchez-Valenzuela, & Jensen, 2011; Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010; Romo, et al., 2008; Rosvoll, et al., 2010). Table 2 provides an updated list of enterococcal replication initiators that cluster into the above six families. As reflected in the table, a number of plasmids encode more than one replicon (up to three different initiator sequences), which hampers their definitive classification.

While sometimes multiple replication initiators are encoded, plasmids rarely carry multiple determinants for mobilization. Thus, a categorization based on the latter could serve as an alternate classification scheme. Mobile plasmids are characterized by the presence or absence of: i) a MOB region, which is involved in DNA processing and provides the relaxase, accessory proteins, and an origin of transfer (*oriT*); and ii) a MPF (mating pair formation) region that includes construction of the mating channel. Self-transmissible elements carry both MOB and MPF regions, whereas mobilizable elements encode just the MOB module and need the MPF genes from a co-resident conjugative element for mobilization. As relaxases are crucial for initiation of DNA transfer, their amino acid sequences can serve as the basis of a classification criterion. Seven families of relaxases have thus far been described: MOB_P, MOB_F, MOB_H, MOB_Q, MOB_V, MOB_C, and MOB_T (Francia, Varsaki, Garcillán-Barcia, Latorre, Drainas, & de la Cruz, 2004; Garcillán-Barcia, Francia, & de la Cruz, 2009; Smillie, Garcillán-Barcia, Francia, Rocha, & de la Cruz, 2010). It is fitting that this classification extends to the entire MOB region (which also includes the *oriT* and the nicking accessory proteins).

A grouping of the enterococcal mobile elements whose DNA sequences were deposited in GenBank by the end of December 2011 into mobilization families was performed, based on PSI-BLAST searches using the N-terminal 300 amino acids of prototype relaxases, as previously described (Garcillán-Barcia, Francia, & de la Cruz, 2009; Guglielmini, Quintais, Garcillán-Barcia, de la Cruz, & Rocha, 2011). Relaxases encoded in contigs derived from enterococcal genome sequencing projects were not included, unless a VirB4-like and/or a T4SS coupling protein (T4CP) gene were also identified in its proximity. Close co-occurring relaxase-like, T4CP-like and VirB4-like hits were indicative of putative conjugative elements (Guglielmini, Quintais, Garcillán-Barcia, de la Cruz, & Rocha, 2011), and in some cases, the determinants were related to integrative elements (ICEs) rather than plasmids. Relaxases from mobile plasmids, as well as ICEs, in enterococci were found to cluster into five families: MOB_P (17 plasmids and 4 ICEs), MOB_Q (6 plasmids), MOB_V (8 plasmids), MOB_C (6 plasmids and 1 ICE), and MOB_T (12 ICEs and 1 plasmid) (Table 3). MOB_F or MOB_H relaxases were not found in enterococci, while the MOB_P family was clearly the most represented with 21 members. MOB_Q, MOB_C, and MOB_T were found only in conjugative elements. In contrast, MOB_V was present only in mobilizable plasmids. MOB_P is found in both conjugative and mobilizable elements. MOB_P is the most diverse family and can be found associated with RepA_N, Inc18-like and small theta-replicating plasmids, including Rep_3 elements, while MOB_C comprises RepA_N and Inc18-like plasmids. MOB_Q is associated with a group of broad host range Inc18-like plasmids and MOB_V includes RCR and small theta plasmids. MOB_T relaxases are actually members of the Rep_trans superfamily of replication initiation factors.

This classification scheme can be easily applied to any newly sequenced plasmid, with only BLAST alignments needed to assign the relaxase to one of the MOB families. The related multiplex-PCR approach (Goicoechea, et al., 2012) has been used to identify the relaxase determinants in different collections of enterococcal isolates. Only three examples of more than one relaxase have been identified thus far: pAMα1, pCF10, and pMG2200. As previously described, pAMα1 is a cointegrate of two mobilizable plasmids (Francia & Clewell, 2002) while pCF10 and pMG2200 are pheromone-responding plasmids that contain a copy of a tetracycline resistance Tn*916*-like element and a vancomycin-resistance Tn*1549*-like element, both of which encode relaxases, respectively (Hirt, et al., 2005; Zheng B. , Tomita, Inoue, & Ike, 2009).

The implementation of these typing schemes has revealed a significant degree of mosaicism, which mainly involves the Inc18 and RepA_N families of plasmids (Freitas, et al., 2013). It shows a variable content of replication and relaxase determinants that deserve attention, as these elements carry important adaptive traits such as antibiotic resistance, bacteriocins, or virulence (see Table 1).

Epidemiology and the Flow of Mobile Genetic Elements: Opportunities and Limitations

Enterococci are recovered from a diversity of environments that include dairy products, foodstuffs, biofilms on food-processing and medical equipment, and the gastrointestinal tracts and oral cavities of animals and humans —locations where horizontal gene transfer (HGT) between groups of bacterial species readily occurs (Aarestrup, Butaye, & Witte, 2002; Palmer, Kos, & Gilmore, 2010). The ever-increasing numbers of reports of enterococcal strains that have acquired new adaptive traits that enable them to survive in different conditions (such as resistance to antibiotics, biocides, heavy metals, and different metabolic capabilities, or the ability to colonize different epithelial tissue or cause infection) illustrates the role of HGT in the combinatorial evolution of these microorganisms. Phylogenomic analyses have revealed the influence of HGT in the evolutionary trajectories of some sequence types (ST) within major clonal complexes of *E. faecium* and *E. faecalis*, and also in determining strain-specific properties (de Regt, et al., 2012; van Schaik, et al., 2010). Although other enterococcal species have not been studied in such detail, we might consider each one as constituted by a variety of populations with individual properties, which enables adaptation to particular conditions and therefore likely to have been influenced by HGT. The number of acquired genes that enterococci share with other species and genera from related habitats supports this notion (see Enterococcus Diversity, Origins in Nature, and Gut Colonization and Enterococcal Genomics).

Two main post-transfer barriers protect a given host cell from invasion by foreign DNA: *restriction-modification* (RM) *systems,* and *clustered, regularly interspaced short palindromic repeats* (CRISPR). Little is known about the diversity of RM and anti-RM systems associated with enterococci. To date, the presence of anti-RM systems is confined to analogs of ArdA (alleviation of restriction of DNA) proteins that act against Type I restriction systems (detected in Tn*916* and CTn*6000,*) and other genes predicted to be involved in methylation (e.g. in CTn*6000*) (Brouwer, Mullany, & Roberts, 2010; Serfiotis-Mitsa, et al., 2008). Interestingly, the presence of RM in pathogens such as *S. aureus* has been observed to limit the transfer of enterococcal DNA to this species (Corvaglia, François, Hernandez, Perron, Linder, & Schrenzel, 2010; Sung & Lindsay, 2007; Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008). CRISPRs are defence systems that provide a type of acquired immunity against specific sequences [for a more complete review, see Marraffini & Sontheimer, 2010]. There is evidence that the presence of complete CRISPR loci is inversely related to the presence of MGE (Palmer & Gilmore, 2010), a relationship that, in retrospect, is not surprising.

Whereas plasmids with different mechanisms of replication, conjugation abilities, and host ranges have been described in enterococci, their occurrence varies between species and strain origin. For example, while Inc18 plasmids appear to be widely distributed among different species, other plasmid types seem to be confined to either *E. faecium* (e.g. RCR pRI1-like, small theta pCIZ2-like, and pRUM-like plasmids, as well as a variety of

megaplasmids) or *E. faecalis* (e.g. pheromone-responsive plasmids) (Freitas, et al., 2013; Freitas, et al., 2010; Leon-Sampedro, et al., 2012). Antibiotic resistance is frequently associated with Inc18, pRUM-like, and pheromone-responsive plasmids. In addition, mosaics are often reported, and relevant epidemiological differences are common (Table 1).

The various sources of enterococcal plasmids indicated in Table 1 illustrate only the "tip of the iceberg" when considering the vast populations of organisms with the potential to colonize and adapt to specific environments. The presence of plasmid-borne passenger genes should reflect an epidemiological "history" of sorts. Traits carried on transferable elements can be kept within the host bacterium ("private" traits") or secreted ("public traits"), based on whether they make their hosts "helpers" or "harmers" of neighboring cells, respectively (Rankin, Rocha, & Brown, 2011). "Private" traits would comprise a wide diversity of genes involved in basic and adaptive functions, while "public traits" would comprise secreted proteins that are involved in capturing resources (such as siderophores), metabolic expansion (such as the catabolism of emerging energy resources, such as complex carbohydrates and polycyclic aromatic hydrocarbons), biofilm formation (traits that enhance adhesion), killing of competing lineages (such as bacteriocins), exploitation of hosts (virulence factors), or addictive systems (such as toxin-antitoxin systems). Such characteristics, which are commonly associated with plasmids, can play important roles as enterococci evolve. Indeed, many of the related determinants are able to move between plasmids and between chromosome and plasmid because of their association with site-specific recombination systems. Such elements correspond to transposons that are able to move from one site to another, and, in some cases, that use flanking insertion sequences to facilitate movement. Of course, insertion sequences themselves are an important component of the bacterial mobilome, in that they can "jump" independently or serve as portable regions of homology able to facilitate homologous recombination, which enables integration or excision of plasmids into or from other replicons. As discussed below, all of these elements are ubiquitous in enterococci.

Movement between replicons

Insertion sequences and composite transposons

ISs have been identified in different enterococcal species that belong to 10 IS families that are widespread among *Firmicutes* (Table 4). Some ISs that are multiply represented in the chromosomes, and MGEs of both *E. faecalis* and *E. faecium* [e.g. IS*1216* and IS*257* (IS*6* family), IS*Ef1*/IS*6770* (IS*30* family) and IS*16* and IS*256* (IS*256* family)] have been useful for typing purposes (Leavis H. L., Willems, van Wamel, Schuren, Caspers, & Bonten, 2007; Rice & Thorisdottir, 1994; Thorisdottir, et al., 1994; Werner, Fleige, Geringer, van Schaik, Klare, & Witte, 2011). Additionally, a role in the formation of functional promoters for biofilm production in virulent *E. faecalis* strains has been suggested for IS*1191* (Coburn, Baghdayan, Dolan, & Shankar, 2008).

Composite transposons that are flanked by terminal IS elements and encode antibiotic resistance are common among enterococci, and range in size from 4.7 to 65 kb (Table 5a). Examples of widespread composite transposons are Tn*5405*, which encodes aminoglycoside and streptothricin resistance, Tn*1547,* which confers vancomycin resistance, and elements that encode resistance to high levels of gentamicin (Tn*5281*, which contains a tandem duplication of IS*256* at one terminus of Tn*4001*, a Tn*4001*-IS*257* hybrid, and different Tn*4001*-truncated elements that can also be included in larger platforms such as Tn*5384*, Tn*5385*, or Tn*924*). While Tn*5405* and Tn*4001* derivatives have been identified in commensal enterococci, staphylococci, and streptococci from pets, farm animals, and humans (mainly associated with worldwide-spread elements such as Inc18 plasmids), Tn*1547* has been identified in a few *E. faecium* strains from the USA and Europe. ISs are frequently involved in the genesis of novel IS-based composite transposons in enterococci that yield composite mosaic platforms (Bonafede, Carias, & Rice, 1997; Rice & Carias, 1998; Rice & Marshall, 1994).

Tn*3*- and Tn*7*-related elements

Among enterococci, Tn*3*-family tranposons are represented by Tn*1546* and Tn*917*, which closely resemble Tn*551* in staphylococci (Arthur, Molinas, Depardieu, & Courvalin, 1993; Grindley, 2001; Shaw & Clewell, 1985). Although they have a highly related transposase (TnpA), they differ in transposon organization, particularly in the relative orientation of the *tnpR*, with Tn*917* resembling the Tn*501* organization and Tn*1546* being similar to that of Tn*3* (Grindley, 2001). Tn*1546* has an atypical *res* site related to the β recombination systems of Inc18 plasmids pSM19035 and pAMβ1 (Arthur, Molinas, Depardieu, & Courvalin, 1993; Grindley, 2001).

Tn*1546* (10.8-kb) contains a cluster of 7 *van* genes (*vanRSHAXYZ*) that code for resistance to vancomycin and teicoplanin (Arthur, Molinas, Depardieu, & Courvalin, 1993; Werner, et al., 2008). A large number of Tn*1546* variants that include deletions, rearrangements and/or insertions have been described, and their differences have been exploited for epidemiological purposes (Jensen, Ahrens, Dons, Jones, Hammerum, & Aarestrup, 1998; López, et al., 2010; Novais, et al., 2008; Willems, et al., 1999; Woodford, Adebiyi, Palepou, & Cookson, 1998). They are often found on members of Inc18 and RepA_N, plasmids, which has probably facilitated their wide distribution among enterococci and non enterococcal species isolated from animals, humans, and soil (Clark, Weigel, Patel, & Tenover, 2005; Flannagan, et al., 2003; Freitas, et al., 2013; Freitas, et al., 2012; Guardabassi & Agersø, 2006; Guardabassi, Perichon, van Heijenoort, Blanot, & Courvalin, 2005).

Tn*917* (5.3-kb) and its highly related variant Tn*3871*, contain the *ermB* gene that encodes macrolides-lincosamide-streptogramin B (MLS_B) resistance (Banai & LeBlanc, 1984; Shaw & Clewell, 1985; Tomich, An, & Clewell, 1980), and its transposition, as well as the expression of resistance, is inducible by erythromycin (Tomich P. K., An, Damle, & Clewell, 1979). These transposons seem to be widely spread among humans and farm animals since the early 1980s (LeBlanc, Inamine, & Lee, 1986; Rollins, Lee, & LeBlanc, 1985).

An example of production of beta-lactamase among enterococci (Murray, 1990; Sarti, Campanille, Sabia, Santagati, Gargiulo, & Stefani, 2012) is due to the *blazZ-blaI-blaR1* operon located on Tn*552*, a transposon that is widely spread among staphylococcal plasmids. Tn*552* is a Tn*7*-like element with an atypical transposition module that includes a TnsB-like transposase homologous with DNA integrases of eukaryotic retroviruses and retrotransposons, a putative ATP-binding protein similar to the B protein of phage Mu, and a co-integrate resolution system homologous with those of Tn*3* family *resL binL* (Rowland & Dyke, 1990). Although Tn*552* has been identified among globally spread CC2 *E. faecalis* and CC17 *E. faecium* clinical isolates that belong to main human lineages ST17, ST18, and ST78 previously considered as CC17, its occurrence remains rare among enterococci (75, 251, 312, 393). A Tn*552* variant that lacks regulatory *blaI* and *blaR1* genes due to an IS*256* insertion has been identified on plasmids of *E. faecalis* isolates that belong to CC2 (ST6) recovered in the mid-1980s from certain American states and Argentina (Murray, 1990). A full copy of the Tn*552* element surrounded by sequences related to the α-family of staphylococcal plasmids was identified on a large composite structure designated Tn*5384*, located on the chromosome of *E. faecalis* isolates of the clonal complex 9, that caused an outbreak in Boston in the early 1980s (Murray, 1990; Rice L. B., Carias, Marshall, & Bonafede, 1996). Only two reports have documented the plasmid location of the *blaZ-blaI-blaR1* operon among *E. faecium* isolates. One describes a single isolate in an American hospital with endemic presence of *E. faecalis* beta-lactamase producers (Coudron, Markowitz, & Wong, 1992); the other documents eight *E. faecium* clinical isolates that belong to the main human lineages ST17, ST18, and ST78, previously considered as CC17, which were recovered in a single Italian hospital during 2010 (Willems, et al., 2012).

Conjugative transposons /integrative conjugative elements

A diversity of conjugative transposons (CTns) have been identified among enterococci since the description of Tn*916* in the early 1980s (see earlier section and Table 5 for details). They display a common synteny, but differ in their integrase/excisionase sequences and the specificity for the insertion site (Roberts & Mullany, 2009; Roberts & Mullany, 2011). Many of them encode resistance to tetracycline (Tn*916-like,* Tn*6000,* Tn*5801,*

Tn*5397*), kanamycin and erythromycin (Tn*1545*), or glycopeptides (Tn*1549*/Tn*5382*) (Carias, Rudin, Donskey, & Rice, 1998; Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000; Rice L. B., 1998; Roberts & Mullany, 2011).

A large number of Tn*916*/Tn*1545* family members (*tetM*) have been detected in different enterococcal species recovered from humans, pets, farm animals, wild boars, house flies from food settings, and foods (Hegstad, Mikalsen, Coque, Werner, & Sundsfjord, 2010; Roberts & Mullany, 2011) (see Table 5C). Besides the prototype Tn*916,* characterized enterococcal Tn*916*-like transposons are Tn*918*, Tn*925,* Tn*3702* (Horaud, Delbos, & de Cespédès, 1990), Tn*5031/5032/5033*, Tn*5381* and Tn*5383*, Tn*6084*, Tn*6085*a, and Tn*6085*b (Rice L. B., Carias, Rudin, Hutton, & Marshall, 2010) or Tn*6009*, which is a Tn*916*-like transposon that is directly linked to a functional staphylococcal mercury resistance operon located upstream of the Tn*916* conjugation module. Tn*1549* (*vanB2*) is a Tn*916*-like element that comprises a central part, with the *vanB2* operon replacing the *tet(M)* gene (Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000).

Other CTns that code for resistance to tetracycline (*tetM*) encode integrases that are highly homologous to those of certain *S. aureus* PAIs (Tn*6000*) or the large serine recombinase *tndX* gene of *Clostridium difficile* (Tn*5397*), which is responsible for the excision and insertion of the element (Brouwer, Mullany, & Roberts, 2010; Hegstad, Mikalsen, Coque, Werner, & Sundsfjord, 2010; Novais, Freitas, Sousa, Baquero, Coque, & Peixe, 2008; Roberts & Mullany, 2011). Tn*6000,* formerly EfcTn1 (Brouwer, Mullany, & Roberts, 2010; Roberts, Davis, Seville, Villedieu, & Mullany, 2006), is a mosaic element whose integrase is highly homologous with those of SaPIbov and SaPIbov2, two bovine staphylococcal pathogenicity islands (42% and 41% identity with Int_{Tn916}, respectively). It also encodes: i) a group II intron that is identical to that of Tn*5397* from *C. difficile*; ii) the *vap* and *hel* genes which are homologous to genes coding for a virulence associated protein; iii) a DEAD helicase of the Superfamily 2 within the virulence-related transposon from *Dichelobacter nodosus* (which seems to be transferred horizontally between bacteria and is possibly mediated by bacteriophages); and iv) a putative type I restriction/modification system (Brouwer, Mullany, & Roberts, 2010). Recent studies have detected both $TndX_{5397}$ and $Int_{5801/CW459}$ among enterococcal species of different origins, although $TndX_{5397}$ is enriched among *E. faecium* isolates of poultry origin (Agersø, Pedersen, & Aarestrup, 2006; Novais, et al., 2008). Tn*6000* appears to be spread among enterococci from community based humans, animals, and soil since at least the early 1980s (Novais, et al., 2012).

Enterococcal CTns that lack antibiotic resistance genes have also been described (Burrus, Pavlovic, Decaris, & Guédon, 2002; Fujimoto, Tomita, Wakamatsu, Tanimoto, & Ike, 1995; Rice L. B., Carias, Marshall, Rudin, & Hutton-Thomas, 2005) (Table 5). A Tn*916*-like element, *efaB5*, which consists of EF_1846 to EF_1897, which is ~49.5 kb in *E. faecalis* strain V583, seems to be enriched among CC2 isolates, and plays a role in niche adaptation (Galloway-Peña, Bourgogne, Qin, & Murray, 2011; Solheim, Brekke, Snipen, Willems, Nes, & Brede, 2011). ICE*Efm1,* previously designated as an *esp* pathogenicity island in *E. faecium* (*esp*$PAI_{E. faecium}$) is now classified as an ICE element, as it has recently been shown that it is self-transmissible and requires its IntA integrase for excision (Top, Sinnige, Majoor, Bonten, Willems, & van Schaik, 2011). The element is flanked by two 54-bp-direct imperfect repeats, with only the 54-bp DR at the 5′ end being present at the junction site, which suggests that the *esp* PAI is similarly transferred as described for the ICE-like element Tn*916*. The *esp* gene is involved in biofilm formation and infection in a mouse model (Leavis, et al., 2004; van Schaik, et al., 2010).

Enterococcal isolates that contain different CTns and/or multiple copies of a given CTn have been described (see previous section). Besides the emblematic example of the widespread VanB *E. faecium* C68 clone widely disseminated in Cleveland, Ohio (USA) in the early 90s, which harbors three Tn*916*-like transposons (Tn*6084*, Tn*6085*a and Tn*6085*b, and Tn*5382* (*vanB2, pbp5*), enterococcal isolates from humans and animals that contain different CTns (different combinations of Tn*916*, Tn*5397*, Tn*6000* and other ICEs) have been frequently reported (Agersø, Pedersen, & Aarestrup, 2006; Novais, et al., 2012).

Some CTns have an extraordinary ability to undergo genetic exchange processes within a diversity of genomes, which often result in mosaic platforms that carry fragments of transposons, plasmids, RM systems, and self-

splicing elements, as demonstrated for the Tn*6000* (Brouwer, Mullany, & Roberts, 2010; Novais, et al., 2012). It has been suggested that they might also contribute to genome evolution by favoring large deletions (Rice L. B., Carias, Marshall, Rudin, & Hutton-Thomas, 2005). Mosaic platforms that contain Tn*916-like* transposons, such as Tn*5385* (Tn*5381*-Tn*5384*-Tn*552*), Tn*5382* (*pbp5*-CTn*1549)*, or Tn*6009* (Tn916-mer) have also been described (Carias, Rudin, Donskey, & Rice, 1998; Rice L. B., 1998; Soge, Beck, White, No, & Roberts, 2008).

Self-splicing elements

Group II introns are active mobile elements that relate to a family of self-splicing RNAs. Different introns have been identified in chromosomal DNA, conjugative plasmids, CTns, and pathogenicity islands of *E. faecalis* and *E. faecium* (Coburn, et al., 2010; Rice L. B., Carias, Rudin, Hutton, & Marshall, 2010; Sletvold, Johnsen, Wikmark, Simonsen, Sundsfjord, & Nielsen, 2010) (Table 6). These belong to what has been designated as class B, and thus far have been observed only in enterococci**.** A class A intron Ll.ltrB located on the *L. lactis* plasmid pRS01 has been observed to target conjugative elements of other Gram-positive bacteria, including enterococci (Belhocine, Yam, & Cousineau, 2005).

Integrons

Data related to the spread of integrons in enterococci are scarce. In 1998, the antiseptic resistance gene *qacE* delta 1, frequently encoded in the 3′conserved region of integrons, was identified in *Enterococcus*, and bore a nucleotide sequence identical to that of *qacE* delta 1 from Integron InC from *Pseudomonas* (Kazama, Hamashima, Sasatsu, & Arai, 1998). In 1999, the presence in *E. faecalis* of an integron-related antibiotic-resistance cassette *aadA* (identical to *aadA* genes from integrons in several *E. coli* plasmids) was observed (Clark, Olsvik, Swenson, Spiegel, & Tenover, 1999). While these two reports suggested the appearance of integrons in enterococci, the presence of intact class 1 and class 2 integrons in hospital-associated *E. faecalis* and *E. faecium* isolates has recently been reported in China (Xu, et al., 2010). The appearance of identical cassettes in *Staphylococcus*, *Pseudomonas,* and *E. coli* suggested the intergeneric dissemination of integrons in that hospital.

Bacteriophages

Most of the bacteriophages currently described in *Enterococcus* spp. belong to tailed families of *Podoviridae*, *Siphoviridae*, and *Myoviridae* (Mazaheri Nezhad Fard, Barton, & Heuzenroeder, 2010). Data related to the spread of virulence traits or antibiotic resistance by lysogenic bacteriophages among enterococci are limited (Mazaheri Nezhad Fard, Barton, & Heuzenroeder, 2011; van Schaik, et al., 2010; Yasmin, et al., 2010). Similar to the case in other MGEs, the incidence of prophage elements is mainly detected in the genomes of *E. faecium* and *E. faecalis* (V583) that are poor in CRISPR systems (van Schaik, et al., 2010) and seem to be more predominant in human isolates than in those from animal origin (Lepage, et al., 2006). Nevertheless, the high incidence of phage-specific genes in *E. faecium* and *E. faecalis* suggests that they play an important role in the genome diversity observed among members of these species (Leavis H. L., Willems, van Wamel, Schuren, Caspers, & Bonten, 2007; McBride, Fischetti, Moellering, Jr., & Gilmore, 2007; van Schaik, et al., 2010). See Enterococcal bacteriophages and genome defense for more extensive coverage of enterococcal bacteriophages.

Concluding Remarks

It is evident that the employment of mobile genetic elements by enterococci in the spread of clinically significant traits, such as antibiotic resistance and virulence, is a greatly used and varied process. The efficiency with which certain conjugation systems can operate is highly evolved, which suggests that features of the enterococcal cell surface may be particularly suitable for this type of DNA transfer. Enterococci are especially hardy organisms, and their widespread distribution in the environment has undoubtedly facilitated the collection of traits important to their eventual establishment as human commensals, as well as made them one of the most common organisms involved in nosocomial infections. As members of the normal intestinal flora, their conjugation

proficiency likely makes them a significant reservoir of genetic information for other bacteria, and they likely contribute to the passage of a variety of survival traits to other genera. The recent focus on the molecular biology and epidemiology of these organisms and their involvement in horizontal DNA transfer has begun to shed significant light on the role these organisms play in human health and disease.

Appendix

Table 1. Enterococcal plasmids

Plasmid family	Plasmid[b]	Size (kb)	Original host (country)	Origin	Host range	Genetic markers (pheromone response)	Reference
ROLLING-CIRCLE REPLICATING PLASMIDS							
Rep_trans	pJS42	4.1	*E. faecium* JH95		*E. faecium*	cryptic	Garcia-Migura, Hasman, & Jensen, 2009
(pRI1-like)	pRI1	6.0	*E. faecium* 9631160-1	F (chicken), A (poultry), H F (Japanese rice bran paste)	*E. faecium, E. hirae*	cryptic	Freitas, et al., 2012; Garcia-Migura, Hasman, & Jensen, 2009; Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010; Ridenhour, Fletcher, Mortensen, & Daneo-Moore, 1996
	pEFNP1	ND	*E. faecium* N15		*E. faecium*	cryptic	
	pKq10	ND	*E. faecium*		*E. faecium*	*tet(U)*	
Rep_2	pMV158	5.5	*S. agalactiae*	H	*S. agalactiae, E. faecalis*	*tet(L)*	Burdett, 1980; van der Lelie, Bron, Venema, & Oskam, 1989
	pJB01	2.2	*E. faecium*	H	*E. faecium*	Cryptic	Kim, et al., 2006
Rep_1	pNJAKD	3.8	*E. faecium*	F (milk)	*E. faecium*	cryptic	Kumar, Ponnaluri, Putarjunan, Ranganathan, Roy, & Das, 2012
THETA REPLICATING PLASMIDS							
Rep_3	pCIZ2	7.4	*E. faecium* L50	F (sausage, chicken), H	*E. faecium*	cryptic	Criado, et al., 2006
	p200B[c]	12.5	*E. faecium*	HH	*E. faecium*	*bac32(also enterocin IT)*	Inoue, Tomita, & Ike, 2006
	pHY	6,037	*E. faecium*	HH	*E. faecium*	*bac51*	Yamashita, Tomita, Inoue, & Ike, 2011
	pB82	6.2	*E. faecium* VR82	H, F (chicken), SW	*E. faecium, E. hirae, E. durans*	*bac43*	Todokoro, Tomita, Inoue, & Ike, 2006
	pEF1071	9.3	*E. faecalis* BFE 1071 (Germany)	A (pig)	*E. faecalis*	bacteriocins (1071A and 1071B)	Balla & Dicks, 2005

Table 1. continued from previous page.

	Plasmid	>3.2[b]	*E. faecalis* FAIR-E 309 (Argentina)	F (cheese)	*E. faecalis*	bacteriocin	Unpublished
	pGL (also pDGL)	8.3	*E. durans* 41D	F (cheese)	*E. durans*	bacteriocin	Du, Somkuti, & Renye, Jr., 2012
	pEFR	3.2	*E. faecium*	NA	*E. faecium*	cryptic	Unpublished
	pAMα1	9.8	*E. faecalis* DS5	H	*E. faecalis*	*tet(L)*	Francia & Clewell, 2002
	pS86	5.1	*E. faecalis* S-86	H	*E. faecalis,*	cryptic	Martínez-Bueno, Valdivia, Gálvez, & Maqueda, 2000
	pEF47	5.5	*E. faecalis* 47	A (cow)	*E. faecalis, other Gram positives*	cryptic	Sprincova, Stovcik, Javorsky, & Pristas, 2005
	p703/5	ND	*E. faecalis* KBL703	F (milk)	*E. faecalis*	NA	Cha, Lim, Jang, Lim, Kim, & Chang, 2007
	pEF418[d]	>15.9[j]	*E. faecalis* 418	HH	*E. faecalis, E. faecium*	*aadE*	GenBank AF408195.1125
	EF62pA	5.1	*E. faecalis* 62	HH	*E. faecalis*	cryptic	Brede, Snipen, Ussery, Nederbragt, & Nes, 2011
	pJS33	3.1	*E. faecium* JH95	NA	*E. faecium*	cryptic	unpublished
	pMBB1	>1.9[j]	*E. faecium*	NI	*E. faecium*	cryptic	Wyckoff, Barnes, Gillies, & Sandine, 1996
Inc18[a]	pIP501	30.6	*S. agalactiae* B96	H, A, SW	*E. faecalis, E. faecium*	*cat, erm(B)*	Thompson & Collins, 2003
	pRE25	50.2	*E. faecalis* RE25	F (sausage, chicken), H, HH, A (poultry, pig), SW	*E. faecalis, E. faecium*	*cat, erm(B), sat4, aph(3′)-III, aadK*	Schwarz, PerretenV., & Teuber, 2001
	pAMβ1	27.8	*E. faecalis* DS5	H	*E. faecalis, E. faecium*	*erm(B)*	Clewell & Francia, 2004; Clewell, Yagi, Dunny, & Schultz, 1974
	pTEF3	17.9	*E. faecalis* V583	H	*E. faecalis*	*UV*	Paulsen, et al., 2003
	pEF-01	35,9	*E. faecalis* EF-01	A (bovine)	*E. faecalis*	*cfr fex(B)*	Liu, et al., 2011
	pEH-1[e]	>18.1	*E. hirae*	A (pig)	*E. hirae*	*fex(B)*	Liu, et al., 2012
	pEFM-1	14.8	*E. faecium*	A (pig)	*E. faecium*	*fex(B)*	Liu, et al., 2012)
	pPPM1000	ND	*E. faecium*	A (monkey)	Unknown	Sm^r, Hg^r	Davis, Roberts, Ready, Richards, Wilson, & Mullany, 2005
	pM7M2	19.5	*E. faecium* (USA)	F (cheese)	*E. faecium, S. mutans*	*tet(M), tet(L)*	Li, Alvarez, Harper, & Wang, 2011

Table 1. continued from previous page.

	pIP816 [f]	34.6	*E. faecium* BM4147	H	*E. faecium*	*vanA, str*	Sletvold, Johnsen, Wikmark, Simonsen, Sundsfjord, & Nielsen, 2010
	pVEF1 [f]	39.6	*E. faecium* 399/F99/H8 (Norway)	HH, F (chicken), A (poultry)	*E. faecium, E. hirae, E. durans*	Tn*1546*(*vanA*)	Sletvold, Johnsen, Simonsen, Aasnæs, Sundsfjord, & Nielsen, 2007
	pVEF2 [f]	39.7	*E. faecium* 399/F99/A9 (Norway)	F (chicken), A (poultry)	*E. faecium, E. hirae, E. durans*	Tn*1546*(*vanA*)	Sletvold, Johnsen, Simonsen, Aasnæs, Sundsfjord, & Nielsen, 2007
	pVEF3 [f]	63.1	*E. faecium* 399/S99/A7 (Norway)	A (poultry)	*E. faecium*	Tn*1546*(*vanA*)	Sletvold, Johnsen, Hamre, Simonsen, Sundsfjord, & Nielsen, 2008
	pVEF4 [f]	>44[j]	*E. faecium* 399/F98/A4 (Norway)	A (poultry)	*E. faecium*	Tn*1546*(*vanA*)	Sletvold, Johnsen, Wikmark, Simonsen, Sundsfjord, & Nielsen, 2010
	pWZ1668 [f]	48.3	*E. faecalis*	HH	*E. faecalis*	Tn*1546*(*vanA*)	Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008
	pWZ909 [f]	42.6	*E. faecalis*	HH	*E. faecalis*	Tn*1546*(*vanA*)	Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008
	pWZ7140 [f]	47.2	*E. faecalis*	HH	*E. faecalis*	Tn*1546*(*vanA*)	Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008
	pEF1[g]	21.3	*E. faecium* 6T1a	F (olive), H, HH, F (chicken), A (pig), SW	*E. faecium*	*uvrA*	Ruiz-Barba, Floriano, Maldonado-Barragán, & Jiménez-Díaz, 2007
	pLG2 [h]	62.6	*E. faecalis* UW3114	H	*E. faecalis*	*ant(6)-Ia, sat, aph(3′)-IIIa, tet(M)*	Laverde-Gomez, et al., 2011
	pHTβ	52.9	*E. faecium* FH	H, HH, F (chicken)	*E. faecium, E. avium, E. durans*	Tn*1546*(*vanA*)	Tomita & Ike, 2008
	pMG1 and closely related plasmids	65.0	*E. faecium*	H, HH, F (chicken)	*E. faecium, E. durans*	Tn*4001,* Tn*1546*	Tanimoto & Ike, 2008

Table 1. continued from previous page.

	pZB18	68	*E. faecium*	H	*E. faecium*	Tn*1546*(*vanA*)	Zheng, Tomita, Xiao, Wang, Li, & Ike, 2007
RepA_N	pAD1	59.3	*E. faecalis* DS16	H, F (chicken), A (pig), SW	*E. faecalis*	*uvrB* (cAD1), Hly/cyl	Colmar & Horaud, 1987; Francia, et al., 2001
	pTEF1	66.3	*E. faecalis* V583	H	*E. faecalis*	Tn*4001, erm(B), qacZ* (cAD1)	Paulsen, et al., 2003
	pTEF2	57.7	*E. faecalis* V583	H	*E. faecalis*	(cCF10)	Paulsen, et al., 2003
	pJH2	59	*E. faecalis* JH1	H	*E. faecalis*	*Hyl/bac*(cAD1)	Clewell & Francia, 2004; Clewell & Weaver, 1989; Colmar & Horaud, 1987; Galli & Wirth, 1991
	pIP964	65	*E. faecalis*	H	*E. faecalis*	*Hyl/bac* (cAD1)	Clewell & Francia, 2004; Colmar & Horaud, 1987
	pAMγ1	60	*E. faecalis* DS5	H	*E. faecalis*	*Hyl/bac , uvr* (cAD1)	Clewell & Francia, 2004; Clewell, Yagi, Dunny, & Schultz, 1974; Colmar & Horaud, 1987
	pAMγ2	~60	*E. faecalis* DS5	H	*E. faecalis*	(cAMγ2)	Clewell & Francia, 2004; Galli & Wirth, 1991
	pAMγ3	~60	*E. faecalis* DS5	H	*E. faecalis*	(cAMγ3)	Clewell & Francia, 2004; Galli & Wirth, 1991
	pBEM10	70	*E. faecalis* HH2	H	*E. faecalis*	*bla,* Tn*4001* (cAD1)	Murray, An, & Clewell, 1988
	pCF10	67.7	*E. faecalis* SF-7	H, F (chicken)	*E. faecalis*	*tet(M)*-CTn*925, uvrA* (cCF10)	Hirt, et al., 2005
	pAMS1	130	*E. faecalis* MC4	HH	*E. faecalis*	(cCF10)	Sedgley, Clewell, & Flannagan, 2009
	pMB1	90	*E. faecalis* S-48	HH	*E. faecalis*	(cCF10)	Clewell & Francia, 2004
	pMB2	56	*E. faecalis* S-48	HH	*E. faecalis*	(cPD1)	Clewell & Francia, 2004
	pPD1	59	*E. faecalis* 39-5	HH	*E. faecalis*	(cPD1)	Fujimoto, Tomita, Wakamatsu, Tanimoto, & Ike, 1995
	pYI14	61	*E. faecalis* 39-5	H	*E. faecalis*	(cPD1)	Tomita, Kamei, & Ike, 2008

Table 1. continued from previous page.

	pEJ97-1	11.3	*E. faecalis* EJ97	SW	*E. faecalis*	(cPD1)	Sánchez-Hidalgo, Magueda, Gálvez, Abriouel, Valdivia, & Martínez-Bueno, 2003
	pAM373	36.8	*E. faecalis* RC73	H, F (chicken)	*E. faecalis*	(cAM373)	De Boever, Clewell, & Fraser, 2000
	pAM368	107	*E. faecalis* 368	H	*E. faecalis*	(cAM373); Tn*1546* (*vanA*)	Showsh, De Boever, & Clewell, 2001
	pBEE99	80.6	*E. faecalis* E99	H	*E. faecalis*	*bee, uvr* (UK pheromone)	Coburn, et al., 2010
	pMG2200 [i]	106.5	*E. faecalis* NKH15	H	*E. faecalis*	*vanB2_Tn1549, uvr,* Bac41 (cCF10)	Zheng, Tomita, Inoue, & Ike, 2009
	pMG2201	65.7	*E. faecalis* NKH15	H	*E. faecalis*	ermB, Hly/cyl, (cAD1)	Zheng, Tomita, Inoue, & Ike, 2009
	pAM323	66	*E. faecalis* HH2	H	*E. faecalis*	(cAM323)	Murray, An, & Clewell, 1988
	pAM324	53	*E. faecalis* HH2	H	*E. faecalis*	(cAM324)	Murray, An, & Clewell, 1988
	pHKK100	55	*E. faecalis* 228	H	*E. faecalis, E. faecium*	(cHKK100), Tn*1546* (*vanA*)	Handwerger, Pucci, & Kolokathis, 1990
	pOB1	71	*E. faecalis* 5952	H	*E. faecalis*	(cOB1)	Galli & Wirth, 1991; Nakayama, Abe, Ono, Isogai, & Suzuki, 1995
	pYI1/2	58/56	*E. faecalis*	H	*E. faecalis*	(cOB1/cYI2)	Ike & Clewell, 1992; Nakayama, Abe, Ono, Isogai, & Suzuki, 1995
	pYI17	57.5	*E. faecalis* YI717	H	*E. faecalis*	(cYI17)	Tomita, Fujimoto, Tanimoto, & Ike, 1996
	pSL1/2	128.1	*E. faecalis* KV1	H/A (chicken)	*E. faecalis*	(cSL1) Tn*1546* (*vanA*), *ermB, aac(6)-aph(2), ant(6)-Ia, aph(3 ′)-IIIa*	Lim, Tanimoto, Tomita, & Ike, 2006
	EF62pC	55.3	*E. faecalis* 62	HH	*E. faecalis*	*bac, uvr*	Brede, Snipen, Ussery, Nederbragt, & Nes, 2011
	EF62pB	51.1	*E. faecalis* 62	HH	*E. faecalis*	*bac, uvr*	Brede, Snipen, Ussery, Nederbragt, & Nes, 2011
	pTW9	85	*E. faecalis*	H, A (poultry)	*E. faecalis*	Tn*1546* (*vanA*), *erm(B),* bac, IS*4*-b-lactamase B-IS*4*	unpublished AB563188
	pHKK703	55	*E. faecium* R7	H	*E. faecalis, E. faecium*	(cCF10)	Heaton, Discotto, Pucci, & Handwerger, 1996

Table 1. continued from previous page.

	pBRG1	50	*E. faecium* LS10	H	*E. faecalis, E. faecium*	(cCF10-like), Tn*1546* (*vanA*)	Magi, et al., 2003
	pRUM [i]	24.9	*E. faecium* U37	H, HH, F (poultry), A (pig)	*E. faecium*	*Cat (orf25), Δ*Tn*5405, erm(B), uvr, Axe-Tse*	Grady & Hayes, 2003
	pS177	39	*E. faecium* S177	H	*E. faecium*	Tn*1546* (*vanA*),	Halvorsen, Williams, Bhimani, Billings, & Hergenrother, 2011
	p5753B	56.7	*E. faecium* 5753c	H, HH	*E. faecium*	*aac(6´)-aph(2´´), tetM, Δ*Tn*5405, erm(B)*	GenBank: GQ900487.1
	pRUM-like [j]	30-70	*E. faecium*	H, HH	*E. faecium*	Tn*1546* (*vanA*)	Freitas, et al., 2012
	pLG1 [k] pDO2 pDO3 pNB2354	281.0 66.2 251.9 214.3	*E. faecium* UW2774 *E. faecium* DO *E. faecium* DO *E. faecium* B-2354	H, HH, A (pig), F (chicken), SW H H H	*E. faecium* *E. faecium* *E. faecium* *E. faecium*	Tn*1546* (*vanA*), *erm(B), tcrYAZB, ctpA, pilA, aac(6´)Ie-aph(2˝)Ia aadE, sat4,aph2 hly* adhesion *fimH* Type I RM system, diverse traits, Cd, Co, Mn	Laverde-Gomez, et al., 2011; Rosvoll, et al., 2012 Qin, et al., 2012 Qin, et al., 2012 GenBank CP004064.1

Table 1. continued from previous page.

	Untypeable megaplasmid k	>150kb-350kb	*E. faecium*	H, HH, A, SW	*E. faecium, E. casseliflavus* and *E. gallinarum, E. durans, E. hirae*	Tn*1546* (*vanA*), *erm(B), hyl, aac(6´)-aph(2´´),, crYAZB, different metabolic* (mannitol, glycerol, sorbitol, raffinose, complex carbohydrates) , adhesion fimH	Biavasco, et al., 2007; Freitas, et al., 2011; Freitas, Novais, Cunha, Silveira, Peixe, & Coque, 2010; Freitas, et al., 2010; Hasman, Villadsen, & Aarestrup, 2005; Laverde-Gomez, et al., 2011

Abbreviations: Erm, erythromycin; Gm, high level of resistance to gentamicin; Km, kanamycin; Sm, streptomycin; St, streptothricin; Tet, tetracycline; Van, vancomycin: H, Hospital; HH, healthy humans; HHc, Healthy humans (children); A, animals (farm or healthy animals); WA, wild animals; F, foods; P, pets; SW, sewage.NA: Not available; NI: not identified.

a Plasmid size and backbone of putative Inc18 members are highly variable (30-50kb), due to the frequent acquisition of insertion sequences and transposons and DNA losses which seem to occur by spontaneous deletion after conjugal transfer to different hosts. Cointegration with theta plasmids of different families as pRUM, pheromone responsive, and pLG1-like plasmids from VRE and VSE strains is frequently observed (103, 125, 127, 308, 331, 333).

b Plasmids with unavailable sequences were assigned to a specific family based on homology or incompatibility with other known plasmids.

c p200B (GenBank acc number AB158402) contains a bac32 gene, which is associated with plasmid pTI1 (12.5 kb) (Inoue, Tomita, & Ike, 2006). Bac32 seems to be identical to EntTI (178). p200B and pTI1 are linked to the same publication and the same bacteriocin, so they might be the same plasmid, which is widespread among clinical isolates of E. faecium. To date, it is one of the two known bacteriocins linked to nosocomial E. faecium isolates.

d pEF418b was originally found in E. faecalis (GenBank AF408195.1) but epidemiological studies suggest it is much more frequent among E. faecium (Freitas, et al., 2013; Qin, et al., 2012).

e pEH-1 contains three Rep proteins, one identical to the Inc18 plasmids pVEF1-3 and other two 45 and 87% homologous to pWV05 from lactic bacteria. This plasmid is able to replicate in S. aureus RN4220 (Liu, et al., 2012).

f Inc18::Tn1546 plasmids from Europe are associated with E. faecium isolates (such as pVEF1, pEVF2, pVEF3, and pVEF4) lack a transfer system, and seem to have evolved from pIP816 isolated in France in 1987 (Freitas, et al., 2012; Rosvoll, et al., 2010; Sletvold, Johnsen, Simonsen, Aasnæs, Sundsfjord, & Nielsen, 2007; Sletvold, Johnsen, Wikmark, Simonsen, Sundsfjord, & Nielsen, 2010). Inc18::Tn1546 plasmids from the USA are linked to E. faecalis isolates (pWZ909, pWZ1668, pWZ7140) and contain a complete transfer system (Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008; Zhu, et al., 2010). Differences among enterococcal clonal backgrounds and Inc18 plasmids seem to be important in the spread of Tn1546 to non-enterococcal species (Fitzgerald & Clewell, 1985; Zhu, Clark, McDougal, Hageman, McDonald, & Patel, 2008; Zhu, et al., 2010).

g pEF1 carry a rep identical to that of pRE25 and a relaxase detected among most human E. faecium isolates (more than 90% in different collections analyzed) (Freitas, et al., 2011; Freitas, et al., 2012). Relaxase originally identified in pEF1 (pEF1_p34) (Garcillán-Barcia, Francia, & de la Cruz, 2009) has been detected in plasmids of different RepA_N subfamilies (Freitas, et al., 2013).

h pLG2 contains a rep similar to that of Inc18 plasmids and relaxases related to pAD1 (Laverde-Gomez, et al., 2011).

i pMG2200 is the first pheromone-responsive highly conjugative plasmid that encodes vanB resistance. It is a chimeric plasmid with a prgX-prgQ-traE1 genetic organization in the regulatory region of the pheromone response (prgX and prgQ being the key negative regulatory elements for plasmid pCF10 and TraE1, a key positive regulator of plasmid pAD1), a functional oriT region, and a putative relaxase gene member of the MOBP family, which is found in pheromone-independent pMG1-like plasmids.

j pRUM-related plasmids (such as pRUM, p5373c, pS177, or pDO2) are mosaic plasmids of variable size (30->60kb) that are comprised of sequence fragments from mobile genetic elements of different origins (Tns, ISs, small tetha replicating plasmids, bacteriocin clusters). Members of this group differ in the sequence of RepA_N, the mobilization system, and the presence of the toxin-antitoxin Axe-Txe locus (Freitas, et al., 2013; Freitas, et al., 2012; Grady & Hayes, 2003; Halvorsen, Williams, Bhimani, Billings, & Hergenrother, 2011; Rosvoll, et al., 2010). Plasmids that contain RepA and Axe-Txe from pRUM are globally spread among VRE and VSE E. faecium isolates from human and animal origins (Freitas, et al., 2012; Grady & Hayes, 2003; Halvorsen, Williams, Bhimani, Billings, & Hergenrother, 2011; Rosvoll, et al., 2010; Sung, Khan, & Nawaz, 2008). To date, only the original pRUM plasmid (Grady & Hayes, 2003) and another three pRUM-related plasmids [one of them pDO2 (Qin, et al., 2012) and two that carry vanB (Freitas, et al., 2012)] contain a mobilization system, which corresponds to the one encoded on pC223 from S. aureus (Grady & Hayes, 2003). Plasmids with RepA proteins that are 95% homologous to RepA-pRUM and lacking Axe-Txe are detected among a high percentage of VRE and VSE E. faecium from European countries as Portugal, Spain and Norway (Freitas, et al., 2013; Freitas, et al., 2010)]. They carried a relaxase originally detected in pEF1 (Freitas, et al., 2013; Freitas, et al., 2012; Garcillán-Barcia, Francia, & de la Cruz, 2009; Ruiz-Barba, Floriano, Maldonado-Barragán, & Jiménez-Díaz, 2007). These pRUM-like plasmids frequently contain additional replicons that belong to other RepA_N plasmid families (Freitas, et al., 2013; Freitas, et al., 2012).

k pLG1 contains a Rep protein similar to RepApAD1 (Jensen, Garcia-Migura, Valenzuela, Løhr, Hasman, & Aarestrup, 2010; Laverde-Gomez, et al., 2011)

Table 2. Replication initiator proteins in enterococci

Rep family	Plasmid name	Plasmid GenBank Acc. nº	Bacterial Host	Rep name	Rep initiator protein Genbank Acc. Nº
Rep_trans (RCR)	pJS42	NC_010291	*E. faecium*	RepA	YP_001654986.1
	pRI1	NC_010330.1	*E. faecium*	Rep	YP_001672021.1
	pRE25	NC_008445	*E. faecalis*	pRE25p11	YP_783895.1
	pEFNP1	AB038522.1	*E. faecium*	Orf1	BAA92162.1
	pRUM	NC_005000	*E. faecium*	RepM	NP_863166.1
	pTG9790	NC_015845.1	*E. hirae*	NicK	YP_004739228.1
	pKQ10	U01917.1	*E. faecium*	Orf1	AAB08924.1
Rep_2 (RCR)	pMV158	X15669	*Streptococcus, Enterococcus*	RepB	CAA33711.1
	pJB01	NC_006427.1	*E. faecium*	RepB	YP_138502.1
Rep_1 (RCR)	pNJAKD	NC_015849.1	*E. faecium*	pNJAKD_p5	YP_004747351.1
	pTEF1	NC_004669	*E. faecalis* V583	Rep	NP_816941.1
	pM7M2	NC_016009.1	*E. faecium*	Rep	YP_004849398.1
	pAMα1	NC_005013	*E. faecalis* DS5	RepB	NP_863351.1
RepA_N (θ)	pAD1	L01794.1	*E. faecalis* DS16	RepA	AAB00503.1
	pTEF1	NC_004669	*E. faecalis* V583	RepA-1	NP_816932.1
	EF62pC	CP002494.1	*E. faecalis* 62	PrgW	ADX81274.1
	pBEE99	NC_013533.1	*E. faecalis*	PrgW	YP_003329050.1
	pTEF2	NC_004671	*E. faecalis* V583	RepA-2	NP_817022.1
	pMG2200	NC_011642.1	*E. faecalis*	RepA	YP_002333458.1
	pTW9	NC_014726.1	*E. faecalis*	RepA	YP_004032946.1
	EF62pB	CP002493.1	*E. faecalis* 62	PrgW	ADX81203.1
	pEJ97-1	AJ490170.1	*E. faecalis*	RepA	CAD35304.1
	pAM373	NC_002630.1	*E. faecalis*	pAM373_p02	NP_071995.1
	pCF10	NC_006827	*E. faecalis*	PrgW	YP_195765.1
	pPD1	D78016.1	*E. faecalis*	RepA	BAA11194.1
	pLG1	HM565192.1	*E. faecium*	RepA	ADO66907.1
	pRUM	NC_005000	*E. faecium*	pRUM_p29	NP_863172.1
	pS177	NC_014959.1	*E. faecium*	RepA	YP_004172632.1
	p5753cB	GQ900487.1	*E. faecium*	RepA	ADA62751.1
Inc18 (θ)	pIP501	X17655.1	*Streptococcus, Enterococcus*	RepR	CAA35647.1
	pTEF1	NC_004669	*E. faecalis* V583	RepE	NP_816981.1
	pIP816	NC_011140.1	*E. faecium*	RepE	YP_002128409.1

Table 2. continued from previous page.

	pAMβ1	NC_013514.1	*E. faecalis* DS5	pAMbeta1_p03	YP_003305348.1
	pTW9	NC_014726.1	*E. faecalis*	RepE	YP_004032996.1
	pRE25	NC_008445	*E. faecalis*	RepS	YP_783890.1
	pWZ1668	NC_014475.1	*E. faecalis*	RepE	YP_003864107.1
	pWZ909	GQ484954.1	*E. faecalis*	RepE	ADM24822.1
	pWZ7140	GQ484955.1	*E. faecalis*	RepE	ADM24861.1
	pVEF1	NC_008768.1	*E. faecium*	Rep	YP_976064.1
	pVEF2	NC_008821.1	*E. faecium*	Rep	YP_001019023.1
	pEF-01	NC_014508.2	*E. faecalis*	Rep	YP_003896005.1
	pVEF4	FN424376.1	*E. faecium*	RepE	CAZ67085.1
	pEH-1	JN192453.1	*E. hirae*	RepE	AEV23020.1
	pEFM-1	JN201336.1	*E. faecium*	RepE	AEV23037.1
	pVEF3	NC_010980.1	*E. faecium*	Rep	YP_001974773.1
	pRE25	NC_008445	*E. faecalis*	Orf1	Q9AL28
	pVEF1	NC_008768.1	*E. faecium*	RepR	YP_976089.1
	pVEF2	NC_008821.1	*E. faecium*	RepR	YP_001019046.1
	pVEF3	NC_010980.1	*E. faecium*	RepR	YP_001974806.1
	pIP816	NC_011140.1	*E. faecium*	RepR	YP_002128419.1
	pLG2	HQ426650.1	*E. faecalis*	pLG2_0001	AEF32542.1
	pM7M2	NC_016009.1	*E. faecium*	RepR	YP_004849405.1
	p5753cB	GQ900487.1	*E. faecium*	Rep	ADA62715.1
	pEF1	NC_010880.1	*E. faecium*	RepR	YP_001966128.1
	pTEF3	NC_004670.1	*E. faecalis* V583	RepS	NP_817021.1
	pHTβ	NC_007594.1	*E. faecium*	Rep	YP_398701.1
	pZB18	AB611033.1	*E. faecium*	Rep	BAL40904.1
	pMG1	NC_011364.1	*E. faecium*	Rep	YP_002274355.1
Rep_3 (θ)	pAMα1	NC_005013	*E. faecalis* DS5	RepE	NP_863355.1
	pS86	AJ223161	*E. faecalis*	Rep	CAA11136.1
	pEFC1	D85392.1	*E. faecalis*	Rep	BAA12801.1
	p703/5	AF109375.1	*E. faecalis*	RepA	AAD16983.1
	EF62pA	CP002492.1	*E. faecalis* 62	RepE	ADX81198.1
	pEF-01	NC_014508.2	*E. faecalis*	RepB	YP_003896028.1
	pCIZ2	NC_008259	*E. faecium*	Orf8	YP_691718.1
	pGL	HQ696461.1	*E. durans*	Orf1	ADW93773.1
	pJS33	NC_010290.1	*E. faecium*	pJS33_01	YP_001654983.1
	pEF1071	NC_005010	*E. faecalis*	RepA	NP_863270.1
	p200B	AB158402	*E. faecium*	RepA	BAF44066.1

Table 2. continued from previous page.

	pEF47	AY842500.1	*E. faecalis*	RepE	AAX44237.1
	pB82	AB178871	*E. faecium*	RepA	BAF36632.1
	pHY	AB570326.1	*E. faecium*	RepA	BAK74730.1
	Plasmid	AY063485.1	*E. faecalis* FAIR-E 309	RepA	AAL39167.1
	pEF418	AF408195.1	*E. faecalis*	RepA	AAL05545.1
	pEFR	AF511037.1	*E. faecium*	Rep	AAM44830.1
	pMBB1	U26268.1	*E. faecium*	RepB	AAC44119.1

Neither replication initiators encoded in contigs derived from enterococcal genome sequencing projects, nor replication proteins from cloning vectors, are included.

Table 3. Enterococcal Relaxases

MOB family	Plasmid or Mobile element (ME) name	Plasmid or ME GenBank Acc. Nº	Bacterial Host	Relaxase name	Relaxase GenBank Acc. Nº
MOB_C	pAD1	AF343837	*E. faecalis* DS16	Orf57	AAL59457
	PAI_V583	NC_004668	*E. faecalis* V583	EF0505	NP_814286
	pTEF1	NC_004669	*E. faecalis* V583	EFA0025	NP_816951
	pAM373	NC_002630	*E. faecalis*	Orf8	NP_072012
	pLG2	HQ426665.1	*E. faecalis*	pLG2_0032	AEF32592.1
	pTW9	AB563188.1	*E. faecalis*	Orf39	BAJ34866.1
	EF62pC	CP002494.1	*E. faecalis* 62	EF62pC_0039	ADX81304.1
MOB_Q	pRE25	NC_008445	*E. faecalis*	MobA	YP_783908
	pIP501	L39769	*Streptococcus, Enterococcus*	MobA	AAA99466
	pAMβ1	NC_013514.1	*E. faecalis* DS5	pAMbeta1_p18	YP_003305363.1
	pWZ1668	NC_014475.1	*E. faecalis*	TrsA	YP_003864133.1
	pWZ7140	GQ484955.1	*E. faecalis*	TrsA	ADM24887.1
	pWZ909	GQ484954.1	*E. faecalis*	TrsA	ADM24846.1
MOB_P	pB82	AB178871	*E. faecium*	MobA	BAF36629
	pRUM	NC_005000	*E. faecium*	Rlx protein	NP_863170
	pCIZ2	NC_008259	*E. faecium*	Mob	YP_691715
	p200B	AB158402	*E. faecium*	MobA	BAF44062
	pEF1071	NC_005010	*E. faecalis*	MobA	NP_863268
	Tn*1549*_pIP834	AF192329	*E. faecalis*	Rlx-like protein	AAF72355
	pTEF2	NC_004671	*E. faecalis* V583	relaxase	NP_817049
	pCF10	NC_006827	*E. faecalis*	PcfG	YP_195793
	pGL	HQ696461.1	*E. durans*	MobA	ADW93779.1
	pHY	AB570326.1	*E. faecium*	MobA	BAK74727.1
	pEF1	NC_010880.1	*E. faecium*	pEF1_p34	YP_001966134.1
	pMG1	NC_011364.1	*E. faecium*	pMG1_p45	YP_002274390.1

Table 3. continued from previous page.

	pHTβ	NC_007594.1	*E. faecium*	pHTbeta_34	YP_398676.1
	pZB18	AB611033.1	*E. faecium*	TraI	BAL40943.1
	pLG1	HM565192.1	*E. faecium*	pLG1_0177	ADO66931.1
	pBEE99	NC_013533.1	*E. faecalis*	pBEE99_p32	YP_003329081.1
	EF62pB	CP002493.1	*E. faecalis* 62	EF62pB_0031	ADX81233.1
	pMG2200	NC_011642.1	*E. faecalis*	pMG2200_46	YP_002333406.1
	Tn*1549*_pMG2200	NC_011642.1	*E. faecalis*	pMG2200_26	YP_002333387.1
	BM4518_vanG	AY271782.1	*E. faecalis* BM4518	OrfG11	AAQ16254.1
	V583_P	NC_004668.1	*E. faecalis* V583	EF2303	NP_815959.1
MOB_V	pMV158	X15669	*Streptococcus, Enterococcus*	MobM	AAA25387
	pS86	AJ223161	*E. faecalis*	Mob, ORF4	CAA11139
	pAMα1	NC_005013	*E. faecalis* DS5	MobE	NP_863358
	pAMα1	NC_005013	*E. faecalis* DS5	MobB	NP_863352.1
	EF62pA	CP002492.1	*E. faecalis* 62	MobE	ADX81202.1
	pJS42	NC_010291	*E. faecium*	MobA	YP_001654987
	pRI1	NC_010330.1	*E. faecium*	Disrupted by ISEfa4	
	pNJAKD	NC_015849.1	*E. faecium*	pNJAKD_p4	YP_004747350.1
	pM7M2	NC_016009.1	*E. faecium*	pM7M2_p07	YP_004849396.1
MOB_T	Tn*916*	NC_006372.1	*E. faecalis* DS16	Orf20	YP_133675.1
	Tn*925*_pCF10	NC_006827	*E. faecalis*		
	Tn*5397*	AF333235.1	*Clostridium, Enterococcus*	Orf20	AAO24811.1
	Tn*5386*	DQ321786.1	*E. faecium*	Orf7	ABC48880.1
	Tn*6000*	JN208881.1	*E. faecalis*	Orf20	AEP33205.1
	Tn*6000*	FN555436.1	*E. casseliflavus*	Orf20	CBG92849.1
	OG1RF_CTn	CP002621.1	*E. faecalis*	OG1RF_10792	AEA93479.1
	V583_1	NC_004668.1	*E. faecalis* V583	EF0143	NP_813946.1
	V583_2	NC_004668.1	*E. faecalis* V583	EF1886	NP_815569.1
	V583_3	NC_004668.1	*E. faecalis* V583	EF2338	NP_815993.1
	V583_4	NC_004668.1	*E. faecalis* V583	EF2528	NP_816171.1
	EF62_CTn	CP002491.1	*E. faecalis* 62	EF62_0521	ADX78807.1
	pTG9790	NC_015845.1	*E. hirae*	NicK	YP_004739228.1

Table 4. Insertion sequences among enterococcal genomes and mobile genetic elements

Family group[a]	Nature of the catalytic site	Insertion Sequence	Genetic Element

Table 4. continued from previous page.

IS*6*	DDE	IS*1216*	Tn*1546*, Tn*5405,* Tn*5385* pIP816, pRE25, pRUM, pTEF1, pTEF3, pVEF1-2-3-4, pLG1, pS177, p5753cA, p5753cB, pUW1965, pUW786, pTW9, pSL1, pBEE99, pEF418, pEF-01, Efs-PAI, pA17Sv1, pJH1, pEJ97-1, pJM01, pHKK701, pEF-01, pEH-1, pEFM-1, pM7M2, pMG1-like, pDO1, pDO2, pDO3
		IS*257*	Tn*5384,* Tn*5385,* Tn*924*
IS*256*	DDE	IS*256*	Tn*4001*, Tn*5384*, Tn*5382*, Tn*1547*, p5753cB
			pTEF1, pTEF2, pTEF3, pVEF1-2-3, pWZ1668, pGM1
		IS*16*	Tn*1546*, Tn*1547,* pRUM, pDO2, pDO3
		IS*1191*/IS*905*[c]	Efs-PAI
		IS*1542*	Tn*1546*
IS*3*	DDE	IS*3*/IS*911*	pWZ1668, pBEE99, pLG1, pDO1, pDO2, pDO3, pVEF4, pRE25, pEF1
		IS*150*	Tn*5382*, pUW786
		IS*Enfa3*	Tn*5382*
		IS*153*	
		IS*1485* IS*Efa8*	Tn*1546*, pRE25, p5753cB, pM7M2, pMG1, pBE99, pDO1, pDO3, pVEF4, pWZ1668, pWZ140, pWZ909 pDO1, pDO3, pLG1
		IS*981*	pEF1, pRE25[e], p5753cB
IS*982*	DDE	IS*19* (IS*Efm1*)[d]	Tn*1546*, *vanD* cluster
IS*30*	DDE	IS*6770* (IS*Ef1*)[c]	Tn*1546*, Tn*1549*, pMRC01, pLG1, pBEE99
		IS*1252*	pHKK701, pHKK702, pIP816
		IS*1678*	Tn*1546*, pS177
		IS*1062*	pRE25, pPD1
IS*5* IS*66*	DDE DDE	IS*Efa5* IS*66*	Tn*1546* pLG1, pDO3
IS*110*	Site-specific	IS*110*/*116*/*902*	pEF01-1, pLG1, pDO2
	recombinase	IS*Enfa110*	Tn*5382*
IS*605*	Complex	IS*Efa4*	*vanD* cluster, pRI1
IS*200*	organization*	IS*Enfa200*	Tn*5382*
IS*L3*	Unknown*	IS*L3*	
		IS*204*/IS*1001*/	Tn*1546*, pLG1, pDO3
		IS*1096*/IS*1165*	
		IS*1251*	Tn*1546*, pS177
		IS*1476* IS*Efa11* (IS*1167*)	Tn*1546,* pLG1, pDO3

Table 4. continued from previous page.

IS*1380*	Unknown*	IS*Ecp1*	
IS*NYC*	Unknown*	IS*1182*	Tn*5405*, pRUM, pUW786, pS177, p5753cB

Abbreviations: PAI, Pathogenicity island.
[a] An IS family is defined as a group of ISs with related transposases, strong conservation of the catalytic site, conservation of organization, and similar IRs.
[b] The active sites of IS*200*/IS*605*, IS*L3*, IS*1380*, and IS*NYC* families have not been defined.
[c] IS*905* is an isoform of IS*1191*.
[d] IS*19* and IS*Efm1* and IS*6770* and IS*Ef1* are synonyms.
[e] The truncated version of IS*981* is present on pRE25.

Table 5. Enterococcal Transposons

Transposon	Flanking insertion sequences (Tn backbone)	Size (kb)	Characterized function	Host range	Origin	Comments	Reference
Composite Transposons							
Tn*4001(5281)* and variants	IS*256*-//-IS*256* (IS*256-aac6´-aph2´´*-IS*256*)	4.7	AB^R, Gm (*aac6´-aph2´´*)	*Enterococcus, S. aureus*, *Streptococcus agalactiae*, *Mycoplasma*	H, P	Tn*5381* is almost identical to Tn*4001* from *S. aureus* and Tn*3706* from *Streptococcus* Larger elements identified areTn*5384*, Tn*5385* in enterococci and *SCCmec in Staphylococci*	Hodel-Christian & Murray, 1991; Leelaporn, Yodkamol, Waywa, & Pattanachaiwit, 2008; Simjee, Fraise, & Gill, 1999; Simjee, et al., 2002
Tn*924*	IS*257*-//-IS*257* (IS*257-aac6´-aph2´´*-IS*257*)	27	AB^R, Gm (*aac6´-aph2´´*)	*E. faecalis*	H		Thal, Chow, Clewell, & Zervos, 1994
Tn*5384*	IS*256*-//-IS*256* (IS*256*-Tn*4001*-ΔTn*917-mer*-IS*256*)	26	AB^R, Gm, Erm (*aac6´-aph2´´*, *ermB*) Mercuric chloride (*merX*)	*E. faecalis*	H	Directly repeated copies of IS*256* Larger element identified: Tn*5385*	Bonafede, Carias, & Rice, 1997; Rice, Carias, & Marshall, 1995
Tn*5385*	IS*1216*-//-IS*1216* (IS*1216*-Tn*5381-Tn5384-Tn552*-IS*1216*)	65	AB^R, Gm, Erm, Sm, Tet, Pen (*aac6´-aph2´´*, *ermB, aadE, tetM, blaZ*) Mercuric chloride (*mer*)	*E. faecalis*	H	Directly repeated copies of IS*1216*	Rice & Carias, 1998
Tn*5405* and variants	IS*1182*-//-IS*1182* (IS*1182-aadE-sat4-aphA-3*-IS*1182*)	12	AB^R, Sm, St, Km (*aadE-sat4-aphA-3*)	*Enterococcus, Staphylococcus*, *Streptococcus, Campylobacter*	H, HH, P, A, F, SW	Inverted repeated copies of IS*1182* Larger element identified*:* Tn*4001*-Tn*5405,* Tn*5405*::Tn*5404, ermB*-Tn*5405*	Boerlin, Burnens, Frey, Kuhnert, & Nicolet, 2001; Derbise, Dyke, & el Solh, 1996; Werner, Hildebrandt, & Witte, 2003

Table 5. continued from previous page.

Tn*1547*	IS*16*-//-IS*256*-like (IS*16*-*vanB1*-IS*256*-like)	64	AB^R, Van *(vanB1)*	*Enterococcus*	H	Direct orientation of IS*16* and IS*256*-like	Quintillani, Jr. & Courvalin, 1996
Tn*5482*	IS*1216*-//-IS*1216* (IS*1216*-ΔIS*3*-like-Tn*1546*::*IS1251*-IS*1216*)	26-30	AB^R, Van *(vanA)*	*E. faecium, E. faecalis*	H		Handwerger & Skoble, 1995
Tn*5506*	IS*1216*-//-IS*1252*-*IS1216* (IS*1216V2*-*vanA*-IS*1252*-*IS1216V1*)	39	AB^R, Van *(vanA)*	*E. faecium*	H	IS*1216V2* in inverted direction of IS*1216V1* with IS*1252* insertion	Heaton, Discotto, Pucci, & Handwerger, 1996

Table 5b. Tn*3* and Tn*7* derivatives

Transposon	Family	Integrase type	Size (kb)	Characterized function[a] (genotype)	Host range	Origin	Reference
Tn*917* and variants (Tn*3871*)	Tn*3* (Tn*551*)	NA	5.1-5.4	AB^R, Erm *(ermB)*	*Enterococcus, Staphylococcus, Lactococcus, Streptococcus, Bacillus, Listeria, Paenibacillus*	H, HH, A, F, SW	McDougal, et al., 1998; Novais, et al., 2008; Tomich, An, & Clewell, 1980
Tn*917* and variants (Tn*3871*)	Tn*3* (Tn*551*)	NA	5.1-5.4	AB^R, Erm *(ermB)*	*Enterococcus, Staphylococcus, Lactococcus, Streptococcus, Bacillus, Listeria, Paenibacillus*	H, HH, A, F, SW	McDougal, et al., 1998; Novais, et al., 2008; Tomich, An, & Clewell, 1980
Tn*1546* and variants[d]	Tn*3* (Tn*3*)	NA	10.8	AB^R, Van *(vanA)*	*Enterococcus, Bacillus, Staphylococcus, Oeskorvia, Streptococcus, Rhodococcus, Arcanobacterium haemolyticum, Paenibacillus*	H, HH, A, WA, P, F, SW	Arthur, Molinas, Depardieu, & Courvalin, 1993; Guardabassi, Perichon, van Heijenoort, Blanot, & Courvalin, 2005; López, et al., 2010; Novais, Freitas, Sousa, Baquero, Coque, & Peixe, 2008; Weigel, et al., 2003
Tn552 and variantsd	Tn7	NA	10.8	ABR, beta-lactams (blaZ-blaI-blaR1)	Enterococcus, Staphylococcus	HH	Coudron, Markowitz, & Wong, 1992; Murray, 1990; Rice & Thorisdottir, 1994; Sarti, Campanille, Sabia, Santagati, Gargiulo, & Stefani, 2012

Table 5c. Conjugative transposons/ICEs

Transposon	Family	Integrase type	Size (kb)	Characterized function[a] (genotype)	Host range	Origin	Integration sites	Reference
Conjugative Transposons								
Tn916 [a] and closely related variants showing insertions or deletions	Tn916	Tyr	18	AB^R, Tet (tetM)	Enterococcus, Staphylococcus, Streptococcus, Lactococcus, Lactobacillus,	H, HH, FA, WA, F, SW	A+T rich	Flannagan, Zitzow, Su, & Clewell, 1994

Table 5c. continued from previous page.

					Bacillus, Clostridium, Leuconostoc, Listeria, Mycoplasma, Actinobacillus, Acholeplasma, Acinetobacter, Alcaligenes, Butyrivibria, Citrobacter, Erysipelothrix, Escherichia, Fusobacterium, Granulicatella, Haemophilus, Neisseria, Pseudomonas, Thermus, Ureaplasma, Veillonella, anaerobes			
Tn918	Tn916	Tyr	16	AB^R, Tet (tetM)	Enterococcus faecalis, staphylococci	H	A+T rich	Clewell & Francia, 2004
Tn919 [b]	Tn916	Tyr	15.4	AB^R, Tet (tetM)	S. sanguis, E. faecalis, Lactoccus lactis	HH	A+T rich	Fitzgerald & Clewell, 1985
Tn920 [b]	Tn916	Tyr	23	AB^R, Tet (tetM)	Enterococcus faecalis	H	A+T rich	Murray, An, & Clewell, 1988
Tn925*	Tn916	Tyr	18	AB^R, Tet (tetM)	Enterococcus faecalis, Leuconostoc	H, HH, F	A+T rich	Christie, Korman, Zahler, Adsit, & Dunny, 1987; Hirt, et al., 2005
Tn3702	Tn916	Tyr	18	AB^R, Tet (tetM)	Enterococcus faecalis	H	A+T rich	Horaud, Delbos, & de Cespédès, 1990
Tn5031, Tn5032, Tn5033	Tn916	Tyr	NK	AB^R, Tet (tetM)	Enterococcus faecium	H	A+T rich	Fletcher, Marri, & Daneo-Moore, 1989
Tn5381	Tn916	Tyr	18	AB^R, Tet (tetM)	Enterococcus faecalis	H	A+T rich	Rice & Carias, 1998; Rice, Marshall, & Carias, 1992
Tn5383	Tn916	Tyr	18	AB^R, Tet (tetM)	Enterococcus faecalis	H	A+T rich	Rice, Marshall, & Carias, 1992

Table 5c. continued from previous page.

Tn1545 and variants	Tn916	Tyr	ca. 25	AB^R, Tet, Erm, Kan (tetM, ermB, aphA-3)	Streptococcus, Enterococcus, Staphylococcus, Bacillus, Bacteroides, Clostridium, Lactococcus, Listeria, Escherichia, Eubacterium, Neisseria, Roseburia, Ureaplasma	H, F	A+T rich	Clewell & Francia, 2004; Weaver, Rice, & Churchward, 2002
Tn1549	Tn916	Tyr	30	AB^R, Van (vanB2)	E. faecium, anaerobes	H, HH	A+T rich	Ballard, Pertile, Lim, Johnson, & Grayson, 2005; Garnier, Taourit, Glaser, Courvalin, & Galimand, 2000; Launay, Ballard, Johnson, Grayson, & Lambert, 2006
Tn5382 [c]	Tn916	Tyr	34	AB^R, Van, Amp (vanB2, pbp5)	Enterococcus (E. faecium, E. faecalis, E. gallinarum, E. hirae), Streptococcus, anaerobes	H, A, F	A+T rich	Ballard, Pertile, Lim, Johnson, & Grayson, 2005; Carias, Rudin, Donskey, & Rice, 1998; López, et al., 2009; Torres, et al., 2003
Tn6084, Tn6085a Tn6085b [b]	Tn916	Tyr		AB^R, Tet (tetM)	Enterococcus faecium	H, HH	A+T rich	Rice L. B., Carias, Rudin, Hutton, & Marshall, 2010
Tn5386	Tn916	Tyr	29	Lantibiotics (spa)	Enterococcus faecium	H	A+T rich	Rice L. B., Carias, Marshall, Rudin, & Hutton-Thomas, 2005
Tn6009	Tn916	Tyr	18	AB^R, Tet (tetM) Mercury (mer)	Enterococcus, Streptococcus, Pseudomonas, Klebsiella, Serratia	HHc	Not identified	Soge, Beck, White, No, & Roberts, 2008

Table 5c. continued from previous page.

ICEEfm1 [e] ($espPAI_{Efaecium}$)	Tn916	Tyr	64-104	Esp, partial copy of Tn916 including relaxase	Enterococcus faecium	H, HH, A, SW	Single site, Identified downstream rpsI gene	Leavis, et al., 2004; Top, Sinnige, Majoor, Bonten, Willems, & van Schaik, 2011; van Schaik, et al., 2010
Tn5397	Tn916	Ser	21	AB^R, Tet (tetM)	Enterococcus (E. faecium, E. hirae), Streptococcus, Clostridium difficile, Bacillus subtilis	H, HH, A, WA, F, SW	Single site	Agersø, Pedersen, & Aarestrup, 2006; Novais, Freitas, Baquero, Peixe, & Coque, 2010; Poeta, et al., 2007
Tn5801 [f], CW459 (Tn6086, Tn6014)	Tn916		25	AB^R, Tet (tetM)	Staphylococcus, Enterococcus, Streptococcus mitis, Clostridium perfringens	H, HH, A, F, SW	3′ of GMP synthase gene	de Vries, Christensen, Skov, Aarestrup, & Agersø, 2009; Denapaite, et al., 2010; Novais, Freitas, Baquero, Peixe, & Coque, 2010
Tn6000	Tn916	Related to that of S. aureus PAI SaPIbov and SaPIbov2	33.2	AB^R, Tet (tetS)	Enterococcus (E. faecium, E. faecalis, E. casseliflavus)	H, HH, A	3′ end of gene encoding ribosomal protein L31	Brouwer, Mullany, & Roberts, 2010; Novais, Freitas, Baquero, Peixe, & Coque, 2010; Roberts, Davis, Seville, Villedieu, & Mullany, 2006
Tn950	NI	Not identified	47	AB^R, Erm (ermB)	Enterococcus faecium	H	Not identified	Takeuchi, Tomita, Fujimoto, Kudo, Kuwano, & Ike, 2005
EfaC1	NI	Tyr	25.3	None identified	Enterococcus faecalis	H	3′ end of gene encoding tRNA	Burrus, Pavlovic, Decaris, & Guédon, 2002; Paulsen, et al., 2003

Table 5c. continued from previous page.

EfaC2	NI	Tyr	32.7	None identified	Enterococcus faecalis	H	3′ of GMP synthase gene	Burrus, Pavlovic, Decaris, & Guédon, 2002; Paulsen, et al., 2003
EfaD2	NI	Tyr	ND	None identified	Enterococcus faecalis	H	Unknown	Burrus, Pavlovic, Decaris, & Guédon, 2002; Paulsen, et al., 2003
OG1RF-CTn homologue	NI	Putative phage-related integrase	49	Putative adhesin proteins	*Enterococcus faecalis*	H	Unknown	Bourgogne, et al., 2008

Abbreviations: AB^r, antibiotic resistance; Amp, ampicillin; Erm, erythromycin; Gm, high level of resistance to gentamicin; Km, kanamycin; Sm, streptomycin; St, streptothricin; Tet, tetracycline; Van, vancomycin: Tyr, tyrosine recombinase; Ser, serine recombinase; H, Hospital; HH, healthy humans; HHc, Healthy humans (children); A, animals (farm or healthy animals); WA, wild animals; F, foods; P, pets; SW, sewage.NA: Not available; NI: not identified

[a] Multiple variants that contain insertions or deletions have been described for these CTns. The previously published CTn*916S* (*tetS*), identified in a *Streptococcus*
intermedius human isolate (Genbank accession no.AY534326.1), is in fact an hybrid of CTn*6000* and CTn*916* carrying a mosaic tet(S/M) (Novais, et al., 2012).

[b] Tn*919* and Tn*920* have not been completely characterized.

[c] Tn*5382* is basically Tn*1549* with a cluster of genes that contain *pbp5* inserted upstream of the Tn.

[d] Tn*6084*, Tn*6085a*, and Tn*6085b* contain a 2.7 kb putative group II intron inserted at nucleotide 3913 at the beginning of orf19 in Tn916, whose function remains unknown. Tn*6084* has an *ISEfa11* insertion. Tn*6085a* and Tn*6085b* are identical and therefore were given the designations Tn*6085a* and Tn*6085b*. All three have been identified in the same isolate *E. faecium* strain C68, which also harbors Tn*5382* (*vanB*).

[e] Previously described as a genetic island (Leavis, et al., 2004; van Schaik, et al., 2010). It shares the ***esp*** gene itself and a 10-kb completely conserved gene cluster with the PAI of E. *faecalis* (Top, Sinnige, Majoor, Bonten, Willems, & van Schaik, 2011).

[f] Tn*5801* has been completely characterized in *E. faecium* and *E. faecalis* (Novais, et al., 2013, T.M. Coque personal communication), only integrases have been detected in natural isolates (Novais, et al., 2013).

Table 6. Enterococcal Introns

Name	ORF Name	Host gene	Locus	Locus	Size	Type	GenBank accession nº (nt position)	Species
E.f.I1	RT	Unknown	Plasmid	pHTbeta	638	B	AB105543 (1-2748)	*E. faecalis, E. faecium*
E.f.I2	IepA like protein	TraG like protein	PAI	PAI	653	B	AF454824 (13115-15951)	*Enterococcus faecalis*
E.f.I3	Group II intron RT	MAFF-like protein	Chromosome	Chromosome	628	B	AE016954 (115179-117948)	*Enterococcus faecalis*
E.f.I4	Unnamed	FtsK/ SpoIIIE family protein	Chromosome	Chromosome	584	B	AE016830 (1833893-1836490)	*Enterococcus faecalis*

Table 6. continued from previous page.

En.fm.I1-1	EfaeDRAFT_2438	ORF19	CTn, plasmid	Tn*916*, Tn*6085*a, Tn*6085*, pCF10	638	B	NZ_AAAK03000007 (10877-13634) NC_006372.1	*Enterococcus faecium Enterococcus faecalis, Streptococcus, Staphylococci, Clostridium*
En.fm.I1-2	ORF16	None	CTn, plasmid	CTn*5386*, Inc18 plasmids (pVEF1-4, pIP186, pWZ909, pTW9,	608	B	DQ321786 (12459-15138)	*Enterococcus faecium Enterococcus faecalis, Streptococcus, Staphylococci, Lactococcus*
C.d.I1	ORF16	Unnamed	ORF14	CTn*5397*, CTn*6000*	609	B	X98606 (13-2658)	*Clostridium difficile, Enterococcus casseliflavus, E. faecium,*
L.l.I1[a]	ltrA	ltrB, mobA	Conjugative plasmid	pRS01	599	A1	U50902 (2854-5345)	*Lactococcus lactis*

Updated and completed on the information available at http://www.fp.ucalgary.ca/group2introns/
Abbreviations: PAI, pathogenicity island; CTn, conjugative transposon
[a] L.l.I1 has not been identified in natural enterococcal isolates (see text).

References

Aarestrup, F. M., Butaye, P., & Witte, W. (2002). Nonhuman reservoirs of enterococci. In M. S. Gilmore, D. B. Clewell, P. Courvalin, G. M. Dunny, B. E. Murray, & L. B. Rice (Eds.), *The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance* (pp. 55-100). Washington, D. C.: ASM Press.

Abajy M. Y., Kopeć J., Schiwon K., Burzynski M., Döring M., Bohn C., et al. A type IV-secretion-like system is required for conjugative DNA transport of broad-host-range plasmid pIP501 in gram-positive bacteria. Journal of Bacteriology. 2007;189(6):2487–2496. PubMed PMID: 17209024.

Agersø Y., Pedersen A. G., Aarestrup F. M. Identification of Tn5397-like and Tn916-like transposons and diversity of the tetracycline resistance gene tet(M) in enterococci from humans, pigs and poultry. Journal of Antimicrobial Chemotherapy. 2006;57(5):832–839. PubMed PMID: 16565159.

Alonso J. C., Ayora S., Canosa I., Weise F., Rojo F. Site-specific recombination in gram-positive theta-replicating plasmids. FEMS Microbiology Letters. 1996;142(1):1–10. PubMed PMID: 8759784.

Alvarez-Martinez C. E., Christie P. J. Biological diversity of prokaryotic type IV secretion systems. Microbiology and Molecular Biology Reviews. 2009;73(4):775–808. PubMed PMID: 19946141.

An F. Y., Clewell D. B. Characterization of the determinant (traB) encoding sex pheromone shutdown by the hemolysin/bacteriocin plasmid pAD1 in Enterococcus faecalis. Plasmid. 1994;31(2):215–221. PubMed PMID: 8029329.

An F. Y., Clewell D. B. The origin of transfer (oriT) of the enterococcal, pheromone-responding, cytolysin plasmid pAD1 is located within the repA determinant. Plasmid. 1997;37(1):87–94. PubMed PMID: 9073585.

An F. Y., Clewell D. B. Identification of the cAD1 sex pheromone precursor in Enterococcus faecalis. Journal of Bacteriology. 2002;184(7):1880–1887. PubMed PMID: 11889094.

An F. Y., Sluvaik M. C., Clewell D. B. Identification and characterization of a determinant (eep) on the Enterococcus faecalis chromosome that is involved in production of the peptide sex pheromone cAD1. Journal of Bacteriology. 1999;181(19):5915–5921. PubMed PMID: 10498702.

Arthur M., Molinas C., Depardieu F., Courvalin P. Characterization of Tn1546, a Tn3-related transposon conferring glycopeptide resistance by synthesis of depsipeptide peptidoglycan precursors in Enterococcus faecium BM4147. Journal of Bacteriology. 1993;175(1):117–127. PubMed PMID: 8380148.

Bae T., Kozlowicz B. K., Dunny G. M. Characterization of cis-acting prgQ mutants: evidence for two distinct repression mechanisms by Qa RNA and PrgX protein in pheromone-inducible enterococcal plasmid pCF10. Molecular Microbiology. 2004;51(1):271–281. PubMed PMID: 14651627.

Balla E., Dicks L. M. Molecular analysis of the gene cluster involved in the production and secretion of enterocins 1071A and 1071B and of the genes responsible for the replication and transfer of plasmid pEF1071. International Journal of Food Microbiology. 2005;99(1):33–45. PubMed PMID: 15718027.

Ballard S. A., Pertile K. K., Lim M., Johnson P. D., Grayson M. L. Molecular characterization of vanB elements in naturally occurring gut anaerobes. Antimicrobial Agents and Chemotherapy. 2005;49(5):1688–1694. PubMed PMID: 15855482.

Banai M., LeBlanc D. J. Streptococcus faecalis R plasmid pJH1 contains an erythromycin resistance transposon (Tn3871) similar to transposon Tn917. Journal of Bacteriology. 1984;158(3):1172–1174. PubMed PMID: 6327631.

Barillà D., Rosenberg M. F., Nobbmann U., Hayes F. Bacterial DNA segregation dynamics mediated by the polymerizing protein ParF. The EMBO Journal. 2005;24(7):1453–1464. PubMed PMID: 15775965.

Belhocine K., Yam K. K., Cousineau B. Conjugative transfer of the Lactococcus lactis sex factor and pRS01 plasmid to Enterococcus faecalis. FEMS Microbiology Letters. 2005;187(3):930–939. PubMed PMID: 17263841.

Benachour A., Auffray Y., Hartke A. Construction of plasmid vectors for screening replicons from gram-positive bacteria and their use as shuttle cloning vectors. Current Microbiology. 2007;54(5):342–347. PubMed PMID: 17486410.

Biavasco F., Foglia G., Paoletti C., Zandri G., Magi G., Guaglianone E., et al. VanA-Type Enterococci from Humans, Animals, and Food: Species Distribution, Population Structure, Tn1546 Typing and Location, and Virulence Determinants. Applied and Environmental Microbiology. 2007;73(10):3307–3319. PubMed PMID: 17351100.

Bidnenko V., Ehrlich S. D., Jannière L. In vivo relations between pAMβ1-encoded type I topoisomerase and plasmid replication. Molecular Microbiology. 1998;28(5):1005–1016. PubMed PMID: 9663686.

Blower T. R., Fineran P. C., Johnson M. J., Toth I. K., Humphreys D. P., Salmond G. P. Mutagenesis and functional characterization of the RNA and protein components of the toxIN abortive infection and toxin-antitoxin locus of Erwinia. Journal of Bacteriology. 2009;191(19):6029–6039. PubMed PMID: 19633081.

Blower T. R., Pei X. Y., Short F. L., Fineran P. C., Humphreys D. P., Luisi B. F., et al. A processed noncoding RNA regulates an altruistic bacterial antiviral system. Nature Structural & Molecular Biology. 2011;18(2):185–190. PubMed PMID: 21240270.

Boerlin P., Burnens A. P., Frey J., Kuhnert P., Nicolet J. Molecular epidemiology and genetic linkage of macrolide and aminoglycoside resistance in Staphylococcus intermedius of canine origin. Veterinary Microbiology. 2001;79(2):155–169. PubMed PMID: 11230937.

Bonafede M. E., Carias L. L., Rice L. B. Enterococcal transposon Tn5384: evolution of a composite transposon through cointegration of enterococcal and staphylococcal plasmids. Antimicrobial Agents and Chemotherapy. 1997;41(9):1854–1858. PubMed PMID: 9303373.

Bourgogne A., Garsin D. A., Qin X., Singh K. V., Sillanpää J., Yerrapragada S., et al. Large scale variation in Enterococcus faecalis illustrated by the genome analysis of strain OG1RF. Genome Biology. 2008;9(7):R110. PubMed PMID: 18611278.

Brantl S., Behnke D. Copy number control of the streptococcal plasmio plP501 occurs at three levels. Nucleic Acids Research. 1991;20(3):395–400. PubMed PMID: 1741273.

Brantl S., Wagner E. G. An unusually long-lived antisense RNA in plasmid copy number control: in vivo RNAs encoded by the streptococcal plasmid pIP501. Journal of Molecular Biology. 1996;255(2):275–288. PubMed PMID: 8551520.

Brantl S., Wagner E. G. Dual function of the copR gene product of plasmid pIP501. Journal of Bacteriology. 1997;179(22):7016–7024. PubMed PMID: 9371448.

Brantl S., Behnke D., Alonso J. C. Molecular analysis of the replication region of the conjugative Streptococcus agalactiae plasmid pIP501 in Bacillus subtilis. Comparison with plasmids pAMβ1 and pSM 19035. Nucleic Acids Research. 1990;18(16):4783–4790. PubMed PMID: 2118624.

Brantl S., Birch-Mirschfeld E., Behnke D. RepR protein expression on plasmid pIP501 is controlled by an antisense RNA-mediated transcription attenuation mechanism. Journal of Bacteriology. 1993;175(13):4052–4061. PubMed PMID: 8320221.

Brede D. A., Snipen L. G., Ussery D. W., Nederbragt A. J., Nes I. F. Complete genome sequence of the commensal Enterococcus faecalis 62, isolated from a healthy Norwegian infant. Journal of Bacteriology. 2011;193(9):2377. PubMed PMID: 21398545.

Brinkman C. L., Bumgarner R., Kittichotirat W., Dunman P. M., Kuechenmeister L. J., Weaver K. E. Characterization of the effects of an rpoC mutation that confers resistance to the Fst peptide toxin-antitoxin system. Journal of Bacteriology. 2013;195(1):156–166. PubMed PMID: 23104812.

Broome-Smith J. RecA independent, site-specific recombination between ColE1 or ColK and a miniplasmid they complement for mobilization and relaxation: implications for the mechanism of DNA transfer during mobilization. Plasmid. 1980;4(1):51–63. PubMed PMID: 6100903.

Brouwer M. S., Mullany P., Roberts A. P. Characterization of the conjugative transposon Tn6000 from Enterococcus casseliflavus 664.1H1 (formerly Enterococcus faecium 664.1H1). FEMS Microbiology Letters. 2010;309(1):71–76. PubMed PMID: 20528943.

Brown M. S., Ye J., Rawson R. B., Goldstein J. L. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell. 2000;100(4):391–398. PubMed PMID: 10693756.

Bruand C., Ehrlich S. D. Transcription-driven DNA replication of plasmid pAMβ1 in Bacillus subtilis. Molecular Microbiology. 1998;30(1):135–145. PubMed PMID: 9786191.

Bruand C., Le Chatelier E., Ehrlich S. D., Jannière L. A fourth class of theta-replicating plasmids: the pAM beta 1 family from gram-positive bacteria. Proceedings of the National Academy of Sciences. 1993;90(24):11668–11672. PubMed PMID: 8265606.

Burdett V. Identification of tetracycline-resistant R-plasmids in Streptococcus agalactiae (group B). Antimicrobial Agents and Chemotherapy. 1980;18(5):753–760. PubMed PMID: 7004347.

Burrus V., Pavlovic G., Decaris B., Guédon G. Conjugative transposons: the tip of the iceberg. Molecular Microbiology. 2002;46(3):601–610. PubMed PMID: 12410819.

Byzov B. A., Claus H., Tretyakova E. B., Ryabchenko N. F., Mozgovaya I. N., Zvyagintsev D. G., et al. Plasmid transfer between introduced and indiginous bacteria in leaf litter, soil and vermicompost as affected by soil invertebrates. Biology and Fertility of Soils. 1999;28(2):169–176.

Camacho A. G., Misselwitz R., Behlke J., Ayora S., Welfle K., Meinhat A., et al. In vitro and in vivo stability of the epsilon2zeta2 protein complex of the broad host-range Streptococcus pyogenes pSM19035 addiction system. The Journal of Biological Chemistry. 2002;383(11):1701–1713. PubMed PMID: 12530535.

Campbell C. S., Mullins R. D. In vivo visualization of type II plasmid segregation: bacterial actin filaments pushing plasmids. The Journal of Cell Biology. 2007;179(5):1059–1066. PubMed PMID: 18039937.

Caparon M. G., Scott J. R. Excision and insertion of the conjugative transposon Tn916 involves a novel recombination mechanism. Cell. 1989;59(6):1027–1034. PubMed PMID: 2557157.

Carias L. L., Rudin S. D., Donskey C. J., Rice L. B. Genetic linkage and cotransfer of a novel, vanB-containing transposon (Tn5382) and a low-affinity penicillin-binding protein 5 gene in a clinical vancomycin-resistant Enterococcus faecium isolate. Journal of Bacteriology. 1998;180(17):4426–4434. PubMed PMID: 9721279.

Cascales E., Christie P. J. The versatile bacterial type IV secretion systems. Nature Reviews Microbiology. 2003;1(2):137–149. PubMed PMID: 15035043.

Ceglowski P., Boitsov A., Karamyan N., Chai S., Alonso J. C. Characterization of the effectors required for stable inheritance of Streptococcus pyogenes pSM19035-derived plasmids in Bacillus subtilis. Molecular Genetics and Genomics. 1993;241(5-6):579–585. PubMed PMID: 8264532.

Cercenado, E., Marín, M., Insa, R., & Bouza, E. (2010). Emergence of linezolid-resistant Gram-positive clinical isolates due to cfr-methylase gene production associated withG2576T mutation in the 23S rRNA. *20th European Congress of Clinical Microbiology and Infectious Diseases.* Vienna: European Society of Clinical Microbiology and Infectious Diseases.

Cha K. I., Lim K., Jang S., Lim W., Kim T., Chang H. Molecular interactions of a replication initiator protein, RepA, with the replication origin of the enterococcal plasmid p703/5. Journal of Microbiology and Biotechnology. 2007;17(11):1841–1847. PubMed PMID: 18092469.

Chandler J. R., Dunny G. M. Characterization of the sequence specificity determinants required for processing and control of sex pheromone by the intramembrane protease Eep and the plasmid-encoded protein PrgY. Journal of Bacteriology. 2008;190(4):1172–1183. PubMed PMID: 18083822.

Chang J. C., LaSarre B., Jimenez J. C., Aggarwal C., Federle M. J. Two Group A Streptococcal Peptide Pheromones Act through Opposing Rgg Regulators to Control Biofilm Development. PLOS Pathogens. 2011;7(8):e1002190. PubMed PMID: 21829369.

Chatterjee A., Johnson C. M., Shu C.-C., Kaznessis Y., Ramkrishna D., Dunny G. M., et al. Convergent transcription confers a bistable switch in Enterococcus faecalis conjugation. Proceedings of the National Academy of Sciences. 2011;108:9721–9726. PubMed PMID: 21606359.

Chattoraj D. K. Control of plasmid DNA replication by iterons: no longer paradoxical. Molecular Microbiology. 2000;37(3):467–476. PubMed PMID: 10931340.

Chen Y., Zhang X., Manias D., Yeo H. J., Dunny G. M., Christie P. J. Enterococcus faecalis PcfC, a spatially localized substrate receptor for type IV secretion of the pCF10 transfer intermediate. Journal of Bacteriology. 2008;190(10):3632–3645. PubMed PMID: 18326569.

Christie P. J., Korman R. Z., Zahler S. A., Adsit J. C., Dunny G. M. Two conjugation systems associated with Streptococcus faecalis plasmid pCF10: identification of a conjugative transposon that transfers between S. faecalis and Bacillus subtilis. Journal of Bacteriology. 1987;169(6):2529–2536. PubMed PMID: 3034859.

Clark N. C., Olsvik Ø., Swenson J. M., Spiegel C. A., Tenover F. C. Detection of a streptomycin/spectinomycin adenylyltransferase gene (aadA) in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1999;43(1):157–160. PubMed PMID: 9869582.

Clark N. C., Weigel L. M., Patel J. B., Tenover F. C. Comparison of Tn1546-like elements in vancomycin-resistant Staphylococcus aureus isolates from Michigan and Pennsylvania. Antimicrobial Agents and Chemotherapy. 2005;49(1):470–472. PubMed PMID: 15616340.

Clewell D. B. Properties of Enterococcus faecalis plasmid pAD1, a member of a widely disseminated family of pheromone-responding, conjugative, virulence elements encoding cytolysin. Plasmid. 2007;58(3):205–227. PubMed PMID: 17590438.

Clewell D. B., Brown B. L. Sex pheromone cAD1 in Streptococcus faecalis: induction of a function related to plasmid transfer. Journal of Bacteriology. 1980;143(2):1063–1065. PubMed PMID: 6782073.

Clewell, D. B., & Dunny, G. M. (2002). Conjugation and genetic exchange in enterococci. In M. S. Gilmore (Ed.), *The Enterococci: Pathogenesis, Molecular Biology and Antibiotic Resistance* (pp. 1880-1887). Washington, D. C.: ASM Press.

Clewell, D. B., & Francia, M. V. (2004). Conjugation in Gram-Positive Bacteria. In B. Funnell, & J. G. Phillips (Eds.), *Plasmid Biology* (pp. 227-256). Washington, D. C.: American Society for Microbiology.

Clewell D. B., Gawron-Burke C. Conjugative transposons and the dissemination of antibiotic resistance in streptococci. Annual Review of Microbiology. 1986;40:635–659. PubMed PMID: 3022640.

Clewell D. B., Weaver K. E. Sex pheromones and plasmid transfer in Enterococcus faecalis. Plasmid. 1989;21(3):175–184. PubMed PMID: 2550976.

Clewell D. B., An F. Y., Flannagan S. E., Antiporta M., Dunny G. M. Enterococcal sex pheromone precursors are part of signal sequences for surface lipoproteins. Molecular Microbiology. 2000;35(1):246–247. PubMed PMID: 10632894.

Clewell D. B., An F. Y., White B. A., Gawron-Burke C. Streptococcus faecalis sex pheromone (cAM373) also produced by Staphylococcus aureus and identification of a conjugative transposon (Tn918). Journal of Bacteriology. 1985;162(3):1212–1220. PubMed PMID: 2987186.

Clewell D. B., Flannagan S. E., Jaworski D. D. Unconstrained bacterial promiscuity: the Tn916 - Tn1545 family of conjugative transposons. Trends in Microbiology. 1995;3(6):229–236. PubMed PMID: 7648031.

Clewell D. B., Yagi Y., Bauer B. Plasmid-determined tetracycline resistance in Streptococcus faecalis: evidence for gene amplification during growth in presence of tetracycline. Proceedings of the National Academy of Sciences. 1975;72(5):1720–1724. PubMed PMID: 807914.

Clewell D. B., Yagi Y., Dunny G. M., Schultz S. K. Characterization of Three Plasmid Deoxyribonucleic Acid Molecules in a Strain of Streptococcus faecalis: Identification of a Plasmid Determining Erythromycin Resistance. Journal of Bacteriology. 1974;117(1):283–289. PubMed PMID: 4202995.

Clewell, D. B., Yagi, Y., Ike, Y., Craig, R. A., Brown, B. L., & An, F. (1982). Sex pheromones in Streptococcus faecalis. Multiple pheromone systems in strain DS5, similarities of pAD1 and pAMgamma1, and mutants of pAD1 altered in conjugative properties. In D. Schlessinger (Ed.), *Microbiology* (pp. 97-100). Washington, D. C.: American Society for Microbiology.

Coburn P. S., Baghdayan A. S., Craig N., Burroughs A., Tendolkar P., Miller K., et al. A novel conjugative plasmid from Enterococcus faecalis E99 enhances resistance to ultraviolet radiation. Plasmid. 2010;64(1):18–25. PubMed PMID: 20307569.

Coburn P. S., Baghdayan A. S., Dolan G. T., Shankar N. Horizontal transfer of virulence genes encoded on the Enterococcus faecalis pathogenicity island. Molecular Microbiology. 2007;63(2):530–544. PubMed PMID: 17163979.

Coburn P. S., Baghdayan A. S., Dolan G. T., Shankar N. An AraC-type transcriptional regulator encoded on the Enterococcus faecalis pathogenicity island contributes to pathogenesis and intracellular macrophage survival. Infection and Immunity. 2008;76(12):5668–5676. PubMed PMID: 18824537.

Colmar I., Horaud T. Enterococcus faecalis hemolysin-bacteriocin plasmids belong to the same incompatibility group. Applied and Environmental Microbiology. 1987;53(3):567–570. PubMed PMID: 3107464.

Condon C. Shutdown decay of mRNA. Molecular Microbiology. 2006;61(3):573–583. PubMed PMID: 16803593.

Corvaglia A. R., François P., Hernandez D., Perron K., Linder P., Schrenzel J. A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical Staphylococcus aureus

strains. Proceedings of the National Academy of Sciences. 2010;107(26):11954–11958. PubMed PMID: 20547849.

Coudron P. E., Markowitz S. M., Wong E. S. Isolation of a beta-lactamase-producing, aminoglycoside-resistant strain of Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 1992;36(5):1125–1126. PubMed PMID: 1510404.

Criado R., Diep D. B., Aakra A., Gutiérrez J., Nes I. F., Hernández P. E., et al. Complete sequence of the enterocin Q-encoding plasmid pCIZ2 from the multiple bacteriocin producer Enterococcus faecium L50 and genetic characterization of enterocin Q production and immunity. Applied and Environmental Microbiology. 2006;72(10):6653–6666. PubMed PMID: 17021217.

Criado R., Gutiérrez J., Budin-Verneuil A., Hernández P. E., Hartke A., Cintas L. M., et al. Molecular analysis of the replication region of the pCIZ2 plasmid from the multiple bacteriocin producer strain Enterococcus faecium L50. Plasmid. 2008;60(3):181–189. PubMed PMID: 18692522.

Davis I. J., Roberts A. P., Ready D., Richards H., Wilson M., Mullany P. Linkage of a novel mercury resistance operon with streptomycin resistance on a conjugative plasmid in Enterococcus faecium. Plasmid. 2005;54(1):26–38. PubMed PMID: 15907536.

De Boever E. H., Clewell D. B., Fraser C. M. Enterococcus faecalis conjugative plasmid pAM373: complete nucleotide sequence and genetic analyses of sex pheromone response. Molecular Microbiology. 2000;37(6):1327–1341. PubMed PMID: 10998166.

de la Hoz A. B., Ayora S., Sitkiewcz I., Fernández S., Pankiewicz R., Alonso J. C., et al. Plasmid copy-number control and better-than-random segregation genes of pSM19035 share a common regulator. Proceedings of the National Academy of Sciences. 2000;97(2):728–733. PubMed PMID: 10639147.

de Regt M. J., van Schaik W., van Luit-Asbroek M., Dekker H. A., van Duijkeren E., Koning C. J., et al. Hospital and Community Ampicillin-Resistant Enterococcus faecium Are Evolutionarily Closely Linked but Have Diversified through Niche Adaptation. PLoS ONE. 2012;7(2):e30319. PubMed PMID: 22363425.

de Smit M. H., van Duin J. Translational Standby Sites: How Ribosomes May Deal with the Rapid Folding Kinetics of mRNA. Journal of Molecular Biology. 2003;331(4):737–743. PubMed PMID: 12909006.

de Vries L. E., Christensen H., Skov R. L., Aarestrup F. M., Agersø Y. Diversity of the tetracycline resistance gene tet(M) and identification of Tn916- and Tn5801-like (Tn6014) transposons in Staphylococcus aureus from humans and animals. Journal of Antimicrobial Chemotherapy. 2009;64(3):490–500. PubMed PMID: 19531603.

Declerck N., Bouillaut L., Chaix D., Rugani N., Slamti L., Hoh F., et al. Structure of PlcR: Insights into virulence regulation and evolution of quorum sensing in Gram-positive bacteria. Proceedings of the National Academy of Sciences. 2007;104(47):18490–18495. PubMed PMID: 17998541.

del Solar G., Giraldo R., Ruiz-Echevarría M. J., Espinosa M., Díaz-Orejas R. Replication and control of circular bacterial plasmids. Microbiology and Molecular Biology Reviews. 1998;62(2):434–464. PubMed PMID: 9618448.

del Solar G., Hernández-Arriaga A. M., Gomis-Rüth F. X., Coll M., Espinosa M. A genetically economical family of plasmid-encoded transcriptional repressors involved in control of plasmid copy number. Journal of Bacteriology. 2002;184(18):4943–4951. PubMed PMID: 12193609.

Denapaite D., Brückner R., Nuhn M., Reichmann P., Henrich B., Maurer P., et al. The Genome of Streptococcus mitis B6 - What Is a Commensal? PLoS ONE. 2010;5(2):e9426. PubMed PMID: 20195536.

Derbise A., Dyke K. G., el Solh N. Characterization of a Staphylococcus aureus transposon, Tn5405, located within Tn5404 and carrying the aminoglycoside resistance genes, aphA-3 and aadE. Plasmid. 1996;35(3):174–188. PubMed PMID: 8812784.

Derman A. I., Becker E. C., Truong B. D., Fujioka A., Tucey T. M., Erb M. L., et al. Phylogenetic analysis identifies many uncharacterized actin-like proteins (Alps) in bacteria: regulated polymerization, dynamic instability and treadmilling in Alp7A. Molecular Microbiology. 2009;73(4):534–552. PubMed PMID: 19602153.

Derome A., Hoischen C., Bussiek M., Grady R., Adamczyk M., Kędzierska B., et al. Centromere anatomy in the multidrug-resistant pathogen Enterococcus faecium. Proceedings of the National Academy of Sciences. 2008;105(6):2151–2156. PubMed PMID: 18245388.

Diaz L., Kiratisin P., Mendes R. E., Panesso D., Singh K. V., Arias C. A. Transferable plasmid-mediated resistance to linezolid due to cfr in a human clinical isolate of Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 2012;56(7):3917–3922. PubMed PMID: 22491691.

Du L., Somkuti G. A., Renye J. A. Jr. Molecular analysis of the bacteriocin-encoding plasmid pDGL1 from Enterococcus durans and genetic characterization of the durancin GL locus. Microbiology. 2012;158(Pt 6):1523–1532. PubMed PMID: 22493306.

Dunny G. M. The peptide pheromone-inducible conjugation system of Enterococcus faecalis plasmid pCF10: cell-cell signalling, gene transfer, complexity and evolution. Philosophical Transactions of the Royal Society B: Biological Sciences. 2007;362(1483):1185–1193. PubMed PMID: 17360276.

Dunny G. M., Johnson C. M. Regulatory circuits controlling enterococcal conjugation: lessons for functional genomics. Current Opinion in Microbiology. 2011;14(2):174–180. PubMed PMID: 21353627.

Dunny G. M., Brown B. L., Clewell D. B. Induced cell aggregation and mating in Streptococcus faecalis: evidence for a bacterial sex pheromone. Proceedings of the National Academy of Sciences. 1978;75(7):3479–3483. PubMed PMID: 98769.

Dunny G. M., Craig R. A., Carron R. L., Clewell D. B. Plasmid transfer in Streptococcus faecalis: production of multiple sex pheromones by recipients. Plasmid. 1979;2(3):454–465. PubMed PMID: 113798.

Ehrenfeld E. E., Kessler R. E., Clewell D. B. Identification of pheromone-induced surface proteins in Streptococcus faecalis and evidence of a role for lipoteichoic acid in formation of mating aggregates. Journal of Bacteriology. 1986;168(1):6–12. PubMed PMID: 3093466.

Espinosa M., del Solar G., Rojo F., Alonso J. C. Plasmid rolling circle replication and its control. FEMS Microbiology Letters. 1995;130(2-3):111–120. PubMed PMID: 7649431.

Fineran P. C., Blower T. R., Foulds I. J., Humphreys D. P., Lilley K. S., Salmond G. P. The phage abortive infection system, ToxIN, functions as a protein–RNA toxin–antitoxin pair. Proceedings of the National Academy of Sciences. 2009;106(3):894–899. PubMed PMID: 19124776.

Firth N., Fink P. D., Johnson L., Skurray R. A. A lipoprotein signal peptide encoded by the staphylococcal conjugative plasmid pSK41 exhibits an activity resembling that of Enterococcus faecalis pheromone cAD1. Journal of Bacteriology. 1994;176:5871–5873. PubMed PMID: 8083183.

Firth N., Fink P. D., Johnson L., Skurray R. A. Enterococcal pheromone-like activity derived from a lipoprotein signal peptide encoded by a Staphylococcus aureus plasmid. Advances in Experimental Medicine and Biology. 1994;176(18):5871–5873.

Fischbach M. A., Walsh C. T. Antibiotics for emerging pathogens. Science. 2009;325(5944):1089–1093. PubMed PMID: 19713519.

Fitzgerald G. F., Clewell D. B. A conjugative transposon (Tn919) in Streptococcus sanguis. Infection and Immunity. 1985;47(2):415–420. PubMed PMID: 2981772.

Flannagan S. E., Clewell D. B. Conjugative transfer of Tn916 in Enterococcus faecalis: trans activation of homologous transposons. Journal of Bacteriology. 1991;173(22):7136–7141. PubMed PMID: 1657880.

Flannagan S. E., Clewell D. B. Identification and characterization of genes encoding sex pheromone cAM373 activity in Enterococcus faecalis and Staphylococcus aureus. Molecular Microbiology. 2002;44(3):803–817. PubMed PMID: 11994160.

Flannagan S. E., Chow J. W., Donabedian S. M., Brown W. J., Perri M. B., Zervos M. J., et al. Plasmid content of a vancomycin-resistant Enterococcus faecalis isolate from a patient also colonized by Staphylococcus aureus with a VanA phenotype. Antimicrobial Agents and Chemotherapy. 2003;47(12):3954–3959. PubMed PMID: 14638508.

Flannagan S. E., Zitzow L. A., Su Y. A., Clewell D. B. Nucleotide sequence of the 18-kb conjugative transposon Tn916 from Enterococcus faecalis. Plasmid. 1994;32(3):350–354. PubMed PMID: 7899523.

Fletcher H. M., Marri L., Daneo-Moore L. Transposon-916-like elements in clinical isolates of Enterococcus faecium. Microbiology. 1989;135(11):3067–3077. PubMed PMID: 2559146.

Folli C., Mangiarotti L., Folloni S., Alfieri B., Gobbo M., Berni R., et al. Specificity of the TraA-DNA interaction in the regulation of the pPD1-encoded sex pheromone response in Enterococcus faecalis. Journal of Molecular Biology. 2008;380(5):932–945. PubMed PMID: 18579153.

Fothergill T. J., Barillà D., Hayes F. Protein diversity confers specificity in plasmid segregation. Journal of Bacteriology. 2005;187(8):2651–2661. PubMed PMID: 15805511.

Fozo E. M., Hemm M. R., Storz G. Small toxic proteins and the antisense RNAs that repress them. Microbiology and Molecular Biology Reviews. 2008;72(4):579–589. PubMed PMID: 19052321.

Fozo E. M., Makarova K. S., Shabalina S. A., Yutin N., Koonin E. V., Storz G. Abundance of type I toxin–antitoxin systems in bacteria: searches for new candidates and discovery of novel families. Nucleic Acids Research. 2010;38(11):3743–3759. PubMed PMID: 20156992.

Franch T., Gerdes K. U-turns and regulatory RNAs. Current Opinion in Microbiology. 2000;3(2):159–164. PubMed PMID: 10744992.

Franch T., Gultyaev A. P., Gerdes K. Programmed cell death by hok/sok of plasmid R1: Processing at the hok mRNA 3′-end triggers structural rearrangements that allow translation and antisense RNA binding. Journal of Molecular Biology. 1997;273(1):38–51. PubMed PMID: 9367744.

Francia M. V., Clewell D. B. Amplification of the Tetracycline Resistance Determinant of pAMα1 in Enterococcus faecalis Requires a Site-Specific Recombination Event Involving Relaxase. Journal of Bacteriology. 2002;184(18):5187–5193. PubMed PMID: 12193637.

Francia M. V., Clewell D. B. Transfer origins in the conjugative Enterococcus faecalis plasmids pAD1 and pAM373: identification of the pAD1 nic site, a specific relaxase and a possible TraG-like protein. Molecular Microbiology. 2002;45(2):375–395. PubMed PMID: 12123451.

Francia M. V., Fujimoto S., Tille P., Weaver K. E., Clewell D. B. Replication of Enterococcus faecalis Pheromone-Responding Plasmid pAD1: Location of the Minimal Replicon and oriV Site and RepA Involvement in Initiation of Replication. Journal of Bacteriology. 2004;186(15):5003–5016. PubMed PMID: 15262938.

Francia M. V., Haas W., Wirth R., Samberger E., Muscholl-Silberhorn A., Gilmore M. S., et al. Completion of the nucleotide sequence of the Enterococcus faecalis conjugative virulence plasmid pAD1 and identification of a second transfer origin. Plasmid. 2001;46(2):117–127. PubMed PMID: 11591137.

Francia M. V., Varsaki A., Garcillán-Barcia M. P., Latorre A., Drainas C., de la Cruz F. A classification scheme for mobilization regions of bacterial plasmids. FEMS Microbiology Reviews. 2004;28(1):79–100. PubMed PMID: 14975531.

Francia M. V., Weaver K. E., Goicoechea P., Tille P., Clewell D. B. Characterization of an active partition system for the Enterococcus faecalis pheromone-responding plasmid pAD1. Journal of Bacteriology. 2007;189(23):8546–8555. PubMed PMID: 17905984.

Frank K. L., Barnes A. M., Grindle S. M., Manias D. A., Schlievert P. M., Dunny G. M. Use of recombinase-based in vivo expression technology to characterize Enterococcus faecalis gene expression during infection identifies in vivo-expressed antisense RNAs and implicates the protease Eep in pathogenesis. Infection and Immunity. 2012;80(2):539–549. PubMed PMID: 22144481.

Franke A. E., Clewell D. B. Evidence for a chromosome-borne resistance transposon (Tn916) in Streptococcus faecalis that is capable of "conjugal" transfer in the absence of a conjugative plasmid. Journal of Bacteriology. 1981;145(1):494–502. PubMed PMID: 6257641.

Franke, A. E., Dunny, G. M., Brown, B., An, F., Oliver, D., Damle, S., et al. (1978). Gene transfer in Streptococcus faecalis: evidence for the mobilization of chromosomal determinants by transmissible plasmids. In D. Schlessinger (Ed.), *Microbiology-1978* (pp. 45-47). Washington, D. C.: American Society for Microbiology.

Freitas A. R., Coque T. M., Novais C., Hammerum A. M., Lester C. H., Zervos M. J., et al. Human and swine hosts share vancomycin-resistant Enterococcus faecium CC17 and CC5 and Enterococcus faecalis CC2 clonal clusters harboring Tn1546 on Indistinguishable plasmids. Journal of Clinical Microbiology. 2011;49(3):925–931. PubMed PMID: 21227995.

Freitas, A. R., Novais, C., Cunha, A., Silveira, E., Peixe, L., & Coque, T. M. (2010). Plasmid analysis of vancomycin-resistant enterococci isolates from Portuguese poultry and healthy humans. *20th European Congress of Clinical Microbiology and Infectious Diseases.* Vienna.

Freitas A. R., Novais C., Tedim A. P., Francia M. V., Baquero F., Peixe L., et al. Microevolutionary events involving narrow host plasmids influences local fixation of vancomycin-resistance in Enterococcus. PLoS ONE. 2013;8(3):e60589. PubMed PMID: 23555993.

Freitas, A. R., Ruiz-Garbajosa, P., Francia, M., Novais, C., Zervos, M. J., Donabedian, S., et al. (2010). Influence of pAD1 and Inc18-plasmids in the worldwide spread of vancomycin-resistant Enterococcus faecalis outbreak strains (1987-2005). *Third ASM Conference on Enterococci* (p. S5). Portland: American Society for Microbiology.

Freitas, A. R., Tedim, A. P., Francia, M. V., Peixe, L., Novais, C., Jensen, L. B., et al. (2012). The spread of vancomycin-resistant Enterococcus faecium (1986-2009): an historic view from the plasmid side. *In editorial revision.*

Freitas A. R., Tedim A. P., Novais C., Ruiz-Garbajosa P., Werner G., Laverde-Gomez J. A., et al. Global spread of the hyl(Efm) colonization-virulence gene in megaplasmids of the Enterococcus faecium CC17 polyclonal subcluster. Antimicrobial Agents and Chemotherapy. 2010;54(6):2660–2665. PubMed PMID: 20385861.

Fujimoto S., Clewell D. B. Regulation of the pAD1 sex pheromone response of Enterococcus faecalis by direct interaction between the cAD1 peptide mating signal and the negatively regulating, DNA-binding TraA protein. Proceedings of the National Academy of Sciences. 1998;95(11):6430–6435. PubMed PMID: 9600983.

Fujimoto S., Tomita H., Wakamatsu E., Tanimoto K., Ike Y. Physical mapping of the conjugative bacteriocin plasmid pPD1 of Enterococcus faecalis and identification of the determinant related to the pheromone response. Journal of Bacteriology. 1995;177(19):5574–5581. PubMed PMID: 7559345.

Galli D., Wirth R. Comparative analysis of Enterococcus faecalis sex pheromone plasmids identifies a single homologous DNA region which codes for aggregation substance. Journal of Bacteriology. 1991;173(9):3029–3033. PubMed PMID: 1673459.

Galloway-Peña J. R., Bourgogne A., Qin X., Murray B. E. Diversity of the fsr-gelE region of the Enterococcus faecalis genome but conservation in strains with partial deletions of the fsr operon. Applied and Environmental Microbiology. 2011;77(2):442–451. PubMed PMID: 21097591.

Garcia-Migura L., Hasman H., Jensen L. B. Presence of pRI1: a small cryptic mobilizable plasmid isolated from Enterococcus faecium of human and animal origin. Current Microbiology. 2009;58(2):95–100. PubMed PMID: 18830745.

Garcia-Migura L., Sanchez-Valenzuela A. J., Jensen L. B. Presence of glycopeptide-encoding plasmids in enterococcal isolates from food and humans in Denmark. Foodborne Pathogens and Disease. 2011;8(11):1191–1197. PubMed PMID: 21793656.

Garcillán-Barcia M. P., Francia M. V., de la Cruz F. The diversity of conjugative relaxases and its application in plasmid classification. FEMS Microbiology Reviews. 2009;33(3):657–687. PubMed PMID: 19396961.

Garnier F., Taourit S., Glaser P., Courvalin P., Galimand M. Characterization of transposon Tn1549, conferring VanB-type resistance in Enterococcus spp. Microbiology. 2000;146(Pt 6):1481–1489. PubMed PMID: 10846226.

Gawron-Burke C., Clewell D. B. A transposon in Streptococcus faecalis with fertility properties. Nature. 1982;300:281–284. PubMed PMID: 6292725.

Gerdes K., Wagner E. G. RNA antitoxins. Current Opinion in Microbiology. 2007;10:117–124. PubMed PMID: 17376733.

Gerdes K., Christensen S. K., Løbner-Olesen A. Prokaryotic toxin-antitoxin stress response loci. Nature Reviews Microbiology. 2005;3(5):371–382. PubMed PMID: 15864262.

Gerdes K., Nielsen A., Thorsted P., Wagner E. G. Mechanism of killer gene activation. Antisense RNA-dependent RNase III cleavage ensures rapid turn-over of the stable Hok, SrnB and PndA effector messenger RNAs. Journal of Molecular Biology. 1992;226(3):637–649. PubMed PMID: 1380562.

Gerdes K., Rasmussen P. B., Molin S. Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. Proceedings of the National Academy of Sciences. 1986;83(10):3116–3120. PubMed PMID: 3517851.

Göbl C., Kosol S., Stockner T., Rückert H. M., Zangger K. Solution Structure and Membrane Binding of the Toxin Fst of the par Addiction Module. Biochemistry. 2010;49(31):6567–6575. PubMed PMID: 20677831.

Goicoechea, P., Romo, M., Coque, T. M., Baquero, F., de la Cruz, F., Martínez-Martínez, L., et al. (2012). Identification of enterococcal plasmids by Multiplex-PCR-based relaxase typing. *In preparation*.

Grady R., Hayes F. Axe-Txe, a broad-spectrum proteic toxin-antitoxin system specified by a multidrug-resistant, clinical isolate of Enterococcus faecium. Molecular Microbiology. 2003;47(5):1419–1432. PubMed PMID: 12603745.

Greenfield T. J., Weaver K. E. Antisense RNA regulation of the pAD1 par post-segregational killing system requires interaction at the 5′ and 3′ ends of the RNAs. Molecular Microbiology. 2000;37(3):661–670. PubMed PMID: 10931359.

Greenfield T. J., Ehli E., Kirshenmann T., Franch T., Gerdes K., Weaver K. E. The antisense RNA of the par locus of pAD1 regulates the expression of a 33-amino-acid toxic peptide by an unusual mechanism. Molecular Microbiology. 2000;37(3):652–660. PubMed PMID: 10931358.

Greenfield T. J., Franch T., Gerdes K., Weaver K. E. Antisense RNA regulation of the par post-segregational killing system: structural analysis and mechanism of binding of the antisense RNA, RNAII and its target, RNAI. Molecular Microbiology. 2001;42(2):527–537. PubMed PMID: 11703673.

Grindley, N. D. (2001). The movement of Tn3-like elements: transposition and cointegrate resolution. In N. Craig, M. Craigie, M. Gellert, & A. M. Lambowitz (Eds.), *Mobile DNA II* (pp. 272-302). Washington, D. C.: ASM Press.

Grohmann E., Muth G., Espinosa M. Conjugative plasmid transfer in gram-positive bacteria. Microbiology and Molecular Biology Reviews. 2003;67(2):277–301. PubMed PMID: 12794193.

Guardabassi L., Agersø Y. Genes homologous to glycopeptide resistance vanA are widespread in soil microbial communities. FEMS Microbiology Letters. 2006;259(2):221–225. PubMed PMID: 16734783.

Guardabassi L., Perichon B., van Heijenoort J., Blanot D., Courvalin P. Glycopeptide resistance vanA operons in Paenibacillus strains isolated from soil. Antimicrobial Agents and Chemotherapy. 2005;49(10):4227–4233. PubMed PMID: 16189102.

Guglielmini J., Quintais L., Garcillán-Barcia M. P., de la Cruz F., Rocha E. P. The repertoire of ICE in prokaryotes underscores the unity, diversity, and ubiquity of conjugation. PLoS Genetics. 2011;7(8):e1002222. PubMed PMID: 21876676.

Halvorsen E. M., Williams J. J., Bhimani A. J., Billings E. A., Hergenrother P. J. Txe, an endoribonuclease of the enterococcal Axe-Txe toxin-antitoxin system, cleaves mRNA and inhibits protein synthesis. Microbiology. 2011;157(Pt 2):387–397. PubMed PMID: 21030436.

Handwerger S., Skoble J. Identification of chromosomal mobile element conferring high-level vancomycin resistance in Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 1995;39(11):2446–2453. PubMed PMID: 8585724.

Handwerger S., Pucci M. J., Kolokathis A. Vancomycin resistance is encoded on a pheromone response plasmid in Enterococcus faecium 228. Antimicrobial Agents and Chemotherapy. 1990;34(2):358–360. PubMed PMID: 2327781.

Hasman H., Villadsen A. G., Aarestrup F. M. Diversity and stability of plasmids from glycopeptide-resistant Enterococcus faecium (GRE) isolated from pigs in Denmark. Microbial Drug Resistance. 2005;11(2):178–184. PubMed PMID: 15910234.

Hayes F. Toxins-antitoxins: plasmid maintenance, programmed cell death, and cell cycle arrest. Science. 2003;301(5639):1496–1499. PubMed PMID: 12970556.

Hayes F., Barillà D. The bacterial segrosome: a dynamic nucleoprotein machine for DNA trafficking and segregation. Nature Reviews Microbiology. 2006;4(2):133–143. PubMed PMID: 16415929.

Hayes F., Van Melderen L. Toxins-antitoxins: diversity, evolution and function. Critical Reviews in Biochemistry and Molecular Biology. 2011;46(5):386–408. PubMed PMID: 21819231.

Heaton M. P., Discotto L. F., Pucci M. J., Handwerger S. Mobilization of vancomycin resistance by transposon-mediated fusion of a VanA plasmid with an Enterococcus faecium sex pheromone-response plasmid. Gene. 1996;171(1):9–17. PubMed PMID: 8675038.

Hedberg P. J., Leonard B. A., Ruhfel R. E., Dunny G. M. Identification and characterization of the genes of Enterococcus faecalis plasmid pCF10 involved in replication and in negative control of pheromone-inducible conjugation. Plasmid. 1996;35(1):46–57. PubMed PMID: 8693026.

Hegstad K., Mikalsen T., Coque T. M., Werner G., Sundsfjord A. Mobile genetic elements and their contribution to the emergence of antimicrobial resistant Enterococcus faecalis and Enterococcus faecium. Clinical Microbiology and Infection. 2010;16(6):541–554. PubMed PMID: 20569265.

Heidrich N., Brantl S. Antisense RNA-mediated transcriptional attenuation in plasmid pIP501: the simultaneous interaction between two complementary loop pairs is required for efficient inhibition by the antisense RNA. Microbiology. 2007;153(Pt 2):420–427. PubMed PMID: 17259613.

Hinerfeld D., Churchward G. Specific binding of integrase to the origin of transfer (oriT) of the conjugative transposon Tn916. Journal of Bacteriology. 2001;183(9):2947–2951. PubMed PMID: 11292817.

Hirt H., Manias D. A., Bryan E. M., Klein J. R., Marklund J. K., Staddon J. H., et al. Characterization of the pheromone response of the Enterococcus faecalis conjugative plasmid pCF10: complete sequence and comparative analysis of the transcriptional and phenotypic responses of pCF10-containing cells to pheromone induction. Journal of Bacteriology. 2005;187(3):1044–1054. PubMed PMID: 15659682.

Hirt H., Wirth R., Muscholl A. Comparative analysis of 18 sex pheromone plasmids from Enterococcus faecalis: detection of a new insertion element on pPD1 and implications for the evolution of this plasmid family. Molecular and General Genetics. 1996;252(6):640–647. PubMed PMID: 8917306.

Hodel-Christian S. L., Murray B. E. Characterization of the gentamicin resistance transposon Tn5281 from Enterococcus faecalis and comparison to staphylococcal transposons Tn4001 and Tn4031. Antimicrobial Agents and Chemotherapy. 1991;35(6):1147–1152. PubMed PMID: 1656854.

Horaud T., Delbos F., de Cespédès G. Tn3702, a conjugative transposon in Enterococcus faecalis. FEMS Microbiology Letters. 1990;60(1-2):189–194. PubMed PMID: 2178139.

Horaud T., Le Bouguenec C., Pepper K. Molecular genetics of resistance to macrolides, lincosamides and streptogramin B (MLS) in streptococci. Journal of Antimicrobial Chemotherapy. 1985;16 Suppl A:111–135. PubMed PMID: 3932298.

Horii T., Nagasawa H., Nakayama J. Functional analysis of TraA, the sex pheromone receptor encoded by pPD1, in a promoter region essential for the mating response in Enterococcus faecalis. Journal of Bacteriology. 2002;184(22):6343–6350. PubMed PMID: 12399504.

Horodniceanu T., Bouanchaud D. H., Bieth G., Chabbert Y. A. R plasmids in Streptococcus agalactiae (group B). Antimicrobial Agents and Chemotherapy. 1976;10(5):795–801. PubMed PMID: 795369.

Ibrahim M., Nicolas P., Bessières P., Bolotin A., Monnet V., Gardan R. A genome-wide survey of short coding sequences in streptococci. Microbiology. 2007;153(Pt 11):3631–3644. PubMed PMID: 17975071.

Igimi S., Ryu C. H., Park S. H., Sasaki Y., Sasaki T., Kumagai S. Transfer of conjugative plasmid pAM beta 1 from Lactococcus lactis to mouse intestinal bacteria. Letters in Applied Microbiology. 1996;23(1):31–35. PubMed PMID: 8679141.

Ike Y., Clewell D. B. Genetic analysis of the pAD1 pheromone response in Streptococcus faecalis, using transposon Tn917 as an insertional mutagen. Journal of Bacteriology. 1984;158(3):777–783. PubMed PMID: 6327637.

Ike Y., Clewell D. B. Evidence that the hemolysin/bacteriocin phenotype of Enterococcus faecalis subsp. zymogenes can be determined by plasmids in different incompatibility groups as well as by the chromosome. Journal of Bacteriology. 1992;174(24):8172–8177. PubMed PMID: 1459967.

Ike Y., Tanimoto K., Tomita H., Takeuchi K., Fujimoto S. Efficient transfer of the pheromone-independent Enterococcus faecium plasmid pMG1 (Gmr) (65.1 kilobases) to Enterococcus strains during broth mating. Journal of Bacteriology. 1998;180(18):4886–4892. PubMed PMID: 9733692.

Inoue T., Tomita H., Ike Y. Bac 32, a novel bacteriocin widely disseminated among clinical isolates of Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2006;50(4):1202–1212. PubMed PMID: 16569830.

Izquierdo E., Wagner C., Marchioni E., Aoude-Werner D., Ennahar S. Enterocin 96, a novel class II bacteriocin produced by Enterococcus faecalis WHE 96, isolated from Munster cheese. Applied and Environmental Microbiology. 2009;75(13):4273–4276. PubMed PMID: 19411428.

Jaffé A., Ogura T., Hiraga S. Effects of the ccd function of the F plasmid on bacterial growth. Journal of Bacteriology. 1985;163(3):841–849. PubMed PMID: 3897195.

Jannière L., Bidnenko V., McGovern S., Ehrlich S. D., Petit M. A. Replication terminus for DNA polymerase I during initiation of pAMβ1 replication: role of the plasmid-encoded resolution system. Molecular Microbiology. 1997;23(3):525–535. PubMed PMID: 9044286.

Jannière, L., Gruss, A., & Ehrlich, S. D. (1993). Plasmids. In A. L. Sonenshein, J. A. Hoch, & R. Losick, *Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physioloogy, and Molecular Genetics.* Washington, D. C.: American Society for Microbiology.

Jaworski D. D., Clewell D. B. Evidence that coupling sequences play a frequency-determining role in conjugative transposition of Tn916 in Enterococcus faecalis. Journal of Bacteriology. 1994;176(11):3328–3335. PubMed PMID: 8195088.

Jaworski D. D., Clewell D. B. A functional origin of transfer (oriT) on the conjugative transposon Tn916. Journal of Bacteriology. 1995;177(22):6644–6651. PubMed PMID: 7592445.

Jensen L. B., Ahrens P., Dons L., Jones R. N., Hammerum A. M., Aarestrup F. M. Molecular analysis of Tn1546 in Enterococcus faecium isolated from animals and humans. Journal of Clinical Microbiology. 1998;36(2):437–442. PubMed PMID: 9466754.

Jensen L. B., Garcia-Migura L., Valenzuela A. J., Løhr M., Hasman H., Aarestrup F. M. A classification system for plasmids from enterococci and other Gram-positive bacteria. Journal of Microbiological Methods. 2010;80(1):25–43. PubMed PMID: 19879906.

Jensen R. B., Gerdes K. Programmed cell death in bacteria: proteic plasmid stabilization systems. Molecular Microbiology. 1995;17(2):205–210. PubMed PMID: 7494469.

Jia Y., Churchward G. Interactions of the integrase protein of the conjugative transposon Tn916 with its specific DNA binding sites. Journal of Bacteriology. 1999;181(19):6114–6123. PubMed PMID: 10498726.

Johnson C. M., Haemig H. H., Chatterjee A., Wei-Shou H., Weaver K. E., Dunny G. M. RNA-mediated reciprocal regulation between two bacterial operons is RNase III dependent. MBio. 2011;2(5):e00189–e00111. PubMed PMID: 21954305.

Johnson C. M., Manias D. A., Haemig H. A., Shokeen S., Weaver K. E., Henkin T. M., et al. Direct evidence for control of the pheromone-inducible prgQ operon of Enterococcus faecalis plasmid pCF10 by a countertranscript-driven attenuation mechanism. Journal of Bacteriology. 2010;192(6):1634–1642. PubMed PMID: 20097859.

Kawano M., Oshima T., Kasai H., Mori H. Molecular characterization of long direct repeat (LDR) sequences expressing a stable mRNA encoding for a 35-amino-acid cell-killing peptide and a cis-encoded small antisense RNA in Escherichia coli. Molecular Microbiology. 2002;45(2):333–349. PubMed PMID: 12123448.

Kazama H., Hamashima H., Sasatsu M., Arai T. Distribution of the antiseptic-resistance gene qacE delta 1 in gram-positive bacteria. FEMS Microbiology Letters. 1998;165(2):295–299. PubMed PMID: 9742702.

Khan S. A. Plasmid rolling-circle replication: highlights of two decades of research. Plasmid. 2005;53(2):126–136. PubMed PMID: 15737400.

Khoo S. K., Loll B., Chan W. T., Shoeman R. L., Ngoo L., Yeo C. C., et al. Molecular and structural characterization of the PezAT chromosomal toxin-antitoxin system of the human pathogen Streptococcus pneumoniae. The Journal of Biological Chemistry. 2007;282(27):19606–19618. PubMed PMID: 17488720.

Kim S. W., Jeong E. J., Kang H. S., Tak J. I., Bang W. Y., Heo J. B., et al. Role of RepB in the replication of plasmid pJB01 isolated from Enterococcus faecium JC1. Plasmid. 2006;55(2):99–113. PubMed PMID: 16188315.

Konieczny I. Strategies for helicase recruitment and loading in bacteria. EMBO Reports. 2003;4(1):37–41. PubMed PMID: 12524518.

Kozlowicz B. K., Dworkin M., Dunny G. M. Pheromone-inducible conjugation in Enterococcus faecalis: a model for the evolution of biological complexity? International Journal of Medical Microbiology. 2006;296(2-3):141–147. PubMed PMID: 16503196.

Krah E. R. 3rd, Macrina F. L. Genetic analysis of the conjugal transfer determinants encoded by the streptococcal broad-host-range plasmid pIP501. Journal of Bacteriology. 1989;171(11):6005–6012. PubMed PMID: 2553667.

Kumar N., Ponnaluri C. V., Putarjunan A., Ranganathan S., Roy U., Das A. Characterization of temperature inducible promoters from a novel rolling circle replicating plasmid of Enterococcus faecium DJ1. Plasmid. 2012;67(3):211–226. PubMed PMID: 22192842.

Kurenbach B., Bohn C., Prabhu J., Abudukerim M., Szewzyk U., Grohmann E. Intergeneric transfer of the Enterococcus faecalis plasmid pIP501 to Escherichia coli and Streptomyces lividans and sequence analysis of its tra region. Plasmid. 2003;50(1):86–93. PubMed PMID: 12826062.

Kurenbach B., Kopeć J., Mägdefrau M., Andreas K., Keller W., Bohn C., et al. The TraA relaxase autoregulates the putative type IV secretion-like system encoded by the broad-host-range Streptococcus agalactiae plasmid pIP501. Microbiology. 2006;152(Pt 3):637–645. PubMed PMID: 16514144.

Kwong S. M., Jensen S. O., Firth N. Prevalence of Fst-like toxin–antitoxin systems. Microbiology. 2010;156(4):975–977. PubMed PMID: 20150240.

Kwong S. M., Lim R., Lebard R. J., Skurray R. A., Firth N. Analysis of the pSK1 replicon, a prototype from the staphylococcal multiresistance plasmid family. Microbiology. 2008;154(Pt 10):3084–3094. PubMed PMID: 18832314.

Kwong S. M., Skurray R. A., Firth N. Staphylococcus aureus multiresistance plasmid pSK41: analysis of the replication region, initiator protein binding and antisense RNA regulation. Molecular Microbiology. 2004;51(2):497–509. PubMed PMID: 14756789.

Kwong S. M., Skurray R. A., Firth N. Replication control of staphylococcal multiresistance plasmid pSK41: an antisense RNA mediates dual-level regulation of Rep expression. Journal of Bacteriology. 2006;188(12):4404–4412. PubMed PMID: 16740947.

Larsen R. A., Cusumano C., Fujioka A., Lim-Fong G., Patterson P., Pogliano J. Treadmilling of a prokaryotic tubulin-like protein, TubZ, required for plasmid stability in Bacillus thuringiensis. Genes & Development. 2007;21(11):1340–1352. PubMed PMID: 17510284.

Launay A., Ballard S. A., Johnson P. D., Grayson M. L., Lambert T. Transfer of vancomycin resistance transposon Tn1549 from Clostridium symbiosum to Enterococcus spp. in the gut of gnotobiotic mice. Antimicrobial Agents and Chemotherapy. 2006;50(3):1054–1062. PubMed PMID: 16495268.

Laverde-Gomez J. A., Hendrickx A. P., Willems R. J., Top J., Sava I., Huebner J., et al. Intra- and interspecies genomic transfer of the Enterococcus faecalis pathogenicity island. PLoS ONE. 2011;6(4):e16720. PubMed PMID: 21559082.

Laverde-Gomez J. A., van Schaik W., Freitas A. R., Coque T. M., Weaver K. E., Francia M. V., et al. A multiresistance megaplasmid pLG1 bearing a hyl(Efm) genomic island in hospital Enterococcus faecium isolates. International Journal of Medical Microbiology. 2011;301(2):165–175. PubMed PMID: 20951641.

Le Chatelier E., Ehrlich S. D., Jannière L. Countertranscript-driven attenuation system of the pAMβ1 repE gene. Molecular Microbiology. 1996;20(5):1099–1112. PubMed PMID: 8809762.

Le Chatelier E., Jannière L., Ehrlich S. D., Canceill D. The RepE Initiator Is a Double-stranded and Single-stranded DNA-binding Protein That Forms an Atypical Open Complex at the Onset of Replication of Plasmid pAMβ1 from Gram-positive Bacteria. The Journal of Biological Chemistry. 2001;276:10234–10246. PubMed PMID: 11124267.

Leavis H. L., Willems R. J., Top J., Spalburg E., Mascini E. M., Fluit A. C., et al. Epidemic and nonepidemic multidrug-resistant Enterococcus faecium. Emerging Infectious Diseases. 2003;9(9):1108–1115. PubMed PMID: 14519248.

Leavis H. L., Willems R. J., van Wamel W. J., Schuren F. H., Caspers M. P., Bonten M. J. Insertion sequence-driven diversification creates a globally dispersed emerging multiresistant subspecies of E. faecium. PLoS Pathogens. 2007;3(1):e7. PubMed PMID: 17257059.

Leavis H., Top J., Shankar N., Borgen K., Bonten M., van Embden J., et al. A novel putative enterococcal pathogenicity island linked to the esp virulence gene of Enterococcus faecium and associated with epidemicity. Journal of Bacteriology. 2004;186(3):672–682. PubMed PMID: 14729692.

LeBlanc D. J., Inamine J. M., Lee L. N. Broad geographical distribution of homologous erythromycin, kanamycin, and streptomycin resistance determinants among group D streptococci of human and animal origin. Antimicrobial Agents and Chemotherapy. 1986;29(4):549–555. PubMed PMID: 3010845.

Leelaporn A., Yodkamol K., Waywa D., Pattanachaiwit S. A novel structure of Tn4001-truncated element, type V, in clinical enterococcal isolates and multiplex PCR for detecting aminoglycoside resistance genes. International Journal of Antimicrobial Agents. 2008;31(3):250–254. PubMed PMID: 18155882.

Leonard B. A., Podbielski A., Hedberg P. J., Dunny G. M. Enterococcus faecalis pheromone binding protein, PrgZ, recruits a chromosomal oligopeptide permease system to import sex pheromone cCF10 for induction of conjugation. Proceedings of the National Academy of Sciences. 1996;93(1):260–264. PubMed PMID: 8552617.

Leon-Sampedro, R., Tedim, P., Del Campo, R., Rodriguez, C., Willems, R., Baquero, F., et al. (2012). Clonal and plasmid diversity among Enterococcus faecalis from wild and migratory birds. *International Plasmid Biology Conference.* Santander.

Lepage E., Brinster S., Caron C., Ducroix-Crepy C., Rigottier-Gois L., Dunny G., et al. Comparative Genomic Hybridization Analysis of Enterococcus faecalis: Identification of Genes Absent from Food Strains. Journal of Bacteriology. 2006;188(19):6858–6868. PubMed PMID: 16980489.

Leplae R., Geeraerts D., Hallez R., Guglielmini J., Drèze P., Van Melderen L. Diversity of bacterial type II toxin–antitoxin systems: a comprehensive search and functional analysis of novel families. Nucleic Acids Research. 2011;39(13):5513–5525. PubMed PMID: 21422074.

Li X., Alvarez V., Harper W. J., Wang H. H. Persistent, toxin-antitoxin system-independent, tetracycline resistance-encoding plasmid from a dairy Enterococcus faecium isolate. Applied and Environmental Microbiology. 2011;77(20):7096–7103. PubMed PMID: 21784909.

Licht A., Freede P., Brantl S. Transcriptional repressor CopR acts by inhibiting RNA polymerase binding. Microbiology. 2011;157(Pt 4):1000–1008. PubMed PMID: 21252280.

Lim S. K., Tanimoto K., Tomita H., Ike Y. Pheromone-responsive conjugative vancomycin resistance plasmids in Enterococcus faecalis isolates from humans and chicken feces. Applied and Environmental Microbiology. 2006;72(10):6544–6553. PubMed PMID: 17021204.

Lioy V. S., Martín M. T., Camacho A. G., Lurz R., Antelmann H., Hecker M., et al. pSM19035-encoded ζ toxin induces stasis followed by death in a subpopulation of cells. Microbiology. 2006;152(8):2365–2379. PubMed PMID: 16849801.

Lioy V. S., Pratto F., de la Hoz A. B., Ayora S., Alonso J. C. Plasmid pSM19035, a model to study stable maintenance in Firmicutes. Plasmid. 2010;64(1):1–17. PubMed PMID: 20403380.

Liu H., Wang Y., Wu C., Schwarz S., Shen Z., Jeon B., et al. A novel phenicol exporter gene, fexB, found in enterococci of animal origin. Journal of Antimicrobial Chemotherapy. 2012;67(2):322–325. PubMed PMID: 22096043.

Liu Y., Wang Y., Wu C., Shen Z., Schwarz S., Du X.-D., et al. First Report of the Multidrug Resistance Gene cfr in Enterococcus faecalis of Animal Origin. Antimicrobial Agents and Chemotherapy. 2011;56(3):1650–1654. PubMed PMID: 22203597.

Llosa M., Bolland S., Grandoso G., de la Cruz F. Conjugation-independent, site-specific recombination at the oriT of the IncW plasmid R388 mediated by TrwC. Journal of Bacteriology. 1994;176(11):3210–3217. PubMed PMID: 8195075.

Llosa M., Gomis-Rüth F. X., Coll M., de la Cruz F. F. Bacterial conjugation: a two-step mechanism for DNA transport. Molecular Microbiology. 2002;45(1):1–8. PubMed PMID: 12100543.

López M., Sáenz Y., Alvarez-Martínez M. J., Marco F., Robredo B., Rojo-Bezares B., et al. Tn1546 structures and multilocus sequence typing of vanA-containing enterococci of animal, human and food origin. Journal of Antimicrobial Chemotherapy. 2010;65(8):1570–1575. PubMed PMID: 20519356.

López M., Sáenz Y., Rojo-Bezares B., Martínez S., del Campo R., Ruiz-Larrea F., et al. Detection of vanA and vanB2-containing enterococci from food samples in Spain, including Enterococcus faecium strains of CC17

and the new singleton ST425. International Journal of Food Microbiology. 2009;133(1-2):172–178. PubMed PMID: 19493581.

Lyon W. R., Gibson C. M., Caparon M. G. A role for trigger factor and an rgg-like regulator in the transcription, secretion and processing of the cysteine proteinase of Streptococcus pyogenes. The EMBO Journal. 1998;17(21):6263–6275. PubMed PMID: 9799235.

Magi G., Capretti R., Paoletti C., Pietrella M., Ferrante L., Biavasco F., et al. Presence of a vanA-carrying pheromone response plasmid (pBRG1) in a clinical isolate of Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2003;47(5):1571–1576. PubMed PMID: 12709324.

Malik A., Celik E. K., Bohn C., Böckelmann U., Knobel K., Grohmann E. Detection of conjugative plasmids and antibiotic resistance genes in anthropogenic soils from Germany and India. FEMS Microbiology Letters. 2008;279(2):207–216. PubMed PMID: 18179584.

Manson J. M., Hancock L. E., Gilmore M. S. Mechanism of chromosomal transfer of Enterococcus faecalis pathogenicity island, capsule, antimicrobial resistance, and other traits. Proceedings of the National Academy of Sciences. 2010;107(27):12269–12274. PubMed PMID: 20566881.

Marraffini L. A., Sontheimer E. J. CRISPR interference: RNA-directed adaptive immunity in bacteria and archaea. Nature Reviews Genetics. 2010;11:181–190. PubMed PMID: 20125085.

Martínez-Bueno M., Valdivia E., Gálvez A., Maqueda M. pS86, a new theta-replicating plasmid from Enterococcus faecalis. Current Microbiology. 2000;41(4):257–261. PubMed PMID: 10977892.

Mashburn-Warren L., Morrison D. A., Federle M. J. A novel double-tryptophan peptide pheromone controls competence in Streptococcus spp. via an Rgg regulator. Molecular Microbiology. 2010;78(3):589–606. PubMed PMID: 20969646.

Masuda Y., Miyakawa K., Nishimura Y., Ohtsubo E. chpA and chpB, Escherichia coli chromosomal homologs of the pem locus responsible for stable maintenance of plasmid R100. Journal of Bacteriology. 1993;175(21):6850–6856. PubMed PMID: 8226627.

Mathy N., Bénard L., Pellegrini O., Daou R., Wen T., Condon C. 5′-to-3′ exoribonuclease activity in bacteria: role of RNase J1 in rRNA maturation and 5′ stability of mRNA. Cell. 2007;129(4):681–692. PubMed PMID: 17512403.

Mazaheri Nezhad Fard R., Barton M. D., Heuzenroeder M. W. Novel Bacteriophages in Enterococcus spp. Current Microbiology. 2010;60(6):400–406. PubMed PMID: 19967374.

Mazaheri Nezhad Fard R., Barton M. D., Heuzenroeder M. W. Bacteriophage-mediated transduction of antibiotic resistance in enterococci. Letters in Applied Microbiology. 2011;52(6):559–564. PubMed PMID: 21395627.

McBride S. M., Coburn P. S., Baghdayan A. S., Willems R. J., Grande M. J., Shankar N., et al. Genetic variation and evolution of the pathogenicity island of Enterococcus faecalis. Journal of Bacteriology. 2009;191(10):3392–3402. PubMed PMID: 19270086.

McBride S. M., Fischetti V. A., Moellering R. C. Jr, Gilmore M. S. Genetic diversity among Enterococcus faecalis. PLoS ONE. 2007;2(7):e582. PubMed PMID: 17611618.

McConnell M. A., Mercer A. A., Tannock G. W. Transfer of plasmid pAMb1 between members of the normal microflora inhabiting the murine digestive tract and modification of the plasmid in a Lactobacillus reuteri host. Microbial Ecology in Health and Disease. 1991;4(6):343–355.

McDougal L. K., Tenover F. C., Lee L. N., Rasheed J. K., Patterson J. E., Jorgensen J. H., et al. Detection of Tn917-like sequences within a Tn916-like conjugative transposon (Tn3872) in erythromycin-resistant isolates of Streptococcus pneumoniae. Antimicrobial Agents and Chemotherapy. 1998;42(9):2312–2318. PubMed PMID: 9736555.

Meinhart A., Alonso J. C., Sträter N., Saenger W. Crystal structure of the plasmid maintenance system ε/ζ: Functional mechanism of toxin ζ and inactivation by ε2ζ2 complex formation. Proceedings of the National Academy of Sciences. 2003;100(4):1661–1666.

Møller-Jensen J., Ringgard S., Mercogliano C. P., Gerdes K., Löwe J. Structural analysis of the ParR/parC plasmid partition complex. The EMBO Journal. 2007;26(20):4413–4422. PubMed PMID: 17898804.

Morelli L., Sarra P. G., Bottazzi V. In vivo transfer of pAM beta 1 from Lactobacillus reuteri to Enterococcus faecalis. Journal of Applied Bacteriology. 1988;65(5):371–375. PubMed PMID: 2976754.

Moritz E. M., Hergenrother P. J. Toxin–antitoxin systems are ubiquitous and plasmid-encoded in vancomycin-resistant enterococci. Proceedings of the National Academy of Sciences. 2007;104(1):311–316. PubMed PMID: 17190821.

Morlot C., Zapun A., Dideberg O., Vernet T. Growth and division of Streptococcus pneumoniae: localization of the high molecular weight penicillin-binding proteins during the cell cycle. Molecular Microbiology. 2003;50(3):845–855. PubMed PMID: 14617146.

Murray B. E. The life and times of the Enterococcus. Clinical Microbiology Reviews. 1990;3(1):46–65. PubMed PMID: 2404568.

Murray B. E., An F. Y., Clewell D. B. Plasmids and pheromone response of the beta-lactamase producer Streptococcus (Enterococcus) faecalis HH22. Antimicrobial Agents and Chemotherapy. 1988;32(4):547–551. PubMed PMID: 3132094.

Mutschler H., Gebhardt M., Shoeman R. L., Meinhart A. A Novel Mechanism of Programmed Cell Death in Bacteria by Toxin–Antitoxin Systems Corrupts Peptidoglycan Synthesis. PLoS Biology. 2011;9(3):e1001033. PubMed PMID: 21445328.

Nakayama J., Abe Y., Ono Y., Isogai A., Suzuki A. Isolation and structure of the Enterococcus faecalis sex pheromone, cOB1, that induces conjugal transfer of the hemolysin/bacteriocin plasmids, pOB1 and pYI1. Bioscience, Biotechnology, and Biochemistry. 1995;59(4):703–705. PubMed PMID: 7772836.

Ni L., Xu W., Kumaraswami M., Schumacher M. A. Plasmid protein TubR uses a distinct mode of HTH-DNA binding and recruits the prokaryotic tubulin homolog TubZ to effect DNA partition. Proceedings of the National Academy of Sciences. 2010;107(26):11763–11768. PubMed PMID: 20534443.

Nisa S., Blokpoel M. C., Robertson B. D., Tyndall J. D., Lun S., Bishai W. R., et al. Targeting the chromosome partitioning protein ParA in tuberculosis drug discovery. Journal of Antimicrobial Chemotherapy. 2010;65(11):2347–2358. PubMed PMID: 20810423.

Novais, C., Freitas, A. R., Baquero, F., Peixe, L., & Coque, T. M. (2010). Enterococci from human, animal and environmental sources are reservoirs of Gram Positive conjugative transposons. *3rd International ASM Conference on Enterococci.* Portland: American Society for Microbiology.

Novais, C., Freitas, A. R., Moreira, L., Francia, M. V., Baquero, F., Peixe, L. V., et al. (2008). Characterisation of Gram-positive conjugative transposons in enterococci from different sources by a multiplex-PCR assay. *18th European Congress of Clinical Microbiology and Infectious Diseases.* Barcelona.

Novais C., Freitas A. R., Silveira E., Baquero F., Peixe L., Roberts A. P., et al. A tet(S/M) hybrid from CTn6000 and CTn916 recombination. Microbiology. 2012;158(Pt 11):2710–2711. PubMed PMID: 22968088.

Novais C., Freitas A. R., Silveira E., Baquero F., Peixe L., Roberts A. P., et al. Different genetic supports for the tet(S) gene in Enterococci. Antimicrobial Agents and Chemotherapy. 2012;56(11):6014–6018. PubMed PMID: 22908170.

Novais C., Freitas A. R., Sousa J. C., Baquero F., Coque T. M., Peixe L. V. Diversity of Tn1546 and its role in the dissemination of vancomycin-resistant enterococci in Portugal. Antimicrobial Agents and Chemotherapy. 2008;52(3):1001–1008. PubMed PMID: 18180362.

Novais, C., Leon, R., Tedim, A., Freitas, A., Peixe, L., Baquero, F., et al. (2013). Spread of CTn5801 among enterococcal species from different origins. *23rd European Congress of Clincal Microbiology and Infections Diseases*. Berlin.

Novick R. P. Plasmid incompatibility. Microbiology and Molecular Biology Reviews. 1987;51(4):381. PubMed PMID: 3325793.

Oancea C., Klare I., Witte W., Werner G. Conjugative transfer of the virulence gene, esp, among isolates of Enterococcus faecium and Enterococcus faecalis. Journal of Antimicrobial Chemotherapy. 2004;54:232–235. PubMed PMID: 15192049.

Ogura T., Hiraga S. Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proceedings of the National Academy of Sciences. 1983;80(15):4784–4788. PubMed PMID: 6308648.

Oliver D. R., Brown B. L., Clewell D. B. Analysis of plasmid deoxyribonucleic acid in a cariogenic strain of Streptococcus faecalis: an approach to identifying genetic determinants on cryptic plasmids. Journal of Bacteriology. 1977;130(2):759–765. PubMed PMID: 122512.

Opdyke J. A., Kang J. G., Storz G. GadY, a Small-RNA Regulator of Acid Response Genes in Escherichia coli. Journal of Bacteriology. 2004;186(20):6698–6705. PubMed PMID: 15466020.

Osborn A. M., Böltner D. When phage, plasmids, and transposons collide: genomic islands, and conjugative- and mobilizable-transposons as a mosaic continuum. Plasmid. 2002;48(3):202–212. PubMed PMID: 12460536.

Ozawa Y., De Boever E. H., Clewell D. B. Enterococcus faecalis sex pheromone plasmid pAM373: analyses of TraA and evidence for its interaction with RpoB. Plasmid. 2005;54(1):57–69. PubMed PMID: 15907539.

Palmer K. L., Gilmore M. S. Multidrug-resistant enterococci lack CRISPR-cas. MBio. 2010;1(4):e00227–e00210. PubMed PMID: 21060735.

Palmer K. L., Kos V. N., Gilmore M. S. Horizontal gene transfer and the genomics of enterococcal antibiotic resistance. Current Opinion in Microbiology. 2010;13(5):632–639. PubMed PMID: 20837397.

Panesso D., Montealegre M. C., Rincón S., Mojica M. F., Rice L. B., Singh K. V., et al. The hylEfm gene in pHylEfm of Enterococcus faecium is not required in pathogenesis of murine peritonitis. BMC Microbiology. 2011;11:20. PubMed PMID: 21266081.

Patel S., Weaver K. E. Addiction Toxin Fst Has Unique Effects on Chromosome Segregation and Cell Division in Enterococcus faecalis and Bacillus subtilis. Journal of Bacteriology. 2006;188(15):5374–5384. PubMed PMID: 16855226.

Paulsen I. T., Banerjei L., Myers G. S., Nelson K. E., Seshadri R., Read T., et al. Role of mobile DNA in the evolution of vancomycin-resistant Enterococcus faecalis. Science. 2003;299(5615):2071–2074. PubMed PMID: 12663927.

Pedersen K., Christensen S. K., Gerdes K. Rapid induction and reversal of a bacteriostatic condition by controlled expression of toxins and antitoxins. Molecular Microbiology. 2002;45(2):501–510. PubMed PMID: 12123459.

Périchon B., Courvalin P. VanA-type vancomycin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2009;53(11):4580–4587. PubMed PMID: 19506057.

Perkins J. B., Youngman P. Streptococcus plasmid pAM alpha 1 is a composite of two separable replicons, one of which is closely related to Bacillus plasmid pBC16. Journal of Bacteriology. 1983;155(2):607–615. PubMed PMID: 6307973.

Pinho M. G., Errington J. Dispersed mode of Staphylococcus aureus cell wall synthesis in the absence of the division machinery. Molecular Microbiology. 2003;50(3):871–881. PubMed PMID: 14617148.

Poeta P., Costa D., Igrejas J., Rojo-Bezares B., Sáenz Y., Zarazaga M., et al. Characterization of vanA-containing Enterococcus faecium isolates carrying Tn5397-like and Tn916/Tn1545-like transposons in wild boars (Sus Scrofa). Microbial Drug Resistance. 2007;13(3):151–156. PubMed PMID: 17949300.

Poulsen L. K., Larsen N. W., Molin S., Andersson P. A family of genes encoding a cell-killing function may be conserved in all Gram-negative bacteria. Molecular Microbiology. 1989;3(11):1463–1472. PubMed PMID: 2693900.

Qin X., Galloway-Peña J. R., Sillanpää J., Roh J. H., Nallapareddy S. R., Chowdhury S., et al. Complete genome sequence of Enterococcus faecium strain TX16 and comparative genomic analysis of Enterococcus faecium genomes. BMC Microbiology. 2012;12:135. PubMed PMID: 22769602.

Quintillani R. Jr, Courvalin P. Characterization of Tn1547, a composite transposon flanked by the IS16 and IS256-like elements, that confers vancomycin resistance in Enterococcus faecalis BM4281. Gene. 1996;172(1):1–8. PubMed PMID: 8654967.

Rankin D. J., Rocha E. P., Brown S. P. What traits are carried on mobile genetic elements, and why? Heredity. 2011;106:1–10. PubMed PMID: 20332804.

Rice L. B. Tn916 family conjugative transposons and dissemination of antimicrobial resistance determinants. Antimicrobial Agents and Chemotherapy. 1998;42(8):1871–1877. PubMed PMID: 9687377.

Rice L. B., Carias L. L. Transfer of Tn5385, a composite, multiresistance chromosomal element from Enterococcus faecalis. Journal of Bacteriology. 1998;180(3):714–721. PubMed PMID: 9457879.

Rice L. B., Marshall S. H. Insertions of IS256-like element flanking the chromosomal beta-lactamase gene of Enterococcus faecalis CX19. Antimicrobial Agents and Chemotherapy. 1994;38(4):693–701. PubMed PMID: 8031032.

Rice L. B., Thorisdottir A. S. The prevalence of sequences homologous to IS256 in clinical enterococcal isolates. Plasmid. 1994;32(3):344–349. PubMed PMID: 7899522.

Rice L. B., Carias L. L., Marshall S. H. Tn5384, a composite enterococcal mobile element conferring resistance to erythromycin and gentamicin whose ends are directly repeated copies of IS256. Antimicrobial Agents and Chemotherapy. 1995;39(5):1147–1153. PubMed PMID: 7625803.

Rice L. B., Carias L. L., Hutton-Thomas R., Rudin S. Interaction of related Tn916-like transposons: analysis of excision events promoted by Tn916 and Tn5386 integrases. Journal of Bacteriology. 2007;189(10):3909–3917. PubMed PMID: 17322310.

Rice L. B., Carias L. L., Marshall S. H., Bonafede M. E. Sequences found on staphylococcal beta-lactamase plasmids integrated into the chromosome of Enterococcus faecalis CH116. Plasmid. 1996;35(2):81–90. PubMed PMID: 8700969.

Rice L. B., Carias L. L., Marshall S., Rudin S. D., Hutton-Thomas R. Tn5386, a novel Tn916-like mobile element in Enterococcus faecium D344R that interacts with Tn916 to yield a large genomic deletion. Journal of Bacteriology. 2005;187(19):6668–6677. PubMed PMID: 16166528.

Rice L. B., Carias L. L., Rudin S., Hutton R. A., Marshall S. Multiple copies of functional, Tet(M)-encoding Tn916-like elements in a clinical Enterococcus faecium isolate. Plasmid. 2010;64(3):150–155. PubMed PMID: 20600284.

Rice L. B., Marshall S. H., Carias L. L. Tn5381, a conjugative transposon identifiable as a circular form in Enterococcus faecalis. Journal of Bacteriology. 1992;174(22):7308–7315. PubMed PMID: 1331026.

Ridenhour M. B., Fletcher H. M., Mortensen J. E., Daneo-Moore L. A novel tetracycline-resistant determinant, tet(U), is encoded on the plasmid pKq10 in Enterococcus faecium. Plasmid. 1996;35(2):71–80. PubMed PMID: 8700968.

Ringgaard S., van Zon J., Howard M., Gerdes K. Movement and equipositioning of plasmids by ParA filament disassembly. Proceedings of the National Academy of Sciences. 2009;106(46):19369–19374. PubMed PMID: 19906997.

Roberts A. P., Mullany P. A modular master on the move: the Tn916 family of mobile genetic elements. Trends in Microbiology. 2009;17(6):251–258. PubMed PMID: 19464182.

Roberts, A. P., & Mullany, P. (2010). The conjugative transposons: integrative gene transfer elements. In P. Mullany (Ed.), *The Dynamic Bacterial Genome* (pp. 207-233). New York, New York: Cambridge University Press.

Roberts A. P., Mullany P. The Tn916-like genetic elements; a diverse group of modular mobile elements. FEMS Microbiology Reviews. 2011;35(5):856–871. PubMed PMID: 21658082.

Roberts A. P., Davis I. J., Seville L., Villedieu A., Mullany P. Characterization of the ends and target site of a novel tetracycline resistance-encoding conjugative transposon from Enterococcus faecium 664.1H1. Journal of Bacteriology. 2006;188(12):4356–4361. PubMed PMID: 16740942.

Robertson H. D. Escherichia coli ribonuclease III cleavage sites. Cell. 1982;30(3):669–672. PubMed PMID: 6754088.

Rocco J. M., Churchward G. The integrase of the conjugative transposon Tn916 directs strand- and sequence-specific cleavage of the origin of conjugal transfer, oriT, by the endonuclease Orf20. Journal of Bacteriology. 2006;188(6):2207–2213. PubMed PMID: 16513750.

Rocha-Estrada J., Aceves-Diez A. E., Guarneros G., de la Torre M. The RNPP family of quorum-sensing proteins in Gram-positive bacteria. Applied Microbiology and Biotechnology. 2010;87(3):913–923. PubMed PMID: 20502894.

Rollins L. D., Lee L. N., LeBlanc D. J. Evidence for a disseminated erythromycin resistance determinant mediated by Tn917-like sequences among group D streptococci isolated from pigs, chickens, and humans. Antimicrobial Agents and Chemotherapy. 1985;27(4):439–444. PubMed PMID: 2988428.

Romo, M., Goicoechea, P., Coque, T. M., Baquero, F., de la Cruz, F., Martínez-Martínez, L., et al. (2008). Molecular typing of the resistance plasmids of a VRE Nosocomial Outbreak in Spain. *18th European Congress of Clinical Microbiology and Infectious Diseases.* Barcelona: European Society of Clinical Microbiology and Infectious Diseases.

Rosch J., Caparon M. A microdomain for protein secretion in Gram-positive bacteria. Science. 2004;304(5676):1513–1515. PubMed PMID: 15178803.

Rosvoll T. C., Lindstad B. L., Lunde T. M., Hegstad K., Aasnaes B., Hammerum A. M., et al. Increased high-level gentamicin resistance in invasive Enterococcus faecium is associated with aac(6′)le-aph(2")la-encoding transferable megaplasmids hosted by major hospital-adapted lineages. FEMS Immunology and Medical Microbiology. 2012;66(2):166–176. PubMed PMID: 22672387.

Rosvoll T. C., Pedersen T., Sletvold H., Johnsen P. J., Sollid J. E., Simonsen G. S., et al. PCR-based plasmid typing in Enterococcus faecium strains reveals widely distributed pRE25-, pRUM-, pIP501- and pHTβ-related replicons associated with glycopeptide resistance and stabilizing toxin–antitoxin systems. FEMS Immunology & Medical Microbiology. 2010;58(2):254–268. PubMed PMID: 20015231.

Rowland S. J., Dyke K. G. Tn552, a novel transposable element from Staphylococcus aureus. Molecular Microbiology. 1990;4(6):961–975. PubMed PMID: 2170815.

Ruiz-Barba J. L., Floriano B., Maldonado-Barragán A., Jiménez-Díaz R. Molecular analysis of the 21-kb bacteriocin-encoding plasmid pEF1 from Enterococcus faecium 6T1a. Plasmid. 2007;57(2):175–181. PubMed PMID: 16893567.

Sánchez-Hidalgo M., Magueda M., Gálvez A., Abriouel H., Valdivia E., Martínez-Bueno M. The genes coding for enterocin EJ97 production by Enterococcus faecalis EJ97 are located on a conjugative plasmid. Applied and Environmental Microbiology. 2003;69(3):1633–1641. PubMed PMID: 12620853.

Sarti M., Campanille F., Sabia C., Santagati M., Gargiulo R., Stefani S. Polyclonal diffusion of beta-lactamase-producing Enterococcus faecium. Journal of Clinical Microbiology. 2012;50(1):169–172. PubMed PMID: 22075588.

Schreiter E. R., Drennan C. L. Ribbon-helix-helix transcription factors: variations on a theme. Nature Reviews Microbiology. 2007;5:710–720. PubMed PMID: 17676053.

Schumacher M. A. Structural biology of plasmid partition: uncovering the molecular mechanisms of DNA segregation. Biochemical Journal. 2008;412(1):1–18. PubMed PMID: 18426389.

Schumacher M. A. Bacterial plasmid partition machinery: a minimalist approach to survival. Current Opinion in Structural Biology. 2012;22(1):72–79. PubMed PMID: 22153351.

Schumacher M. A., Funnell B. E. Structures of ParB bound to DNA reveal mechanism of partition complex formation. Nature. 2005;438:516–519. PubMed PMID: 16306995.

Schumacher M. A., Glover T. C., Brzoska A. J., Jensen S. O., Dunham T. D., Skurray R. A., et al. Segrosome structure revealed by a complex of ParR with centromere DNA. Nature. 2007;450(7173):1268–1271. PubMed PMID: 18097417.

Schwarz F. V., Perreten V., Teuber M. Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from Enterococcus faecalis RE25. Plasmid. 2001;46(3):170–187. PubMed PMID: 11735367.

Scott J. R., Churchward G. G. Conjugative transposition. Annual Review of Microbiology. 1995;49:367–397. PubMed PMID: 8561465.

Sedgley C. M., Clewell D. B., Flannagan S. E. Plasmid pAMS1-encoded, bacteriocin-related "Siblicide" in Enterococcus faecalis. Journal of Bacteriology. 2009;191(9):3183–3188. PubMed PMID: 19270087.

Serfiotis-Mitsa D., Roberts G. A., Cooper L. P., White J. H., Nutley M., Cooper A., et al. The Orf18 gene product from conjugative transposon Tn916 is an ArdA antirestriction protein that inhibits type I DNA restriction-modification systems. Journal of Molecular Biology. 2008;383(5):970–981. PubMed PMID: 18838147.

Shankar N., Baghdayan A. S., Gilmore M. S. Modulation of virulence within a pathogenicity island in vancomycin-resistant Enterococcus faecalis. Nature. 2002;417(6890):746–750. PubMed PMID: 12066186.

Shaw J. H., Clewell D. B. Complete nucleotide sequence of macrolide-lincosamide-streptogramin B-resistance transposon Tn917 in Streptococcus faecalis. Journal of Bacteriology. 1985;164(2):782–796. PubMed PMID: 2997130.

Shi K., Brown C. K., Gu Z. Y., Kozlowicz B. K., Dunny G. M., Ohlendorf D. H., et al. Structure of peptide sex pheromone receptor PrgX and PrgX/pheromone complexes and regulation of conjugation in Enterococcus faecalis. Proceedings of the National Academy of Sciences. 2005;102(51):18596–18601. PubMed PMID: 16339309.

Shokeen S., Greenfield T. J., Ehli E. A., Rasmussen J., Perrault B. E., Weaver K. A. An intramolecular upstream helix ensures the stability of a toxin-encoding RNA in Enterococcus faecalis. Journal of Bacteriology. 2009;191(5):1528–1536. PubMed PMID: 19103923.

Shokeen S., Johnson C. M., Greenfield T. J., Manias D. A., Dunny G. M., Weaver K. E. Structural analysis of the Anti-Q-Qs interaction: RNA-mediated regulation of E. faecalis plasmid pCF10 conjugation. Plasmid. 2010;64(1):26–35. PubMed PMID: 20332003.

Shokeen S., Patel S., Greenfield T. J., Brinkman C., Weaver K. E. Translational regulation by an intramolecular stem-loop is required for intermolecular RNA regulation of the par addiction module. Journal of Bacteriology. 2008;190(18):6076–6083. PubMed PMID: 18641135.

Showsh S. A., De Boever E. H., Clewell D. B. Vancomycin resistance plasmid in Enterococcus faecalis that encodes sensitivity to a sex pheromone also produced by Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2001;45(7):2177–2178. PubMed PMID: 11441824.

Simjee S., Fraise A. P., Gill M. J. Plasmid heterogeneity and identification of a Tn5281-like element in clinical isolates of high-level gentamicin-resistant Enterococcus faecium isolated in the UK. Journal of Antimicrobial Chemotherapy. 1999;43(5):625–635. PubMed PMID: 10382883.

Simjee S., White D. G., McDermott P. F., Wagner D. D., Zervos M. J., Donabedian S. M., et al. Characterization of Tn1546 in vancomycin-resistant Enterococcus faecium isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. Journal of Clinical Microbiology. 2002;40(12):4659–4665. PubMed PMID: 12454168.

Sletvold H., Johnsen P. J., Hamre I., Simonsen G. S., Sundsfjord A., Nielsen K. M. Complete sequence of Enterococcus faecium pVEF3 and the detection of an omega-epsilon-zeta toxin-antitoxin module and an ABC transporter. Plasmid. 2008;60(1):75–85. PubMed PMID: 18511120.

Sletvold H., Johnsen P. J., Simonsen G. S., Aasnæs B., Sundsfjord A., Nielsen K. M. Comparative DNA analysis of two vanA plasmids from Enterococcus faecium strains isolated from poultry and a poultry farmer in Norway. Antimicrobial Agents and Chemotherapy. 2007;51(2):736–739. PubMed PMID: 17116680.

Sletvold H., Johnsen P. J., Wikmark O. G., Simonsen G. S., Sundsfjord A., Nielsen K. M. Tn1546 is part of a larger plasmid-encoded genetic unit horizontally disseminated among clonal Enterococcus faecium lineages. Journal of Antimicrobial Chemotherapy. 2010;65(9):1894–1906. PubMed PMID: 20558469.

Smillie C., Garcillán-Barcia M. P., Francia M. V., Rocha E. P., de la Cruz F. Mobility of plasmids. Microbiology and Molecular Biology Reviews. 2010;74(3):434–452. PubMed PMID: 20805406.

Soge O. O., Beck N. K., White T. M., No D. B., Roberts M. C. A novel transposon, Tn6009, composed of a Tn916 element linked with a Staphylococcus aureus mer operon. Journal of Antimicrobial Chemotherapy. 2008;62(4):674–680. PubMed PMID: 18583328.

Solheim M., Brekke M. C., Snipen L. G., Willems R. J., Nes I. F., Brede D. A. Comparative genomic analysis reveals significant enrichment of mobile genetic elements and genes encoding surface structure-proteins in hospital-associated Clonal Complex 2 Enterococcus faecalis. BMC Microbiology. 2011;11(3) PubMed PMID: 21205308.

Sprincova A., Stovcik V., Javorsky P., Pristas P. Occurrence of pS86/pEF47-related plasmids in gram-positive cocci. Current Microbiology. 2005;51(3):198–201. PubMed PMID: 16091849.

Staddon J. H., Bryan E. M., Manias D. A., Dunny G. M. Conserved target for group II intron insertion in relaxase genes of conjugative elements of gram-positive bacteria. Journal of Bacteriology. 2004;186(8):2393–2401. PubMed PMID: 15060042.

Staddon J. H., Bryan E. M., Manias D. A., Chen Y., Dunny G. M. Genetic characterization of the conjugative DNA processing system of enterococcal plasmid pCF10. Plasmid. 2006;56(2):102–111. PubMed PMID: 16774784.

Su Y. A., Clewell D. B. Characterization of the left 4 kb of conjugative transposon Tn916: determinants involved in excision. Plasmid. 1993;30(3):234–250. PubMed PMID: 8302931.

Sulavik M. C., Clewell D. B. Rgg is a positive transcriptional regulator of the Streptococcus gordonii gtfG gene. Journal of Bacteriology. 1996;178(19):5826–5830. PubMed PMID: 8824637.

Sulavik M. C., Tardif G., Clewell D. B. Identification of a gene, rgg, which regulates expression of glucosyltransferase and influences the Spp phenotype of Streptococcus gordonii Challis. Journal of Bacteriology. 1992;174(11):3577–3586. PubMed PMID: 1534326.

Summers D. K., Beton C. W., Withers H. L. Multicopy plasmid instability: the dimer catastrophe hypothesis. Molecular Microbiology. 1993;8(6):1031–1038. PubMed PMID: 8361350.

Sung J. M.-L., Lindsay J. A. Staphylococcus aureus strains that are hypersusceptible to resistance gene transfer from enterococci. Antimicrobial Agents and Chemotherapy. 2007;51(6):2189–2191. PubMed PMID: 17371826.

Sung K., Khan S. A., Nawaz M. S. Genetic diversity of Tn1546-like elements in clinical isolates of vancomycin-resistant enterococci. International Journal of Antimicrobial Agents. 2008;31(6):549–554. PubMed PMID: 18462926.

Takeuchi K., Tomita H., Fujimoto S., Kudo M., Kuwano H., Ike Y. Drug resistance of Enterococcus faecium clinical isolates and the conjugative transfer of gentamicin and erythromycin resistance traits. FEMS Microbiology Letters. 2005;243(2):347–354. PubMed PMID: 15686834.

Takiguchi R., Hashiba H., Aoyama K., Ishii S. Complete nucleotide sequence and characterization of a cryptic plasmid from Lactobacillus helveticus subsp. jugurti. Applied and Environmental Microbiology. 1989;55(6):1653–1655. PubMed PMID: 2764571.

Tanaka T., Ogura M. A novel Bacillus natto plasmid pLS32 capable of replication in Bacillus subtilis. FEBS Letters. 1998;422(2):243–246. PubMed PMID: 9490016.

Tanaka T., Ishida H., Maehara T. Characterization of the Replication Region of Plasmid pLS32 from the Natto Strain of Bacillus subtilis. Journal of Bacteriology. 2005;187(13):4315–4326. PubMed PMID: 15968040.

Tanimoto K., Ike Y. Complete nucleotide sequencing and analysis of the 65-kb highly conjugative Enterococcus faecium plasmid pMG1: identification of the transfer-related region and the minimum region required for replication. FEMS Microbiology Letters. 2008;288(2):186–195. PubMed PMID: 18795955.

Tanimoto K., An F. Y., Clewell D. B. Characterization of the traC determinant of the Enterococcus faecalis hemolysin-bacteriocin plasmid pAD1: binding of sex pheromone. Journal of Bacteriology. 1993;175(16):5260–5264. PubMed PMID: 8349566.

Teuber M., Schwarz F., Perreten V. Molecular structure and evolution of the conjugative multiresistance plasmid pRE25 of Enterococcus faecalis isolated from a raw-fermented sausage. International Journal of Food Microbiology. 2003;88(2-3):325–329. PubMed PMID: 14597005.

Thal L. A., Chow J. W., Clewell D. B., Zervos M. J. Tn924, a chromosome-borne transposon encoding high-level gentamicin resistance in Enterococcus faecalis. Antimicrobial Agents and Chemotherapy. 1994;38(5):1152–1156. PubMed PMID: 8067754.

Thompson J. K., Collins M. A. Completed sequence of plasmid pIP501 and origin of spontaneous deletion derivatives. Plasmid. 2003;50(1):28–35. PubMed PMID: 12826055.

Thorisdottir A. S., Carias L. L., Marshall S. H., Green M., Zervos M. J., Giorgio C., et al. IS6770, an enterococcal insertion-like sequence useful for determining the clonal relationship of clinical enterococcal isolates. The Journal of Infectious Diseases. 1994;170(6):1539–1548. PubMed PMID: 7995994.

Todokoro D., Tomita H., Inoue T., Ike Y. Genetic analysis of bacteriocin 43 of vancomycin-resistant Enterococcus faecium. Applied and Environmental Microbiology. 2006;72(11):6955–6964. PubMed PMID: 17088377.

Tomich P. K., An F. Y., Clewell D. B. Properties of erythromycin-inducible transposon Tn917 in Streptococcus faecalis. Journal of Bacteriology. 1980;141(3):1366–1374. PubMed PMID: 6245068.

Tomich P. K., An F. Y., Damle S. P., Clewell D. B. Plasmid-related transmissibility and multiple drug resistance in Streptococcus faecalis subsp. zymogenes strain DS16. Antimicrobial Agents and Chemotherapy. 1979;15(6):828–830. PubMed PMID: 112916.

Tomita H., Clewell D. B. A pAD1-encoded small RNA molecule, mD, negatively regulates Enterococcus faecalis pheromone response by enhancing transcription termination. Journal of Bacteriology. 2000;182(4):1062–1073. PubMed PMID: 10648533.

Tomita H., Ike Y. Genetic analysis of transfer-related regions of the vancomycin resistance Enterococcus conjugative plasmid pHTbeta: identification of oriT and a putative relaxase gene. Journal of Bacteriology. 2005;187(22):7727–7737. PubMed PMID: 16267297.

Tomita H., Ike Y. Genetic analysis of the Enterococcus vancomycin resistance conjugative plasmid pHTbeta: identification of the region involved in cell aggregation and traB, a key regulator gene for plasmid transfer and cell aggregation. Journal of Bacteriology. 2008;190(23):7739–7753. PubMed PMID: 18835991.

Tomita H., Fujimoto S., Tanimoto K., Ike Y. Cloning and genetic organization of the bacteriocin 31 determinant encoded on the Enterococcus faecalis pheromone-responsive conjugative plasmid pYI17. Journal of Bacteriology. 1996;178(12):3585–3593. PubMed PMID: 8655558.

Tomita H., Kamei E., Ike Y. Cloning and genetic analyses of the bacteriocin 41 determinant encoded on the Enterococcus faecalis pheromone-responsive conjugative plasmid pYI14: a novel bacteriocin complemented by two extracellular components (lysin and activator). Journal of Bacteriology. 2008;190(6):2075–2085. PubMed PMID: 18203826.

Tomita H., Pierson C., Lim S. K., Clewell D. B., Ike Y. Possible connection between a widely disseminated conjugative gentamicin resistance (pMG1-like) plasmid and the emergence of vancomycin resistance in Enterococcus faecium. Journal of Clinical Microbiology. 2002;40(9):3326–3333. PubMed PMID: 12202574.

Tomita H., Tanimoto K., Hayakawa S., Morinaga K., Ezaki K., Oshima H., et al. Highly conjugative pMG1-like plasmids carrying Tn1546-like transposons that encode vancomycin resistance in Enterococcus faecium. Journal of Bacteriology. 2003;185(23):7024–7028. PubMed PMID: 14617670.

Top J., Sinnige J. C., Majoor E. A., Bonten M. J., Willems R. J., van Schaik W. The recombinase IntA is required for excision of esp-containing ICEEfm1 in Enterococcus faecium. Journal of Bacteriology. 2011;193(4):1003–1006. PubMed PMID: 21148730.

Torres C., Tenorio C., Portillo A., García M., Martínez C., Del Campo R., et al. Intestinal colonization by vanA- or vanB2-containing enterococcal isolates of healthy animals in Spain. Microbial Drug Resistance. 2003;9 Suppl 1:S47–S52. PubMed PMID: 14633367.

van der Lelie D., Bron S., Venema G., Oskam L. Similarity of minus origins of replication and flanking open reading frames of plasmids pUB110, pTB913 and pMV158. Nucleic Acids Research. 1989;17(18):7283–7294. PubMed PMID: 2677995.

Van Melderen L. Toxin–antitoxin systems: why so many, what for? Current Opinion in Microbiology. 2010;13(6):781–785. PubMed PMID: 21041110.

van Schaik W., Top J., Riley D. R., Boekhorst J., Vrijenhoek J. E., Schapendonk C. M., et al. Pyrosequencing-based comparative genome analysis of the nosocomial pathogen Enterococcus faecium and identification of a large transferable pathogenicity island. BMC Genomics. 2010;11:239. PubMed PMID: 20398277.

Vickerman M., Flannagan S. E., Jesionowski A. M., Brossard K. A., Clewell D. B., Sedgley C. M. A genetic determinant in Streptococcus gordonii Challis encodes a peptide with activity similar to that of enterococcal sex pheromone cAM373, which facilitates intergeneric DNA transfer. Journal of Bacteriology. 2010;192(10):2535–2545. PubMed PMID: 20233933.

Wang A., Macrina F. L. Streptococcal plasmid pIP501 has a functional oriT site. Journal of Bacteriology. 1995;177(15):4199–4206. PubMed PMID: 7635806.

Wang N. R., Hergenrother P. J. A continuous fluorometric assay for the assessment of MazF ribonuclease activity. Analytical Biochemistry. 2007;371(2):173–183. PubMed PMID: 17706586.

Wang X., Wood T. K. Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. Applied and Environmental Microbiology. 2011;77(16):5577–5583. PubMed PMID: 21685157.

Weaver K. E. Enterococcus faecalis plasmid pAD1 replication and maintenance. Developments in Biological Standardization. 1995;85:89–98. PubMed PMID: 8586250.

Weaver K. E., Clewell D. B. Construction of Enterococcus faecalis pAD1 miniplasmids: Identification of a minimal pheromone response regulatory region and evaluation of a novel pheromone-dependent growth inhibition. Plasmid. 1989;22(2):106–119. PubMed PMID: 2516332.

Weaver K. E., Clewell D. B. Regulation of the pAD1 sex pheromone response in Enterococcus faecalis: effects of host strain and traA, traB, and C region mutants on expression of an E region pheromone-inducible lacZ fusion. Journal of Bacteriology. 1990;172(5):2633–2641. PubMed PMID: 2158976.

Weaver K. E., Tritle D. J. Identification and characterization of an Enterococcus faecalis plasmid pAD1-encoded stability determinant which produces two small RNA molecules necessary for its function. Plasmid. 1994;32(2):168–181. PubMed PMID: 7531349.

Weaver K. E., Clewell D. B., An F. Identification, characterization, and nucleotide sequence of a region of Enterococcus faecalis pheromone-responsive plasmid pAD1 capable of autonomous replication. Journal of Bacteriology. 1993;175(7):1900–1909. PubMed PMID: 8384618.

Weaver K. E., Ehli E. A., Nelson J. S., Patel S. Antisense RNA Regulation by Stable Complex Formation in the Enterococcus faecalis Plasmid pAD1 par Addiction System. Journal of Bacteriology. 2004;186(19):6400–6408. PubMed PMID: 15375120.

Weaver K. E., Jensen K. D., Colwell A., Sriram S. I. Functional analysis of the Enterococcus faecalis plasmid pAD1-encoded stability determinant par. Molecular Microbiology. 1996;20(1):53–63. PubMed PMID: 8861204.

Weaver K. E., Kwong S. M., Firth N., Francia M. V. The RepA_N replicons of Gram-positive bacteria: a family of broadly distributed but narrow host range plasmids. Plasmid. 2009;61(2):94–109. PubMed PMID: 19100285.

Weaver K. E., Reddy S. G., Brinkman C. L., Patel S., Bayles K. W., Endres J. L. Identification and characterization of a family of toxin–antitoxin systems related to the Enterococcus faecalis plasmid pAD1 par addiction module. Microbiology. 2009;155(Pt 9):2930–2940. PubMed PMID: 19542006.

Weaver, K. E., Rice, L. B., & Churchward, G. (2002). Plasmids and Transposons. In M. S. Gilmore, D. B. Clewell, P. Courvalin, & G. M. Dunny (Eds.), *The Enterococci: Pathogenesis, Molecular Biology, and Antibiotic Resistance* (pp. 219-263). Washington, D. C.: ASM Press.

Weaver K. E., Walz K. D., Heine M. S. Isolation of a derivative of Escherichia coli–Enterococcus faecalis shuttle vector pAM401 temperature sensitive for maintenance in E. faecalis and Its use in evaluating the mechanism of pAD1par-dependent plasmid stabilization. Plasmid. 1998;40:225–232. PubMed PMID: 9806859.

Weaver K. E., Weaver D. M., Wells C. L., Waters C. M., Gardner M. E., Ehli E. A. Enterococcus faecalis plasmid pAD1-encoded Fst toxin affects membrane permeability and alters cellular responses to lantibiotics. Journal of Bacteriology. 2003;185(7):2169–2177. PubMed PMID: 12644486.

Weigel L. M., Clewell D. B., Gill S. R., Clark N. C., McDougal L. K., Flannagan S. E., et al. Genetic analysis of a high-level vancomycin-resistant isolate of Staphylococcus aureus. Science. 2003;302(5650):1569–1571. PubMed PMID: 14645850.

Werner G., Coque T. M., Hammerum A. M., Hope R., Hryniewicz W., Johnson A., et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Eurosurveillance. 2008;13(47) PubMed PMID: 19021959.

Werner G., Fleige C., Geringer U., van Schaik W., Klare I., Witte W. IS element IS16 as a molecular screening tool to identify hospital-associated strains of Enterococcus faecium. BMC Infectious Diseases. 2011;11:80. PubMed PMID: 21453466.

Werner G., Hildebrandt B., Witte W. Linkage of erm(B) and aadE-sat4-aphA-3 in multiple-resistant Enterococcus faecium isolates of different ecological origins. Microbial Drug Resistance. 2003;9 Suppl 1:S9–S16. PubMed PMID: 14633362.

Werner G., Klare I., Witte W. Large conjugative vanA plasmids in vancomycin-resistant Enterococcus faecium. Journal of Clinical Microbiology. 1999;37(7):2383–2384. PubMed PMID: 10408957.

Wiedemann I., Breukink E., van Kraaij C., Kuipers O. P., Bierbaum G., de Kruijff B., et al. Specific binding of nisin to the peptidoglycan precursor lipid II combines pore formation and inhibition of cell wall biosynthesis for potent antibiotic activity. The Journal of Biological Chemistry. 2001;276(3):1772–1779. PubMed PMID: 11038353.

Willems R. J., Top J., van den Braak N., van Belkum A., Mevius D. J., Hendriks G., et al. Molecular diversity and evolutionary relationships of Tn1546-like elements in enterococci from humans and animals. Antimicrobial Agents and Chemotherapy. 1999;43(3):483–491. PubMed PMID: 10049255.

Willems R. J., Top J., van Schaik W., Leavis H., Bonten M., Sirén J., et al. Restricted gene flow among hospital subpopulations of Enterococcus faecium. MBio. 2012;3(4):e00151–e00112. PubMed PMID: 22807567.

Woodford N., Adebiyi A.-M. A., Palepou M. F., Cookson B. D. Diversity of VanA glycopeptide resistance elements in enterococci from humans and nonhuman sources. Antimicrobial Agents and Chemotherapy. 1998;42(3):502–508. PubMed PMID: 9517923.

Wozniak R. A., Waldor M. K. Integrative and conjugative elements: mosaic mobile genetic elements enabling dynamic lateral gene flow. Nature Reviews Microbiology. 2010;8(8):552–563. PubMed PMID: 20601965.

Wu L. J., Errington J. Nucleoid occlusion and bacterial cell division. Nature Reviews Microbiology. 2011;10(1):8–12. PubMed PMID: 22020262.

Wu M., Zampini M., Bussiek M., Hoischen C. Segrosome assembly at the pliable parH centromere. Nucleic Acids Research. 2011;39(12):5082–5097. Diekmann, & Hayes, F. PubMed PMID: 21378121.

Wyckoff H. A., Barnes M., Gillies K. O., Sandine W. E. Characterization and sequence analysis of a stable cryptic plasmid from Enterococcus faecium 226 and development of a stable cloning vector. Applied and Environmental Microbiology. 1996;62(4):1481–1486. PubMed PMID: 8919818.

Xu Z., Shirtliff M. E., Peters B. M., Peng Y., Alam M. J., Yamasaki S., et al. First report of class 2 integron in clinical Enterococcus faecalis and class 1 integron in Enterococcus faecium in South China. Diagnostic Microbiology and Infectious Disease. 2010;68(3):315–317. PubMed PMID: 20846812.

Yagi Y., Clewell D. B. Identification and characterization of a small sequence located at two sites on the amplifiable tetracycline resistance plasmid pAMalpha1 in Streptococcus faecalis. Journal of Bacteriology. 1977;129(1):400–406. PubMed PMID: 401496.

Yagi Y., Clewell D. B. Amplification of the tetracycline resistance determinant of plasmid pAM alpha 1 in Streptococcus faecalis: dependence on host recombination machinery. Journal of Bacteriology. 1980;143(2):1070–1072. PubMed PMID: 6782074.

Yagi Y., Clewell D. B., Bauer B. Plasmid-determined tetracycline resistance in Streptococcus faecalis: tandemly repeated resistance determinants in amplified forms of pAMalpha1 DNA. Journal of Molecular Biology. 1976;102:583–600. PubMed PMID: 818395.

Yagi Y., Kessler R. E., Shaw J. H., Lopatin D. E., An F., Clewell D. B. Plasmid content of Streptococcus faecalis strain 39-5 and identification of a pheromone (cPD1)-induced surface antigen. Microbiology. 1983;129(4):1207–1215. PubMed PMID: 6411857.

Yamashita H., Tomita H., Inoue T., Ike Y. Genetic organization and mode of action of a novel bacteriocin, bacteriocin 51: determinant of VanA-type vancomycin-resistant Enterococcus faecium. Antimicrobial Agents and Chemotherapy. 2011;55(9):4352–4360. PubMed PMID: 21709077.

Yarmolinsky M. B. Programmed cell death in bacterial populations. Science. 1995;267(5199):836–837. PubMed PMID: 7846528.

Yasmin A., Kenny J. G., Shankar J., Darby A. C., Hall N., Edwards C., et al. Comparative genomics and transduction potential of Enterococcus faecalis temperate bacteriophages. Journal of Bacteriology. 2010;192(4):1122–1130. PubMed PMID: 20008075.

Zechner, E. L., de la Cruz, F., Eisenbrandt, R., Grahn, A. M., Koraimann, G., Lanka, E., et al. (2000). Conjugative-DNA transfer processes. In *The Horizontal Gene Pool. Bacterial Plasmids and Gene Spread.* (pp. 87-184). Newark: Harwood Academic Publishers.

Zhang X., Vrijenhoek J. E., Bonten M. J., Willems R. J., van Schaik W. A genetic element present on megaplasmids allows Enterococcus faecium to use raffinose as carbon source. Environmental Microbiology. 2011;13(2):518–528. PubMed PMID: 20946531.

Zheng B., Tomita H., Inoue T., Ike Y. Isolation of VanB-type Enterococcus faecalis strains from nosocomial infections: first report of the isolation and identification of the pheromone-responsive plasmids pMG2200, encoding VanB-type vancomycin resistance and a Bac41-type bacteriocin... Antimicrobial Agents and Chemotherapy. 2009;53(2):735–747. PubMed PMID: 19029325.

Zheng B., Tomita H., Xiao Y. H., Wang S., Li Y., Ike Y. Molecular characterization of vancomycin-resistant Enterococcus faecium isolates from mainland China. Journal of Clinical Microbiology. 2007;45(9):2813–2818. PubMed PMID: 17634295.

Zhu W., Clark N. C., McDougal L. K., Hageman J., McDonald L. C., Patel J. B. Vancomycin-resistant Staphylococcus aureus isolates associated with Inc18-like vanA plasmids in Michigan. Antimicrobial Agents and Chemotherapy. 2008;52(2):452–457. PubMed PMID: 18056272.

Zhu W., Murray P. R., Huskins W. C., Jernigan J. A., McDonald L. C., Clark N. C., et al. Dissemination of an Enterococcus Inc18-Like vanA plasmid associated with vancomycin-resistant Staphylococcus aureus. Antimicrobial Agents and Chemotherapy. 2010;54(10):4314–4320. PubMed PMID: 20660665.

Zielenkiewicz U., Kowalewska M., Kaczor C., Cegłowski P. In Vivo Interactions between Toxin-Antitoxin Proteins Epsilon and Zeta of Streptococcal Plasmid pSM19035 in Saccharomyces cerevisiae. Journal of Bacteriology. 2009;191(11):3677–3684. PubMed PMID: 19346303.

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