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MINIREVIEW

Complex Nature of Enterococcal Pheromone-Responsive Plasmids

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Abstract

Pheromone-responsive plasmids constitute a unique group of ~20 plasmids identified, as yet, only among enterococcal species. Several of their representatives, *e.g.* pAD1, pCF10, pPD1 and pAM373 have been extensively studied. These plasmids posses a sophisticated conjugation mechanism based on response to sex pheromones – small peptides produced by plasmid-free recipient cells. Detailed analysis of regulation and function of the pheromone response process revealed its great complexity and dual role – in plasmid conjugation and modulation of enterococcal virulence. Among other functional modules identified in pheromone plasmids, the stabilization/partition systems play a crucial role in stable maintenance of the plasmid molecule in host bacteria. Among them, the *par* locus of pAD1 is one of the exceptional RNA addiction systems. Pheromone-responsive plasmids contribute also to enterococcal phenotype being an important vehicle of antibiotic resistance in this genus. Both types of acquired vancomycin resistance determinants, *vanA* and *vanB*, as well many other resistant phenotypes, were found to be located on these plasmids. They also encode two basic agents of enterococcal virulence, *i.e.* aggregation substance (AS) and cytolysin. AS participates in mating-pair formation during conjugation but can also facilitate the adherence of enterococci to human tissues during infection. The second protein, cytolysin, displays hemolytic activity and helps to invade eukaryotic cells. There are still many aspects of the nature of pheromone plasmids that remain unclear and more detailed studies are needed to understand their uniqueness and complexity.

Key words: pheromone-responsive plasmid, sex pheromone, inhibitory peptide, aggregation substance, vancomycin resistance

Introduction

Enterococci are Gram-positive bacteria, inhabiting gastrointestinal tracts of humans and animals. They are also commonly found in food, soil, sewage and water. During the last three decades enterococci have emerged as opportunistic, multi-resistant pathogens, causing hospital infections, especially in immunocompromised patients with two most prevalent species, *Enterococcus faecalis* and *Enterococcus faecium* (CDC, 2004). Treatment of enterococcal infections is frequently complicated by their resistance to antimicrobials of several classes (Murray, 1998). Acquired resistance in enterococci is often associated with mobile genetic elements (MGE), especially plasmids, *i.e.* autonomously replicating, extrachromosomal DNA molecules, able to be stably maintained in consecutive bacterial generations and to disseminate in the bacterial population.

A wide spectrum of replicons can be distinguished among enterococcal plasmids, ranging from small cryptic plasmids to megaplasmids, composed of different functional modules, responsible for plasmid replication, maintenance, conjugal transfer and phenotypic traits such as resistance to antimicrobials. Three plasmid types are known to replicate in enterococci: broad host range RCR (**r**olling **c**ircle **r**eplicating) and Inc*18* plasmids, and limited to enterococci pheromone-responsive plasmids. This review will focus on the family of ~20 pheromone-responsive plasmids, which display exceptional features, typical only for this group of elements. Pheromone-responsive plasmids described thus far range in size from 37 to 128 kb, have a low copy number (2–4 per cell) and evolved a specific conjugation mechanism based on pheromones, restricted to enterococci (Weaver *et al.*, 2002).

Conjugation system – basic features and regulation mechanism

One of the most extensively studied properties of pheromone-responsive plasmids is their unique conjugation mechanism. The principle of every conjugation

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mechanism is the formation of couples of bacterial cells, which facilitates the transfer of plasmid DNA from donor to recipient cell through a special multiprotein complex formed in the region of cell contact. Conjugation functions are encoded by the *tra* region of a plasmid, which in Gram-positive species exhibits homology to genes encoding type IV secretion systems. Several components encoded by this region are responsible for plasmid DNA binding, ATP-dependent translocation of single-stranded DNA or the formation of mating channel (Grohmann *et al.*, 2003).

Enterococcal pheromone-responsive plasmids constitute one of the most original bacterial MGE. The conjugation mechanism of these plasmids, discovered over 30 years ago (Dunny *et al.*, 1978) is based on the existence of special molecules called sex pheromones – small extracellular peptides specific for donors carrying various conjugative plasmids. Sex pheromones are produced by potential recipients, *i.e.* bacterial cells that lack a plasmid from a particular pheromone group. These signal molecules are chromosomally encoded, whereas genetic determinants for signal detection and response reside on the sex pheromone plasmid (Kozlowicz *et al.*, 2006). Donor cell in the presence of the pheromone produces proteinaceous structures on the cell surface (Yagi *et al*., 1983) called aggregation substance (AS) which binds to enterococcal binding substance (EBS) present on the surface of the recipient. During this process, a mating channel between the donor and the recipient is formed, which enables transfer of the plasmid DNA. After acquiring the plasmid, the recipient cell shuts down the production of pheromone and begins to produce a specific, plasmid-encoded inhibitor peptide which serves to desensitize the bacterial cell to low levels of endogenous pheromone and exogenous pheromones produced by donors (Mori *et al.*, 1986). Special surface exclusion proteins are exposed on the bacterial cells that prevent from acquiring the plasmids already present in the cell (entry-exclusion mechanism). Their probable role is blocking plasmid DNA transfer by disturbing mating-pair formation between donors (Clewell, 1993).

The pheromone-responsive conjugation system has been most extensively examined for plasmids pAD1, pCF10, pPD1 and pAM373 from *E. faecalis*. A schematic representation of conjugation steps of these plasmids and key molecular determinants of each system are listed in Table I. One of the first pheromone-responsive plasmids discovered was highly conjugative 60-kb plasmid pAD1, harboring several resistance and virulence determinants (Clewell, 2007), identified in *E. faecalis* DS16 clinical isolate. More than a half of pAD1 is devoted to mating response and regulation of this process (Francia and Clewell, 2002) and exhibits high level of homology to equivalent regions of other sex pheromone plasmids (Hirt *et al.*, 1996). All major steps, leading to the acquisition of pAD1 by plasmid-free cells, are very similar in most pheromone responsive plasmids characterized so far. Interestingly, plasmid pAM373 exhibits discrepancies in gene content and functions. The AS coded on this plasmid is structurally different and is able to bind to cells defective in EBS (Muscholl-Silberhorn, 1999). Another feature of pAM373 is the absence of the entry-exclusion function (de Boever and Clewell, 2001).

The first evidence for the existence of special molecules inducing cell aggregation and mating has been shown by Dunny *et al.* (1978). Working on *E. faecalis*,

Table I
Schematic representation of conjugation steps of four best-characterized pheromone-responsive plasmids with specification of key molecular determinants of conjugative functions.

Description of conjugation step	Nomenclature of proteins and relevant genes involved in conjugation steps in different plasmids			
	pAD1	pCF10	pPD1	pAM373
1. Secretion of chromosomally-encoded sex pheromones	cAD1 (*cad*)	cCF10 (*ccfA*)	cPD1 (*cpd*)	cAM373 (*camE*)
2. Recognition and internalization of pheromones by recipient cell surface	TraC	PrgZ	TraC	TraC
3. Induction of conjugation system	TraA	PrgX	TraA	TraA
4a. Synthesis of aggregation substance (AS) on the donor cell	Asa1 (*asa1*)	Asc10 (*prgB*)	Asp (*asp*)	Asa373 (*asa373*)
4b. Aggregation of donors and recipients – binding of AS and BS (binding substance on recipient cell)	Sea1 (*sea1*)	PrgA (*Sec10*)	Sep1 (*sep1*)	none
5. Initiation of plasmid transfer	*oriT1*, *oriT2*, TraX, TraW	*oriT*, PcfG	*nk*	*oriT*, TraX and TraW homologs
6. Shut down of conjugation functions in plasmid-acquired recipient	iAD1 (*iad1*)	iCF10 (*prgQ*)	iPD1 (*ipd*)	iAM373 (*iam373*)

Proteins assigned to subsequent steps are as follows: 1 – sex pheromones, 2 – bacterial surface receptors, 3 – negative regulator of conjugation system, 4a – aggregation substance, 4b – binding substance, 5 – origins of transfer, relaxases and TraG-like proteins, 6 – inhibitory peptides (Clewell and Dunny, 2002; Ozawa *et al.*, 2005; Chandler *et al*., 2005; Folli *et al*., 2008; Clewell, 2007); *nk,* not known.

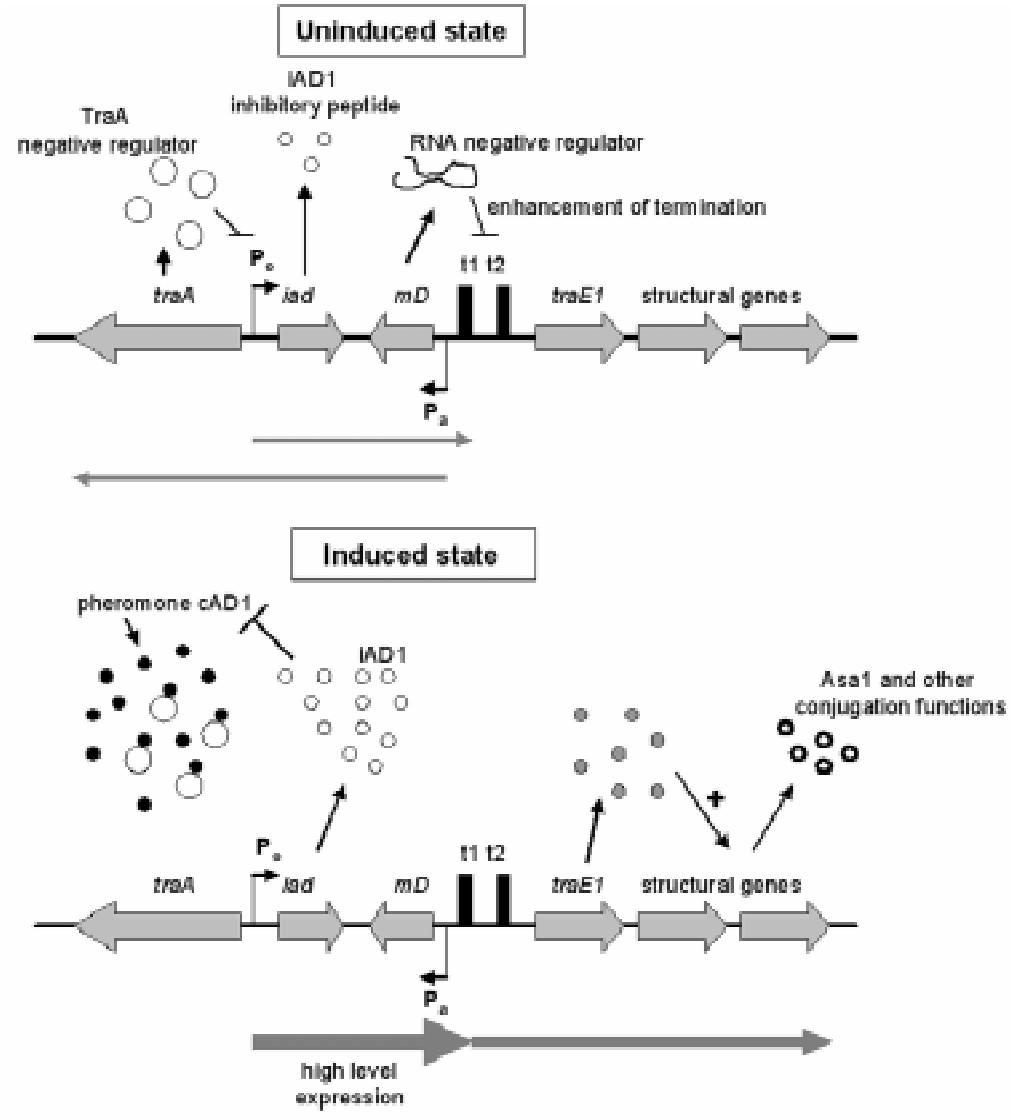

Fig.1. Schematic representation of regulation mechanism of pAD1 pheromone response.

In the absence of pheromone two negative regulators – TraA and mD keep the expression of *iad* on a very low level and block the transcription of TraE1 and structural genes (a). When the pheromone appears it bounds the TraA and negative regulation is abolished, which leads to high level of iAD1 synthesis and expression of TraE1 and conjugation machinery proteins (b). TraA, mD – negative regulators, TraE1 – positive regulator, *iad* – iAD1 gene, t1, t2 – termination sites, P_o, P_a – opposite promoters (according to Clewell and Dunny, 2002; Clewell 2007).

they discovered heat-stable, protease sensitive factors that were called sex pheromones. Different pheromones produced by strains lacking the particular plasmid, exhibit high specificity to the corresponding pheromone-responsive plasmids. One bacterial cell may secrete at least six different pheromones (Clewell and Dunny, 2002). This production results in conjugation and plasmid acquisition, which stops the secretion of a particular pheromone. However, synthesis of other pheromones is continued and thus single bacterial cell is able to accumulate different pheromone-responsive plasmids (Wirth, 1994). Pheromone plasmids can be divided into subgroups on the basis of their pheromone sensitivity, which parallels plasmid incompatibility characteristics (Hirt *et al*., 1996).

Although the structure of sex pheromone molecules was described many years ago, all that was known about their precursors came down to the statement that they must be chromosomally-encoded, since they are excreted by plasmid-free cells (Wirth, 1994). Finally, Clewell *et al.* (2000) identified pheromone determinants. It was revealed that pheromones are part of signal sequences of lipoprotein precursors. In the case of cAD1 (coded by the *cad* determinant), the mature peptide corresponds to the last 8 residues of the 22-amino acid signal sequence of the 309 aa lipoprotein precursor (An and Clewell, 2002). The generation of pheromone molecule involves the action of lipoprotein signal peptidase and the Eep protein similar to certain metaloproteases (Brown *et al.*, 2000). The identification of pheromone inhibitory peptide precursors, coded on plasmids, took place very early, *e.g.* for pAD1, the inhibitor iAD1 is coded by the *iad* determinant. The mature iAD1 peptide corresponds to the last 8 residues of the 22 aa

precursor (Clewell *et al.*, 1990). Inhibitor and corresponding pheromone have the same length and similar sequences, LFSLVLAG and LFVVTLVG, respectively.

The majority of other known pheromones and pheromone inhibitors display great similarity in their processing mechanism and final structure (Clewell *et al*., 2000), except for cAM373, which is produced independently of Eep activity (An *et al*., 1999) and represents the only known example when a very similar peptide is excreted by bacteria other than enterococci, namely by staphylococci (Clewell *et al*., 1985). Nevertheless, there is no relationship between lipoprotein precursors of cAM373 and staph-cAM373 (*camE* and *camS*, respectively), suggesting the similarity between these two peptides is only coincidental (Flannagan and Clewell, 2002).

Potential recipient cells produce constitutively high amounts of pheromone molecules. However, low level of endogenously produced pheromones is observed also for plasmid donors. Strict and complex regulation of conjugation functions prevents self-induction of pheromone-responsive plasmids. As yet, these regulatory mechanisms have been extensively studied for four plasmids – pAD1, pCF10, pPD1 and pAM373 (Clewell and Dunny, 2002). The key players of each system are: (i) two essential intracellular negative regulators, which control the gene expression from two opposite promoters and (ii) positive regulator, controlling the synthesis of conjugation structural genes. In the absence of inducing level of pheromone (Fig. 1a), a negative regulatory protein TraA blocks P_o – the promoter for the *iad* gene (iAD1 determinant). As a result, inhibitory peptide is expressed at a very low level allowing for titration of endogenous pheromone in the uninduced state (Pontius and Clewell, 1992). The transcription from P_o goes until t1 and t2 termination sites but not further (Tomita and Clewell, 2000), due to the presence of the second negative regulator, antisense RNA mD expressed at a relatively high level from the convergent promoter P_a. The expression from this promoter provides also a low level of TraA, essential for P_o activity inhibition (Clewell and Dunny, 2002). mD is the type of transcription termination enhancer at t1, because of its 11 nt sequence complementary to a region spanning the t1 'hairpin loop" transcript (Tomita and Clewell, 2000). This complex negative regulatory circuit, based on the transcription and counter-transcription from two opposing promoters P_o and P_a, seems to be a significant feature of the regulation of pheromone response in general (Callen *et al*., 2004).

When a higher amount of exogenous pheromone appears (Fig. 1b), it is bound by donor cell surface and transported to the cytoplasm, where it binds TraA, switching off its negative regulatory function by release of the protein from the P_o promoter (Fujimoto and Clewell, 1998).The resulting higher level of transcription from P_o leads to the elevated production of inhibitory peptide iAD1, as well as transcriptional read-through of t1 and t2 and the expression of positive regulator TraE1, followed by the expression of conjugation structural genes *e.g. asa1*, encoding the AS. As a result of the above-mentioned events, a recipient cell forms aggregates with the donor and acquires the plasmid. The presence of high amount of iAD1 results in a fast shut down of conjugation functions. This sophisticated control mechanism enables response to a slight change in the balance of the pheromone and inhibitory peptides, ensuring high level of sensitivity towards the presence of recipient cells (Kozlowicz *et al*., 2006).

Other functional modules of pheromone-responsive plasmids

The conjugation system enables efficient plasmid dissemination in a bacterial population. However, there are several other functional modules present on plasmids, essential for their stable maintenance in a bacterial cell including replication, partition and stabilization systems.

Four most studied pheromone plasmids, namely pCF10, pAD1, pAM373 and pPD1, serve as objects for analysis of the replication machinery. Key determinants of pheromone-responsive plasmids are *repA*, *repB* and *repC*. RepA plays a role as a replication initiator protein and resembles a family of proteins encoded by several low-copy plasmids from Gram-positive bacteria which exhibit a theta-type replication (Francia *et al*., 2004). The RepB and RepC proteins represent a partition system (Weaver *et al.*, 2002).

pAD1, as well as other pheromone plasmids (*e.g.* pCF10, pAM373, pPD1, pTEF1, pTEF2) belong to RepA_N family of replicons, known to be broadly distributed in other than enterococci low G+C Gram-positive bacteria, such as staphylococci, lactococci, streptococci and others, as well as in a few phages (Weaver *et al*., 2009). This group exhibits a narrow host range, which indicates that its evolution took place in native hosts with limited number of genetic exchange between different elements belonging to this family. Genes encoding initiator proteins bear a centrally located region of repeats, called *oriV*, which serves as the origin of replication. The *oriV* region of different pheromone responsive plasmids shows sequence variability within the repeats. pCF10 and pPD1 are an interesting example of compatible plasmids with *oriV* sequences differing only by a single nucleotide, which suggests the role of *oriV* as incompatibility determinant and provides a good explanation of coexistence of several related pheromone plasmids in a single cell.

The *repB* and *repC* determinants, located adjacent to the *repA* gene, are required for maximal replicon stabilization (Francia *et al*., 2007). The series of short repeats, called iterons, flank the *repBC* coding region. Their sequence and arrangement vary in different plasmids (Weaver *et al.*, 2002). The basic mechanism of RepBC action is the formation of RepC-iteron complex followed by binding of RepB ATPase, which presumably facilitates the connection between plasmid partition complex and host segregational apparatus as suggested for other partition systems (Funnell and Slavcev, 2004).

Another mechanism, called addiction or toxin-antitoxin system (TAS), encoded by number of plasmids prevents plasmid loss from the bacterial population. Two basic types of TAS can be distinguished depending on the type of their components. So called "proteic" systems consist of stable toxin and labile antitoxin. The second type is RNA – regulated addiction system, in which toxin is a protein while antitoxin is present in the form of the RNA molecule transcribed from the antisense strand. The translation of toxin mRNA is blocked in the presence of homologous antitoxin RNA by formation of duplex, subsequently degraded by bacterial RNAse. Bacterial cell lacking plasmid is killed by toxin translated in the absence of antitoxin RNA (Zielenkiewicz and Ceglowski, 2001). Among TAS of pheromone-responsive plasmids, the *par* locus in pAD1 is best characterized. It represents the unusual type of RNA regulated addiction system in which the binding of two RNAs leads rather to the formation of a highly stable complex than to RNA degradation (Weaver *et al*., 2004). Regulation of the *par* locus is based on the small regions of homology between two transcripts: RNAI (transcript for the Fst toxin) and RNAII (small antisense RNA). They are transcribed convergently and overlap at 3' end at bidirectional transcription terminators and at 5' ends, where similar direct repeats are present. These regions of homology result in the formation of stable RNAI-RNAII complex and inhibition of the Fst expression. Translationally inhibited RNAI is accumulated in the cell. When plasmid replicon is lost, less stable RNAII is degraded and the Fst protein expression leads to membrane permeabilization and cessation of macromolecular synthesis, causing death of the bacterial cell (Weaver *et al*., 2003).

Dissemination of antimicrobial resistance

One of the most threatening features of enterococci is their resistance to multiple antibiotics, which makes enterococcal infections difficult to treat. Among resistant enterococcal strains, VRE (vancomycin-resistant enterococci) are considered alert hospital pathogens (Courvalin, 2005). VRE exhibit high-level resistance to glycopeptides such as vancomycin and teicoplanin, conferred by *vanA* and *vanB* determinants (Courvalin, 2006) usually carried on transposons that can integrate into plasmids.

The most commonly detected vancomycin resistance determinants associated with pheromone-responsive plasmids are the *vanA* operons. *E. faecium* is the species, in which the first vancomycin resistant pheromone responsive plasmid pHKK100 was reported (Handwerger *et al*., 1990). An interesting situation was described in another *E. faecium* strain R7 carrying two plasmids, pHKK702 (41 kb) with integrated Tn*1546* transposon and pheromone-responsive pHKK703 (55 kb; Heaton *et al*., 1996). When R7 strain was used as a donor in mating experiments, a highly conjugative vancomycin-resistant transconjugant was isolated, which harbored 92-kb plasmid pHKK701, a cointegrate of pHKK702 and pHKK703. Yet another VanA pheromone-responsive plasmid, pBRG1 from the *E. faecium* LS10 strain was shown to be transferable to *E. faecalis* and responsive to cCF10 (Magi *et al.* 2003).

VanA pheromone-responsive plasmids have been described also for *E. faecalis*. Good examples are two plasmids – pSL1 and pSL2 – isolated in Korea from a patient and chicken feces, respectively (Lim *et al*., 2006). These plasmids transferred resistance not only to vancomycin but also to gentamicin, kanamycin, streptomycin and erythromycin to *E. faecalis* strains at high frequency. Both plasmids, 128,1 kb in size, exhibited identical restriction patterns while their hosts were unrelated which highlights the possibility of conjugative transfer of vancomycin resistance between chicken and human enterococci. Another *E. faecalis* VanA plasmid, pAM368 (107 kb) conferred a pheromone response to synthetic cAM373, and culture filtrates of *E. faecalis* and *S. aureus* (Showsh *et al*., 2001), providing the first example of enterococcal resistance determinant located on the plasmid that responds to *S. aureus* cAM373-like peptide and suggested that transfer of this resistance from enterococci to staphylococci cannot be neglected.

Very recently, the VanB type of glycopeptide resistance was described on pheromone-responsive plasmids. In two *E. faecalis* NKH15 strains, plasmids pMG2200 (106 kb) and pMG2201 (65,7 kb) were detected (Zheng *et al*., 2009). pMG2200 containing a Tn*1549*-like element responded to cCF10 and harbored the chimeric conjugation regulatory region, consisting of genes from pCF10 and pAD1.

Not only vancomycin resistance disseminates by pheromone plasmid vehicles. Several examples of pheromone plasmids with resistance determinants are presented in Table II. One of the first pheromone-responsive plasmids characterized, pCF10, encodes the resistance to tetracycline on Tn*925* (Christie and

Table II
Representatives of pheromone-responsive plasmids and their resistance determinants.

Plasmid	Original host	Size (kb)	Resistances	References
pCF10	*E. faecalis* SF-7	65	Tc^R	Christie and Dunny, 1986
pBEM10	*E. faecalis* HH2	70	bla^+ Cm^R Km^R Tm^R	Murray *et al*., 1988
pAM323	*E. faecalis* HH2	66	Em^R	Murray *et al*., 1988
pHKK100	*E. faecium* 228	55	VanA	Handwerger *et al*., 1990
pHKK101	*E. faecium* R7	92	VanA	Heaton *et al*., 1996
pBRG1	*E. faecium* LS10	50	VanA	Magi *et al*., 2003
pSL1, pSL2	*E. faecalis* KV1 *E. faecalis* KV2	128	VanA, Gm^R, Km^R, Sm^R, Em^R	Lim *et al*., 2006
pAM368	*E. faecalis* 368	107	VanA	Showsh *et al*., 2001
pMG2200	*E. faecalis* NKH15	106	VanB	Zheng *et al*., 2009
pMG2201	*E. faecalis* NKH15	65,7	Em^R	Zheng *et al*., 2009

bla^+ – β-lactamase production; Cm^R ,Km^R, Tm^R, Gm^R, Sm^R, Em^R – resistance to chloramphenicol, kanamycin, tobramycin, gentamycin, streptomycin and erythromycin, respectively.

Dunny, 1986). Aminoglycoside and beta-lactam resistance genes were also found to be connected with pheromone plasmids. Importantly, they can be both located on the same plasmid as was the case of 70 kb pBEM10 encoding beta-lactamase gene, gentamycin, kanamycin and tobramycin resistance (Murray *et al*., 1988). This plasmid was present in multiresistant strain HH22 of *E. faecalis* together with two other conjugative, probably pheromone-responsive plasmids, pAM323 and pAM324.

Contribution to virulence

Enterococci are the causative agents of several serious infections encountered in hospitals, such as surgical wound infections, bloodstream and urinary tract infections (Gilmore *et al*., 2002) but there is still little information available about pathogenesis of these infections. Virulence traits, encoded on mobile elements can be easily transmitted within bacterial populations. Several of such traits that contribute to the severity of enterococcal infection have been characterized to varying degrees. Two of them, namely cytolysin and AS, are carried on pheromone-responsive plasmids.

Cytolysin is a secreted virulence factor whose gene was identified on pAD1 (Borderon *et al*., 1982). Its discovery was one of the first indications of the possible contribution of pheromone plasmids to virulence, as it significantly enhanced perotinitis in a mouse model (Ike *et al*., 1984). Cytolysin displays both hemolytic and bactericidal activity. Expression of the cytolysin operon is a complex process involving the products of eight genes. Transcription starts from two divergent promoters and leads to the production of two mRNA units (Haas *et al*., 2002). The first transcript encodes structural genes for cytolysin subunits – $cylL_L$ and $cylL_S$, post-transcriptional modification and secretion functions (*cylM*, *cylB* and *cylA*) and immunity determinant (*cylI*). The second transcript comprises regulatory genes – *cylR1* and *cylR2*. $CylL_L$ and $CylL_S$ are synthesized as precursor forms and then post-translationally modified by CylM (Gilmore *et al*., 1994). ATP-binding cassette transporter, the product of *cylB* gene, mediates secretion of the subunits across the cytoplasmic membrane. CylB possesses also a cysteine protease domain and acts as signal peptidase, removing leader sequences from $CylL_L$ and $CylL_S$ precursors and generating $CylL_L$' and $CylL_S$' (Havarstein *et al*., 1995). Externalized subunits undergo the last processing step – removal of six-amino-acid sequence from the N-terminus of each subunit by subtilisin-like serine protease CylA. This leads to the generation of active toxin subunits $CylL_L$'' and $CylL_S$'' (Segarra *et al*., 1991). Regulation of cytolysin expression is dependent on CylR1 and CylR2 negative regulators, as well as on the appropriate concentration of $CylL_S$'', which acts as an autoinducer of operon functions (Haas *et al*., 2002). Mature cytolysin provides an effective tool for enterococci to invade different tissues and to evade the immune response of infected host (Miyazaki *et al*., 1993). Epidemiological data showed it to be associated with lethality in humans (Huycke *et al*., 1991).

The second important agent of enterococcal pathogenicity, the AS, is strictly connected with the conjugation system of pheromone-responsive plasmids. However, it displays some characteristics which can be significant in enterococcal infection mechanism. The AS is a cell wall-associated protein with the integrin-binding Arg-Gly-Asp (RGD) motifs and facilitates the adherence of enterococci to different tissues during infection (Galli *et al*., 1990). Some experiments showed that AS mediates binding to human polimorphonuclear leukocytes (PMN) and interfere with PMN-mediated killing (Vanek *et al*., 1999).

Interestingly, the AS and *cyl* operon occur together on the same plasmids and likely work in concert. The AS mediates adherence to eukaryotic tissues and, as a result, the density of bacterial cells and cytolysin operon inducer $CylL_S$'' increases, which leads to the high-level expression of the toxin (Chow *et al*., 1993), resulting in tissue damage and invasion.

An interesting mechanism has been described in the case of cCF10 and iCF10 activity. It is known that the inactive state of pheromone response mechanism depends on the presence of inhibitory peptides and membrane protein PrgY. These two factors prevent the self-induction by endogenous pheromone and expression of conjugation determinants, including the AS. However, it has been reported that the pCF10 conjugation functions can be activated in the absence of recipient cells when *E. faecalis* pCF10 donors are grown in human or rabbit plasma (Hirt *et al.*, 2002). Some results indicate that albumin in human plasma interacts with iCF10 shifting the ratio of iCF10 to endogenous cCF10 facilitating the latter to self-induce the conjugation mechanism (Chandler *et al*., 2005). Thus, induced synthesis of AS leads to the adherence and invasion of eukaryotic tissues.

Sex pheromone plasmids seem to be an important determinant, conferring virulence traits to enterococcal clinical isolates and significant cause of antibiotic resistance spread among enterococci. Epidemiological studies show that they were more frequently found in isolates from patients with bacteremia and wound infections than from stool specimens of healthy volunteers and hospitalized patients (Coque *et al*., 1995).

Conclusions

Novel details of the complex genetic repertoire, successively discovered in enterococci, force us to treat them as unpredictable opportunistic pathogens with great variability potential. Our knowledge about their diverse genetic elements, among which pheromone-responsive plasmids seem to play one of the critical roles, is still just the tip of the iceberg. The group of pheromone plasmids, with their unique properties, is intriguing and mysterious, with many questions, concerning the structure, mechanism of conjugation, role and evolution of pheromone plasmids remaining open. These plasmids have developed a sophisticated and complex conjugation system restricted, as yet, only to the genus *Enterococcus*. Particular parts of this system often play several different roles, not only limited to conjugation itself. They seem to be responsible in some degree also for the modulation of entococcal virulence. Adding to it the participation of pheromone plasmids in dissemination of antibiotic resistance, enterococci possess sophisticated tools that help them to survive antibiotic pressure and to cause disease (Mundy *et al*., 2000). As a result, highly adapted hospital strains have evolved.

All interesting and complex features of pheromone-responsive plasmids constitute a perfect object to study the nature of mobile genetic elements – their biology and evolution. And finally maybe it will be possible to answer the question: "To what extent are plasmids able to evolve and influence the nature of bacterial hosts?"

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