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MicroCorrespondence

Enterococcal sex pheromone precursors are part of signal sequences for surface lipoproteins

Sir,

The enterococci are among the three most common types of bacteria involved in nosocomial infections in the USA. Pheromone-inducible plasmid transfer is an important mechanism for dissemination of antibiotic resistance and virulence in these organisms. We would like to relate some significant information that has recently become available, concerning the intercellular signals that trigger conjugation. Plasmid-free strains of *Enterococcus faecalis* secrete at least half a dozen peptide sex pheromones – probably many more – that induce a mating response by potential donor strains carrying members of specific families of conjugative plasmids (for recent reviews of this topic, see Clewell, 1999, *Cell–Cell Signaling in Bacteria*. Dunny and Winans (eds). Washington, DC: American Society for Microbiology Press, pp. 47–65; Dunny and Leonard, 1997, *Annu Rev Microbiol* **51:** 527–564). The response is associated with synthesis of a surface 'aggregation substance' that facilitates formation of donor–recipient mating aggregates. When a recipient acquires a given plasmid, the corresponding pheromone becomes shutdown or masked; whereas different pheromones continue to be secreted. Although the structure of at least five of these peptides has been determined (see Table 1), their chromosome-borne genetic determinants have remained elusive, despite a significant effort to identify them.

E. faecalis genome data bases [e.g. The Institute for Genomic Research (TIGR) public domain web site http://www.tigr.org/tdb/mdb/mdb.html] and Peter Barth (AstraZeneca, personal communication) have recently revealed that indeed these determinants are present and exist as components of signal sequences of apparent lipoprotein precursors (Table 1). In the case of the pheromones cAD1, cPD1, cOB1 and cAM373, the peptide corresponds to the last seven or eight residues of a 21- to 22-amino-acid signal sequence immediately preceding a cysteine residue; all four exhibit a leucine and a cysteine residue at the −3 and +1 positions respectively, characteristic of a lipobox processing site (Wu, 1996, E. coli *and* Salmonella, 2nd edn. Neidhardt *et al*. (eds). Washington, DC: American Society for Microbioloby Press, pp. 1005–1014). In the case of cCF10, the cysteine is located four residues downstream of what becomes the C-terminus of the pheromone peptide. With regard to both cPD1 and cCF10, the lipoprotein exhibits some similarity to SpoIIIJ, a protein related to sporulation in *Bacillus subtilis*; some of the precursors have similarities with apparent lipoproteins of unknown function. A coding sequence for cCF10 shows up a second time in the data base, but near the middle of a different open reading frame (ORF); expression from this position seems unlikely. Because the determinants for the different pheromones are present on different contigs, they appear not to be tightly clustered on the chromosome The TIGR data base, which relates to *E. faecalis* V583 (Sahm *et al*., 1989, *Antimicrob Agents Chemother* **33:** 1588–1591), is not yet complete. Partial or complete clones of the pheromone determinants (for example cCF10, cAD1 and cAM373) have now been generated using polymerase chain reaction (PCR) and ligation to appropriate plasmid vectors, and expression of pheromone activity from *E. faecalis* or heterologous hosts has been detected (F. Y. An, S. E. Flanagan and M. Antiporta, work in progress; manuscripts in preparation). The pheromone precursors thus require internal processing at both the C-terminal and N-terminal ends of what ultimately become the active components. The role of the lipoprotein products, devoid of their signal sequences and related pheromones, is unknown; although experiments are under way to generate mutants that may shed light on this question. A chromosome-determined protein that may be involved in processing some of the pheromone precursors is Eep (enhanced expression of pheromone), which is necessary for normal secretion of cAD1, cPD1, cOB1 and cCF10 but not cAM373 (An *et al*., 1999, *J Bacteriol*, **181:** 5915–5921).

The plasmids that confer a pheromone response have been previously shown to encode small peptides that are secreted and act as competitive inhibitors of the corresponding pheromones; this is believed to prevent donors from responding to their own sex pheromone. In addition to utilizing inhibitor peptides to mask endogenously secreted pheromone, some plasmids (for example pAD1) appear to cause a significant reduction in the amount of the peptide produced (see Nakayama *et al*., 1995, *Dev Biol Stand* **85:** 35–38). In the case of the best-studied pheromone-responding plasmids pAD1, pCF10 and pPD1, the precursors of the inhibitors are 21- to 23-amino-acid peptides resembling signal sequences; the C-terminal seven to eight residues constitute the mature inhibitors (see Table 1).

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Table 1. Pheromone, precursor and plasmid-encoded inhibitor structures.

Plasmid: pAD1 (60 kb)	Encodes haemolysin/bacteriocin and UV resistance
Pheromone: cAD1	**LFSLVLAG**
Precursor: cAD1p	MKVNKFVKGFAAIA**LFSLVLAG**CGADKK . . . . . (143 amino acids in total)
Inhibitor: iAD1	**LFVVTLVG**
Precursor: iAD1p	MSKRAMKKIIPLIT**LFVVTLVG**
Plasmid: pCF10 (54 kb)	Encodes tetracycline resistance (Tn*925*)
Pheromone: cCF10	**LVTLVFV**
Precursor: cCF10p	VKKYKRLLLMAG**LVTLVFV**LSACGTAPVS . . . . . (275 amino acids in total)
Inhibitor: iCF10	**AITLIFI**
Precursor: iCF10p	MKTTLKKLSRYIAVVI**AITLIFI**
Plasmid: pPD1 (56 kb)	Encodes bacteriocin (Bac21) essentially identical to AS-48
Pheromone: cPD1	**FLVMFLSG**
Precursor: cPD1p	MRKLNRWLYGSGLL**FLVMFLSG**CVKTGA . . . . . (234 amino acids in total)
Inhibitor: iPD1	**ALILTLVS**
Precursor: iPD1p	MKQQKKHIAALLF**ALILTLVS**
Plasmid: pAM373 (36 kb)	Cryptic
Pheromone: cAM373	**AIFILAS**
Precursor: cAM373p	MLKKPFLLFFSLLG**AIFILAS**CGIGKDAV . . . . . (166 amino acids in total)
Plasmid: pOB1 (71 kb)	Encodes haemolysin/bacteriocin
Pheromone: cOB1	**VAVLVLGA**
Precursor: cOB1p	MKKRTLWSVITVA**VAVLVLGA**CGNKKS . . . . . (272 amino acids in total)

Soluble sex pheromone signals that are secreted by recipients and induce transfer from specific plasmid-bearing donors are still known to occur only in enterococci, despite the fact that more than 20 years have passed since their discovery. *Staphylococcus aureus* and *Streptococcus gordonii* secrete peptides with activities resembling cAM373 in that culture filtrates can induce a clumping response by *E. faecalis* strains harbouring pAM373 (Clewell *et al*., 1985, *J Bacteriol* **162:** 1212–1220). Although there is no evidence yet that these relate to pheromone activities within *S. aureus* or *S. gordonii*, it is conceivable that they could play a role in acquiring genetic information from enterococci. An important clinical concern in this context would be the acquisition of vancomycin resistance determinants.

Finally, it is noteworthy that Firth *et al*. (1994, *J Bacteriol* **176:** 5871–5873) have reported that the *traH* gene of the staphylococcal plasmid pSK41 encodes a lipoprotein precursor bearing a signal sequence with the last eight residues resembling cAD1 (seven of eight identical amino acids). Staphylococci harbouring the plasmid were found to secrete an activity that would induce clumping of an *E. faecalis* strain carrying pAD1. Although there is no evidence that this activity is more than incidental, it is possible that staphylococci carrying pSK41-like plasmids might facilitate targeting by enterococcal donor strains harbouring pAD1-like elements. Conceivably, there are peptides deriving from the signal sequences of many secreted proteins, surface bound (for example lipoproteins) or released, that play a variety of functional or evolutionary roles in bacterial signalling.

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