信息素调控质粒介导的粪肠球菌耐药基因接合转移机制研究进展

杨雨桐，杨晓波，王尚，薛斌，李辰宇，赵辰，张曦，谌志强，王景峰，邱志刚*

军事科学院军事医学研究院环境医学与作业医学研究所，天津 300050

摘要：粪肠球菌是一种在自然环境中广泛存在的革兰氏阳性细菌。由于其特殊的耐药机制及高频率的耐药基因转移方式，导致了环境中耐药粪肠球菌的广泛传播，生态安全形势严峻。其中，信息素应答质粒介导的粪肠球菌耐药基因的接合转移是造成粪肠球菌耐药基因快速扩散的重要方式。本文回顾了近些年关于信息素应答质粒接合转移的研究成果，分析总结了接合转移的必要条件、正负调控信息素对接合转移的调节作用，并以携带四环素抗性的质粒 pCF10 为例简要探讨了接合转移相关基因、蛋白的调控机制，旨在更加全面地揭示粪肠球菌的耐药基因传播机制，为耐药细菌的基因转移机制研究提供参考。
关键词：细菌耐药性；耐药基因；水平转移；质粒
文章编号：1673-5897（2021）5-160-08　　　中图分类号：X171.5　　　文献标识码：A

Mechanism of Pheromone-responsive Plasmids Mediated Resistance Genes Conjugation Transfer in *Enterococcus faecalis*

Yang Yutong, Yang Xiaobo, Wang Shang, Xue Bin, Li Chenyu, Zhao Chen, Zhang Xi, Shen Zhiqiang, Wang Jingfeng, Qiu Zhigang*

Institute of Environmental and Operational Medicine, Academy of Military Medicine, Chinese Academy of Military Science, Tianjin 300050, China

Abstract：*Enterococcus faecalis* is a Gram-positive bacterium that is capable of surviving in a broad range of natural environments. The special resistance mechanism and the high frequency of antibiotic resistance gene transfer cause a wide spread of antibiotic resistance *Enterococcus faecalis* in the environment which makes challenge to the ecological safety. Among the mechanisms of antibiotic resistance transfer, pheromone-responsive plasmid mediated antibiotic resistance genes conjugation transfer is an important way to cause the rapid spread of antibiotic resistance genes in *Enterococcus faecalis*. This review will highlight recent advancement of pheromone-responsive plasmid conjugative transfer, including the necessary conditions of conjugation, the function of positive and negative regula-

tory pheromone on conjugation transfer. And the tetracycline-resistance plasmid pCF10 was taken as an example to expore the regulation mechanism of conjugation transfer related genes and proteins. The review is aiming to reveal the mechanism of antibiotic resistance gene transmission in *Enterococcus faecalis* and provide reference for the gene transfer mechanism of antibiotic resistance bacteria.

Keywords:bacterial resistance; antibiotic resistance genes; conjugative transfer; plasmids

　　粪肠球菌(*Enterococcus faecalis*)是一种革兰氏阳性球菌,广泛分布于在动物、人体胃肠道内以及水体和土壤等环境中。其主要来源是人畜粪便,并通过饮食、呼吸以及皮肤接触途径感染人类,从而引起感染性心内膜炎、菌血症等相关疾病[1-2]。粪肠球菌还可以作为一种指示微生物反映水环境中病原微生物的污染情况,对生态安全的风险控制具有重要意义[3]。粪肠球菌的细胞壁较厚,对氨基糖苷类及头孢类抗生素具有固有耐药性[4-5]。而近年来在畜牧业、水产养殖业以及临床上广泛应用的抗生素,也使得耐药菌株尤其是多重耐药菌株的检出率呈现逐年递增的趋势。更加严重的是,世界各地均检测出了目前仍无特效药的耐万古霉素肠球菌[6]。耐药细菌可通过医院废水、畜牧厂废水等各种途径向环境中扩散,使自然水体或土壤中耐药细菌广泛传播,进而通过食物链等途径影响人类健康[7]。粪肠球菌多重耐药性的急剧发展对人类健康及生态安全都构成了严重威胁,引发了科研人员的广泛关注。

　　粪肠球菌耐药的严峻之处在于其携带抗生素抗性基因的质粒、转座子等可移动遗传元件可通过水平转移在种属内或跨种属的细菌间进行传播,使其他更危险的细菌获得耐药性[8-9]。基因在细菌间的水平转移主要分为转化、转导和接合 3 种方式,其中接合是指通过细菌间接触从而转移质粒的方式,其转移质粒的频率和效率最高[10]。质粒是独立于染色体的能够自我复制的闭合环状 DNA,有研究发现耐药基因及其相关的可移动基因元件存在于质粒上,是引起细菌耐药基因水平转移的主要原因之一[11]。肠球菌质粒一般分为接合质粒和非接合质粒,接合质粒分为信息素调控质粒和非信息素调控质粒[12]。其中,信息素调控质粒在粪肠球菌菌属中非常常见,其大小通常在 37 ~ 128 kb,拷贝数较低,并且具有一种利用多肽信息素作为信号分子且仅限于肠球菌间转移的特殊接合转移机制[13-14]。信息素调控质粒相较于 R 质粒等其他质粒接合转移频率更高,耐药基因扩散速度更快,自然状态下 R 质粒接合频率只有 10^{-6}[15],而粪肠球菌携带质粒接合转移频率却可达

10^{-3},能够造成耐药基因的快速扩散。因此研究信息素调控质粒介导的粪肠球菌耐药基因的接合转移过程对防控耐药基因水平转移具有重要意义。

1 我国部分地区环境中粪肠球菌耐药现状(Current status of drug resistance of *E. faecalis* in the environment in some areas of China)

　　在全国各地的调研中发现,耐药粪肠球菌检出率逐年攀升,耐药问题日趋严峻。不仅耐药的范围和类别逐步扩增,而且多重、交叉耐药的现象也逐渐加剧。易秀丽等[16]从鱼塘、污水沟等环境中分离出共 99 株粪肠球菌,其中污水沟中粪肠球菌对红霉素、土霉素等耐药率可达 89.3%;多重耐药现象也十分严重,其中 21% 的菌株可达五重耐药。吴丽云等[17]从福建省部分河流中分离出粪肠球菌 31 株,发现其对红霉素的耐药率可达 80.65%,对四环素的耐药率也可达 74.19%。陈玉红等[18]从闽江流域的不同水样中分离出 40 株肠球菌菌株,发现其对检测的土霉素、红霉素、环丙沙星、氯霉素和氨苄西林 5 种常用抗生素均具有耐药性,其中对土霉素的耐药率可达 70%。林云琴等[19]在养殖场管网水环境中分离出的 42 株肠球菌样本中,呈多重耐药趋势,其中就包括对万古霉素耐药,其耐药率可达 14.29%。可见当前自然环境中粪肠球菌耐药形势严峻,对耐药细菌蔓延的控制刻不容缓。随着合理使用抗生素的意识逐步普及,以及粪肠球菌的实验室监测进一步深入,粪肠球菌的耐药问题是可以得到控制的,因此深入透彻地研究粪肠球菌的耐药机制也是非常必要的。

2 粪肠球菌耐药基因转移的机制(Drug resistance gene transfer mechanism of *E. faecalis*)

2.1 信息素调控质粒的主要特点

　　在临床粪肠球菌分离株中,已鉴定出多种受到信息素调控的接合质粒,其中包括携带四环素抗性基因的 pCF10 质粒[20-21],编码溶血素的 pAD1 质粒[22],携带万古霉素抗性基因的 pAM373 质粒[23]等等。在信息素调控质粒介导的接合转移过程中,多

肽信息素将作为供、受体菌的配对信号,通过 2 种具有拮抗效应的信息素更高效地调控接合转移过程[24-25]。信息素调控质粒的关键调控区域大部分是高度保守的,例如质粒 pCF10、pAM373 和 pAD1 在 cDNA 链上均携带 2 个 5' 末端存在重叠区域的操纵子,如图 1 所示。这种结构可能导致转录干扰从而产生二者之间的相互负调控,在这个重叠区域内

同时还编码质粒的负调控信息素[26-30]。而正是由于关键调控区域的高度保守,使得信息素调控质粒的接合转移现象和机制高度类似。本文将主要以接合质粒 pCF10 为例,探讨信息素调控质粒介导的耐药基因接合转移的机制。pCF10 可以编码抗四环素的耐药基因,也可以响应供体细胞对多肽信息素 cCF10 的感应从而进行快速且有效地传播[31]。
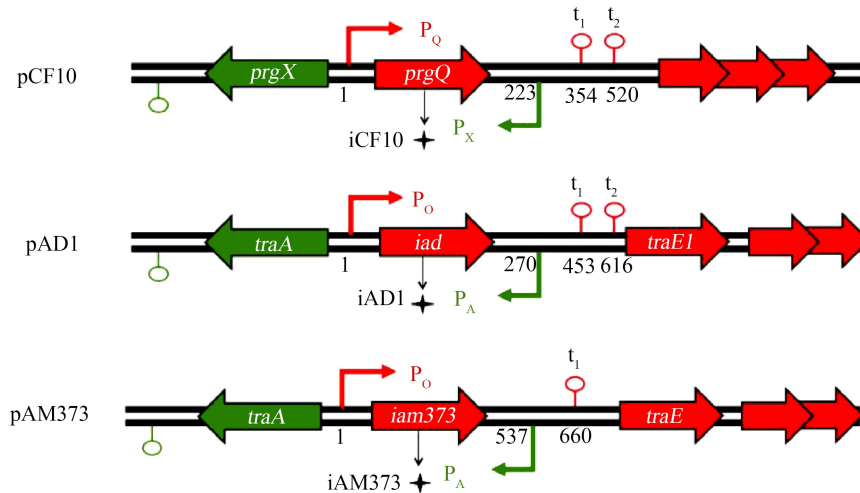

图 1 信息素调控质粒中高度保守的调控区域[32]

注:pCF10 质粒、pAD1 质粒和 pAM373 质粒的信息素调控区域的比较;P_Q、P_O、P_X、P_A 分别表示 prgQ、iad、iam373、prgX 和 traA 的转录起始点,重叠区域始于 P_Q 和 P_O(表示为"1"),终于 P_X 和 P_A(对应数字为重叠区域核苷酸序列长度);重叠区域同时编码负调控信息素 iCF10、iAD1 和 iAM373,在负调控信息素编码基因与下游基因之间的区域,编码调控 RNA,进而控制转录终止子 t_1 下游接合基因的转录。

Fig. 1 Substantial conservation of the critical regulatory regions in pheromone-responsive plasmids [32]

Note: Comparison of the pheromone-responsive region of pCF10 to the sex pheromone plasmids pAD1 and pAM373 in their regulatory regions; P_Q, P_O, P_X, P_A indicated the transcription start sites of prgQ, iad, iam373, prgX and traA; the overlapping region start from P_Q and P_O(indicated as "1"), and terminate in P_X and P_A(the length of nucleotides of the overlapping region indicated as revelant numbers); the overlapping region also encodes the inhibitor pheromone iCF10, iAD1 and iAM373; the region between the inhibitor determinant and the downstream genes is of great regulatory importance, as it is known to encode a small regulatory RNA that participates in controlling the transcription of conjugation genes downstream of the transcriptional terminator t_1.

2.2 信息素调控质粒的主要机制

2.2.1 细胞间紧密接触为接合转移提供条件

粪肠球菌主要通过基因的水平转移获得抗生素抗性基因。粪肠球菌的临床分离株中普遍存在可移动遗传元件(MGEs),如上文所述的 pCF10、pAD1 等接合质粒或转座子[33]。虽然信息素调控质粒可在粪肠球菌中进行高效且高频地水平传播,但该质粒的转移受到分子调控机制中信号分子的严格调控,并且只受到靠近提供质粒的供体细胞的受体细胞诱导[34]。在质粒转移频率较高的供、受体细胞混合培养液中,经常可以观察到细菌细胞有明显的聚集现

象,这是由于接合转移过程中在细菌细胞间紧密接触形成了"接合桥"[35]。前期研究发现,在接合转移过程中存在一种聚集诱导剂,可以诱导聚集形成从而促进细胞配对。相关的实验结果也证实了聚集和质粒转移之间存在正相关关系[36]。接合转移过程中受体细胞将一系列信息素释放到环境中,受到诱导的供体细胞对特异性的信息素做出特异性反应产生聚集诱导剂。聚集诱导剂是接合过程中质粒编码产生的一种称为"聚集性物质"的粘性蛋白,该蛋白可以促进配对形成,进而使得质粒从供体细胞转移到受体细胞[37]。聚集性物质的表达促使供体细胞和受

体细胞间进行紧密的物理接触,使得供体的质粒转移频率高达 10^{-1} [34]。Olmsted 等[38]通过用特定的抗 Asc10 抗体阻止聚集体的形成等实验证实质粒 pCF10 的聚集诱导物质就是 Asc10 蛋白。受体细胞紧密地和供体细胞联系在一起,使得供体细胞受信息素诱导加剧,最终促进质粒的转移,为接合转移提供了先决条件。

2.2.2　拮抗信号分子对接合转移的影响

Clewell[39]在实验中发现,将受体细胞的培养基滤液加入到供体细胞中,在没有受体细胞的情况下也可以观察到细胞间的聚集现象,加入受体细胞后也可以在短时间内发生接合转移;若供体细胞未提前接触受体细胞的培养基滤液,在较短时间内不会观察到明显的转移现象。这使得人们了解到受体细胞分泌的信息素促进了供受体菌间耐药基因的接合转移。质粒 pCF10 的表达由受体细胞分泌的多肽信息素 cCF10 诱导[40],使接合转移基因和毒力基因高表达[32,41]。此外,pCF10 质粒编码的多肽 iCF10 充当 cCF10 的竞争性抑制剂,防止供体细胞分泌的内源信息素进行自我诱导,并且可以抑制紧邻供体的受体细胞所分泌的 cCF10 的活性[32,42]。信息素 cCF10 和 iCF10 都是调节接合转移过程的信号分子,且 cCF10 促进接合转移进程,iCF10 降低接合转移进程,二者互为拮抗作用。而在其他的信息素应答质粒中也存在类似的拮抗信号分子对,例如质粒 pAD1 和 pAM373 同样对各自的正调控信息素 cAD1 和 cAM373 做出应答,促进接合转移,同时分别编码各自的负调控信号分子多肽 iAD1 和 iAM373 作为竞争性抑制剂,防止自我诱导[43-46]。正调控信息素对质粒的接合转移有积极的影响,而负调控信息素对质粒的接合转移则有着负面调节的作用,二者之间相辅相成,互相调节,才使得接合转移过程更加高效,耐药基因扩散更加广泛。

2.2.3　通过质粒编码蛋白调控信息素的机制

信息素在进入细胞后,与一系列受体蛋白结合,从而影响接合转移过程。以质粒 pCF10 为例,cCF10 在进入受体细胞后,将与质粒 pCF10 编码的主要转录调控因子 PrgX 结合[47-48]。当细胞中 cCF10 缺乏时,PrgX 对编码产生抑制剂 iCF10 的 prgQ 操纵子的启动子(P_Q)产生了抑制[49-50]。当供体细胞将 cCF10 导入受体细胞的细胞质,cCF10 与 PrgX 结合后,PrgX 对 P_Q 的抑制将会解除,从而诱导接合转移[31,51-53]。信息素 cCF10 和 iCF10 对诱导受体细胞过程的调节通过对信号肽受体蛋白 PrgX 的结构域调整实现。cCF10 和 iCF10 都与 PrgX 的同一结构域结合,在与 cCF10 和 iCF10 结合形成的络合物中,PrgX 在晶体和溶液中都形成了四聚体,iCF10 将稳定四聚体结构,而 cCF10 与 PrgX 的结合将破坏四聚体的稳定,使 PrgX 的结构发生了改变,其与操纵子 prgQ 结合位点间的相互作用被破坏[48,54]。所以当外源 cCF10 或产生 cCF10 的受体细胞加入,导致细胞内 cCF10 水平的增加,使 PrgX 结构从抑制构象转变为非抑制构象,导致抑制解除,最终诱导接合的发生。pCF10 通过诱导编码 iCF10 的 prgQ 操纵子和质粒转移所启动的接合转移机制如图 2 所示[47]。除此之外,还有其余一些质粒编码的蛋白也能影响接合转移的过程,例如质粒编码的膜蛋白 PrgY,它可以降低由供体细胞所产生的信息素的活性。PrgY 的胞外结构域可以与 cCF10 相互作用并对其进行修饰或降解,从而降低供体细胞的内源信息素活性[55]。又如质粒上 prgU 基因编码的表面粘附素 PrgU,Bhatty 等[56]证明缺少 prgU 基因的细胞,cCF10 的诱导具有高度毒性,会严重损害细胞包膜的完整性;而携带 prgU 基因的细胞过表达粘附素 PrgU 后,细胞对信息素不敏感,cCF10 的诱导毒性降低。因此,PrgU 对 pCF10 接合进程起到另一层负调控作用。总的来说,整个接合转移过程由质粒 pCF10 携带的多个基因所编码的不同种类的蛋白通过协同作用共同调节,通过相关基因蛋白调控信息素对供受体细胞的效应影响整个接合转移进程。质粒 pCF10 是信息素调控质粒家族中的重要代表,研究其接合转移机制对研究信息素调控质粒介导的接合转移机制具有广泛的参考价值,为防止环境中耐药基因的扩散,维护生态安全也具有重要意义。

3　展望(Prospect)

综上所述,粪肠球菌不但具有较强的天然耐药性,更因其特殊的接合转移机制,极易被诱导而发生高效的耐药基因水平转移。粪肠球菌还可以将耐药基因水平传递给其他肠球菌或其他种属的革兰氏阳性菌,包括毒力更强的金黄色葡萄球菌,导致耐药金黄色葡萄球菌的出现。一旦耐药金黄色葡萄球菌出现并造成传播,将会给人类健康和环境生态安全到来巨大的威胁[57]。目前已有研究证明,金属离子、消毒副产物等环境因素在抗生素抗性基因的扩散中产
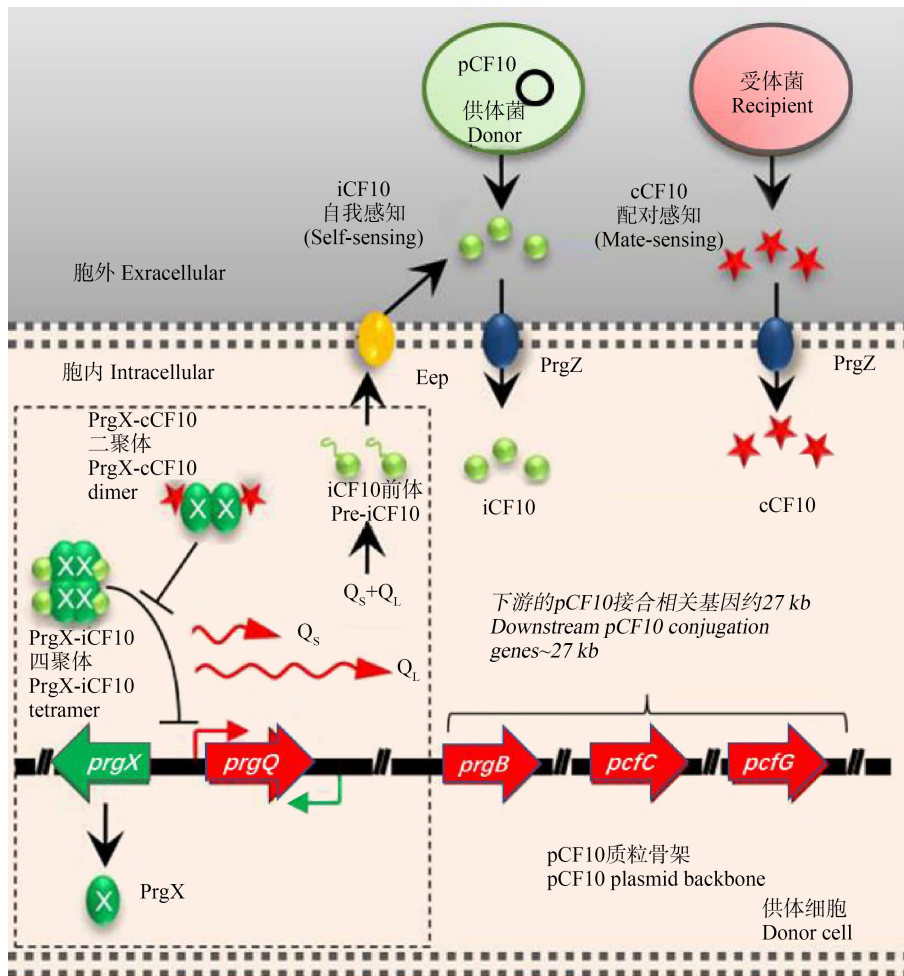
图 2　粪肠球菌中 2 种多肽信号对接合转移的调控机制[47]

注：拮抗信号分子 cCF10 和 iCF10 分别由受体细胞和供体细胞编码，并被供体细胞摄取；在供体细胞中，这些信号分子竞争性地与 PrgX 结合；PrgX 与 iCF10 的复合物抑制 *prgQ* 转录启动，而 PrgX 与 cCF10 的复合物不抑制转录启动；在未诱导的供体细胞中，prgQ 转录产生转录本 Q_S 和 Q_L，二者编码 iCF10 前体，前体被加工成 iCF10 后将分泌到胞外；当 cCF10 浓度足够时，*prgQ* 转录启动，进一步影响下游基因表达。

Fig. 2　Control of conjugation by two peptide signals in *E. faecalis*[47]

Note: Antagonistic signaling molecules cCF10 and iCF10 are released by recipient and donor cells, respectively, and imported into the donor cell; these signals compete for binding to PrgX in donor cells; PrgX-iCF10 complexes repress transcription initiation of *prgQ*, whereas PrgX-cCF10 complexes do not; in uninduced donor cells, transcription of prgQ generates transcripts Q_S and Q_L; both of them encode for the preiCF10 polypeptide, which is processed into iCF10 and secreted into the extracellular; sufficient cCF10 is produced to induce prgQ transcription, encoding downstream genes.

生了巨大影响[58−60]。阐明环境影响因素下耐药基因扩散规律和机制已成为环境科学领域的研究热点，是阻断耐药基因产生和控制耐药基因扩散不可或缺的科学基础。环境因素影响耐药基因扩散揭示了耐药基因水平转移的新途径，同时也提示我们关注环境污染物的生物效应形式和机制，需要我们重新认识其在环境中的行为。例如目前水体环境中广泛存在的具有生物活性或激素效应的环境污染物是否可以模拟信息素效应从而调控粪肠球菌中耐药基因的接合转移过程，还亟待进一步研究。

通讯作者简介：邱志刚(1979—)，男，博士，研究员，主要研究方向为纳米材料生物安全性及细菌耐药基因。

参考文献(References)：

[1]　Madsen K T, Skov M N, Gill S, et al. Virulence factors associated with *Enterococcus faecalis* infective endocarditis: A mini review [J]. The Open Microbiology Journal, 2017, 11: 1-11

[2]　Reigadas E, Rodríguez-Creixems M, Guembe M, et al. Catheter-related bloodstream infection caused by *Enterococcus* spp. [J]. Clinical Microbiology and Infection,

2013, 19(5): 457-461

[3] 陈亚楠, 王亚炜, 魏源送, 等. 不同功能地表水体中病原微生物指示物的标准比较[J]. 环境科学学报, 2015, 35(2): 337-351

Chen Y N, Wang Y W, Wei Y S, et al. Evolution and standard comparison of indicator microorganisms for different surface waters [J]. Acta Scientiae Circumstantiae, 2015, 35(2): 337-351 (in Chinese)

[4] Alipour M, Hajiesmaili R, Talebjannat M, et al. Identification and antimicrobial resistance of *Enterococcus* spp. isolated from the river and coastal waters in northern Iran [J]. The Scientific World Journal, 2014, 2014: 1-5

[5] 李昕, 曾洁, 王岱, 等. 细菌耐药耐受性机制的最新研究进展[J]. 中国抗生素杂志, 2020, 45(2): 113-121

Li X, Zeng J, Wang D, et al. Recent advances in the mechanism of bacterial resistance and tolerance [J]. Chinese Journal of Antibiotics, 2020, 45(2): 113-121 (in Chinese)

[6] Hollenbeck B L, Rice L B. Intrinsic and acquired resistance mechanisms in *Enterococcus* [J]. Virulence, 2012, 3 (5): 421-569

[7] 俞道进, 曾振灵, 陈杖榴. 四环素类抗生素残留对水生态环境影响的研究进展[J]. 中国兽医学报, 2004, 24(5): 515-517

[8] Clewell D B, Victoria Francia M, Flannagan S E, et al. Enterococcal plasmid transfer: Sex pheromones, transfer origins, relaxases, and the *Staphylococcus aureus* issue [J]. Plasmid, 2002, 48(3): 193-201

[9] Wozniak R A F, Waldor M K. Integrative and conjugative elements: Mosaic mobile genetic elements enabling dynamic lateral gene flow [J]. Nature Reviews Microbiology, 2010, 8(8): 552-563

[10] Smillie C, Garcillán-Barcia M P, Francia M V, et al. Mobility of plasmids [J]. Microbiology and Molecular Biology Reviews, 2010, 74(3): 434-452

[11] Partridge S R. Analysis of antibiotic resistance regions in Gram-negative bacteria [J]. FEMS Microbiology Reviews, 2011, 35(5): 820-855

[12] Wardal E, Gawryszewska I, Hryniewicz W, et al. Abundance and diversity of plasmid-associated genes among clinical isolates of *Enterococcus faecalis* [J]. Plasmid, 2013, 70(3): 329-342

[13] Waters C M, Bassler B L. Quorum sensing: Cell-to-cell communication in bacteria [J]. Annual Review of Cell and Developmental Biology, 2005, 21: 319-346

[14] Wardal E, Sadowy E, Hryniewicz W. Complex nature of enterococcal pheromone-responsive plasmids [J]. Polish Journal of Microbiology, 2010, 59(2): 79-87

[15] Inoue D, Sei K, Soda S, et al. Potential of predominant activated sludge bacteria as recipients in conjugative plasmid transfer [J]. Journal of Bioscience and Bioengineering, 2005, 100(6): 600-605

[16] 易秀丽, 俞道进, 黄一帆. 3 种不同水源粪肠球菌耐药情况[J]. 福建农林大学学报: 自然科学版, 2008, 37(1): 92-95

Yi X L, Yu D J, Huang Y F. Drug resistance of *Enterococcus faecalis* in 3 different aquatic environments [J]. Journal of Fujian Agriculture and Forestry University: Natural Science Edition, 2008, 37(1): 92-95 (in Chinese)

[17] 吴丽云, 洪娟, 孙彤, 等. 水源性肠球菌对苯扎溴铵及部分抗菌药的耐药性研究[J]. 中国兽医科学, 2018, 48 (11): 1406-1414

Wu L Y, Hong J, Sun T, et al. Study on resistance of water-borne *Enterococcus* to benzalkonium bromide and antibiotics [J]. Chinese Veterinary Science, 2018, 48 (11): 1406-1414 (in Chinese)

[18] 陈玉红, 肖正润, 林福, 等. 闽江流域水源性肠球菌抗生素耐药与水体氮磷营养盐的相关性研究[C]//中国畜牧兽医学会兽医药理毒理学分会第十一届会员代表大会暨第十三次学术讨论会与中国毒理学会兽医毒理专业委员会第五次学术研讨会论文集. 长沙: 中国畜牧兽医学会, 2015: 221-222

[19] 林云琴, 曾中华, 陈如登. 猪场水源水及管网水肠球菌耐药特性差异研究[J]. 江西农业学报, 2017, 29(8): 99-104

Lin Y Q, Zeng Z H, Chen R D. Difference study on antibiotic-resistant characters of *Enterococcus* from original water and pipe-network water of pig farms [J]. Acta Agriculturae Jiangxi, 2017, 29(8): 99-104 (in Chinese)

[20] Carniol K, Gilmore M S. Signal transduction, quorum-sensing, and extracellular protease activity in *Enterococcus faecalis* biofilm formation [J]. Journal of Bacteriology, 2004, 186(24): 8161-8163

[21] Torres O R, Korman R Z, Zahler S A, et al. The conjugative transposon Tn925: Enhancement of conjugal transfer by tetracycline in *Enterococcus faecalis* and mobilization of chromosomal genes in *Bacillus subtilis* and *E. faecalis* [J]. Molecular and General Genetics, 1991, 225(3): 395-400

[22] Clewell D B. Properties of *Enterococcus faecalis* plasmid pAD1, a member of a widely disseminated family of pheromone-responding, conjugative, virulence elements encoding cytolysin [J]. Plasmid, 2007, 58(3): 205-227

[23] de Boever E H, Clewell D B, Fraser C M. *Enterococcus faecalis* conjugative plasmid pAM373: Complete nucleotide sequence and genetic analyses of sex pheromone re-

sponse [J]. Molecular Microbiology, 2000, 37(6): 1327-1341

[24] 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* [J]. Antimicrobial Agents and Chemotherapy, 2001, 45(7): 2177-2178

[25] Clewell D B. Movable genetic elements and antibiotic resistance in *Enterococci* [J]. European Journal of Clinical Microbiology and Infectious Diseases, 1990, 9(2): 90-102

[26] Johnson C M, Haemig H H A, Chatterjee A, et al. RNA-mediated reciprocal regulation between two bacterial operons is RNase III dependent [J]. mBio, 2011, 2(5): DOI: 10.1128/mbio.00189-11

[27] Shokeen S, Johnson C M, Greenfield T J, et al. Structural analysis of the Anti-Q-Qs interaction: RNA-mediated regulation of *E. faecalis* plasmid pCF10 conjugation [J]. Plasmid, 2010, 64(1): 26-35

[28] Johnson C M, Manias D A, Haemig H A H, et al. Direct evidence for control of the pheromone-inducible prgQ operon of *Enterococcus faecalis* plasmid pCF10 by a countertranscript-driven attenuation mechanism [J]. Journal of Bacteriology, 2010, 192(6): 1634-1642

[29] Ozawa Y, Boever E H D, Clewell D B. *Enterococcus faecalis* sex pheromone plasmid pAM373: Analyses of TraA and evidence for its interaction with RpoB [J]. Plasmid, 2005, 54(1): 57-69

[30] do Carmo de Freire Bastos M, Tomita H, Tanimoto K, et al. Regulation of the *Enterococcus faecalis* pAD1-related sex pheromone response: Analyses of traD expression and its role in controlling conjugation functions [J]. Molecular Microbiology, 1998, 30(2): 381-392

[31] Dunny G M. Enterococcal sex pheromones: Signaling, social behavior, and evolution [J]. Annual Review of Genetics, 2013, 47: 457-482

[32] Bandyopadhyay A, O'Brien S, Frank K L, et al. Antagonistic donor density effect conserved in multiple enterococcal conjugative plasmids [J]. Applied and Environmental Microbiology, 2016, 82(15): 4537-4545

[33] Price V J, Huo W W, Sharifi A, et al. CRISPR-cas and restriction-modification act additively against conjugative antibiotic resistance plasmid transfer in *Enterococcus faecalis* [J]. mSphere, 2016, 1(3): DOI: 10.1128/msphere. 00064-16

[34] Hirt H, Manias D A, Bryan E M, 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 [J]. Journal of Bacteriology, 2005, 187(3): 1044-1054

[35] Kreft B, Marre R, Schramm U, et al. Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured renal tubular cells [J]. Infection and Immunity, 1992, 60(1): 25-30

[36] Dunny G M, Brown B L, Clewell D B. Induced cell aggregation and mating in *Streptococcus faecalis*: Evidence for a bacterial sex pheromone [J]. PNAS, 1978, 75(7): 3479-3483

[37] Varahan S, Harms N, Gilmore M S, et al. An ABC transporter is required for secretion of peptide sex pheromones in *Enterococcus faecalis* [J]. mBio, 2014, 5(5): DOI:10. 1128/mbio.01726-14

[38] Olmsted S B, Kao S M, van Putte L J, et al. Role of the pheromone-inducible surface protein Asc10 in mating aggregate formation and conjugal transfer of the *Enterococcus faecalis* plasmid pCF$_{10}$ [J]. Journal of Bacteriology, 1991, 173(23): 7665-7672

[39] Clewell D B. Tales of conjugation and sex pheromones: A plasmid and enterococcal odyssey [J]. Mobile Genetic Elements, 2011, 1(1): 38-54

[40] Dunny G M, Leonard B A B. Cell-cell communication in Gram-positive bacteria [J]. Annual Review of Microbiology, 1997, 51(1): 527-564

[41] Mori M, Sakagami Y, Ishii Y, et al. Structure of cCF10, a peptide sex pheromone which induces conjugative transfer of the *Streptococcus faecalis* tetracycline resistance plasmid, pCF$_{10}$[J]. The Journal of Biological Chemistry, 1988, 263(28): 14574-14578

[42] Hirt H, Schlievert P M, Dunny G M. *In vivo* induction of virulence and antibiotic resistance transfer in *Enterococcus faecalis* mediated by the sex pheromone-sensing system of pCF$_{10}$ [J]. Infection and Immunity, 2002, 70(2): 716-723

[43] Nakayama J, Ono Y, Suzuki A. Isolation and structure of the sex pheromone inhibitor, iAM373, of *Enterococcus faecalis* [J]. Bioscience, Biotechnology, and Biochemistry, 1995, 59(7): 1358-1359

[44] Flannagan S E, Clewell D B. Identification and characterization of genes encoding sex pheromone cAM373 activity in *Enterococcus faecalis* and *Staphylococcus aureus* [J]. Molecular Microbiology, 2002, 44(3): 803-817

[45] Clewell D B, Pontius L T, An F Y, et al. Nucleotide sequence of the sex pheromone inhibitor (iAD1) determinant of *Enterococcus faecalis* conjugative plasmid pAD1 [J]. Plasmid, 1990, 24(2): 156-161

[46] Mori M, Sakagami Y, Narita M, et al. Isolation and struc-

ture of the bacterial sex pheromone, cAD1, that induces plasmid transfer in *Streptococcus faecalis* [J]. FEBS Letters, 1984, 178(1): 97-100

[47] Chatterjee A, Cook L C C, Shu C C, et al. Antagonistic self-sensing and mate-sensing signaling controls antibiotic-resistance transfer [J]. PNAS, 2013, 110(17): 7086-7090

[48] Chen Y Q, Bandyopadhyay A, Kozlowicz B K, et al. Mechanisms of peptide sex pheromone regulation of conjugation in *Enterococcus faecalis* [J]. MicrobiologyOpen, 2017, 6(4): e00492

[49] Nakayama J, Ruhfel R E, Dunny G M, et al. The prgQ gene of the *Enterococcus faecalis* tetracycline resistance plasmid pCF$_{10}$ encodes a peptide inhibitor, iCF$_{10}$[J]. Journal of Bacteriology, 1994, 176(23): 7405-7408

[50] Bae T, Kozlowicz B, Dunny G M. Two targets in pCF10 DNA for PrgX binding: Their role in production of Qa and *prg*X mRNA and in regulation of pheromone-inducible conjugation[J]. Journal of Molecular Biology, 2002, 315(5): 995-1007

[51] Bensing B A, Meyer B J, Dunny G M. Sensitive detection of bacterial transcription initiation sites and differentiation from RNA processing sites in the pheromone-induced plasmid transfer system of *Enterococcus faecalis* [J]. PNAS, 1996, 93(15): 7794-7799

[52] Leonard B A, Podbielski A, Hedberg P J, et al. *Enterococcus faecalis* pheromone binding protein, PrgZ, recruits a chromosomal oligopeptide permease system to import sex pheromone cCF10 for induction of conjugation [J]. PNAS, 1996, 93(1): 260-264

[53] Fixen K R, Chandler J R, Le T, et al. Analysis of the amino acid sequence specificity determinants of the enterococcal cCF10 sex pheromone in interactions with the pheromone-sensing machinery [J]. Journal of Bacteriology, 2007, 189(4): 1399-1406

[54] Shi K, Brown C K, Gu Z Y, et al. Structure of peptide sex pheromone receptor PrgX and PrgX/pheromone complexes and regulation of conjugation in *Enterococcus faecalis* [J]. PNAS, 2005, 102(51): 18596-18601

[55] Chandler J R, Flynn A R, Bryan E M, et al. Specific control of endogenous cCF10 pheromone by a conserved domain of the pCF10-encoded regulatory protein PrgY in *Enterococcus faecalis* [J]. Journal of Bacteriology, 2005, 187(14): 4830-4843

[56] Bhatty M, Camacho M I, Gonzalez-Rivera C, et al. PrgU: A suppressor of sex pheromone toxicity in *Enterococcus faecalis* [J]. Molecular Microbiology, 2017, 103(3): 398-412

[57] 田英杰, 于慧, 王占黎. 耐万古霉素肠球菌耐药机制及毒力基因分布的研究进展[J]. 现代预防医学, 2018, 45(10): 1895-1899
Tian Y J, Yu H, Wang Z L. Advances in drug resistance mechanism and virulence gene distribution for vancomycin-resistant *Enterococci* [J]. Modern Preventive Medicine, 2018, 45(10): 1895-1899 (in Chinese)

[58] Qiu Z, Yu Y, Chen Z, et al. Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera [J]. PNAS, 2012, 109(13): 4944-4949

[59] Pu Q, Fan X T, Li H, et al. Cadmium enhances conjugative plasmid transfer to a fresh water microbial community [J]. Environmental Pollution, 2021, 268: 115903

[60] Li D, Zeng S Y, He M, et al. Water disinfection byproducts induce antibiotic resistance-role of environmental pollutants in resistance phenomena [J]. Environmental Science & Technology, 2016, 50(6): 3193-3201 ◆