

## *Enterococcus faecalis* Ebp pili are important for cell-cell aggregation and intraspecies gene transfer

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*Enterococcus faecalis* is an opportunistic pathogen that ranks among the leading causes of biofilm-associated infections. We previously demonstrated that the endocarditis- and biofilm-associated pili (Ebp) of *E. faecalis* play a major role in biofilm formation, adherence to abiotic surfaces and experimental infections. In this study, derivatives of *E. faecalis* strain OG1 were engineered to further characterize functions of Ebp pili. Loss of pili resulted in a 36-fold decrease in the number of closely associated cells when OG1RFΔ*ebpABC* was mixed with OG1SSpΔ*ebpABC*, compared with mixing the Ebp<sup>+</sup> parental strains. In addition, using the Ebp<sup>+</sup> parental strains as donor and recipient, we found a statistically significant increase (280–360 %,  $P < 0.05$ ) in the frequency of plasmid transfer versus using Ebp<sup>-</sup> mutants in the conjugation experiments. These results demonstrate a previously unrecognized role of Ebp pili, namely, as important contributors to microscale cell aggregation and horizontal spread of genetic material.

Received 16 December 2015

Revised 25 February 2016

Accepted 10 March 2016

## INTRODUCTION

The endocarditis- and biofilm-associated pili (Ebp) are surface-associated filamentous structures considered to play a pivotal role in *Enterococcus faecalis* virulence (Nallapareddy *et al.*, 2006, 2011b; Kemp *et al.*, 2007; Singh *et al.*, 2007; Nielsen *et al.*, 2012; Sillanpää *et al.*, 2013). Ebp pili are encoded by the ubiquitous *ebpABC-bps* cluster, which codes for the cell wall anchor pilin EbpB, the major shaft pilin EbpC and the fibre tip pilin EbpA. A pilus-specific class C sortase (Bps, for biofilm- and pilus-associated sortase), catalyses the assembly of the structural subunits into pili, before the housekeeping sortase A (SrtA) covalently binds the elongated pili to the cell wall via EbpB (Nallapareddy *et al.*, 2006; Sillanpää *et al.*, 2013). Studies on *ebp* regulation identified the upstream *ebpR* gene (Bourgogne *et al.*, 2007) and the *rnjB* gene (Gao *et al.*, 2010) as activators at the mRNA level, while the Fsr

quorum-sensing system was described as a weak repressor (Bourgogne *et al.*, 2006). In addition, a recent report by Montealegre *et al.* (2015) demonstrated that *E. faecalis* uses the rare initiation codon ATT, found in all *E. faecalis* strains sequenced, as the start codon of the tip pilin EbpA and that this start codon results in reduced expression, at the translational level, relative to an engineered ATG, and negatively affects Ebp-associated functions (Montealegre *et al.*, 2015).

We previously reported that Ebp pili contribute to biofilm formation, adherence to abiotic surfaces (Nallapareddy *et al.*, 2006; Sillanpää *et al.*, 2013) and adherence to platelets (Nallapareddy *et al.*, 2011a), fibrinogen and collagen (Nallapareddy *et al.*, 2011b) of *E. faecalis*, thus supporting the establishment and persistence of this bacterium in clinically important infections. Pili were found to be immunogenic in the human host during infection (Sillanpää *et al.*, 2004), and their contribution to vascular tissue colonization by *E. faecalis* was demonstrated in a rat model of infective endocarditis (Nallapareddy *et al.*, 2006). In addition, deletion of the *ebp* locus resulted in a diminished capacity of *E. faecalis* OG1RF to colonize kidneys and bladders in a murine model of ascending urinary tract

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Abbreviations: Ebp, endocarditis- and biofilm-associated pili; UTI, urinary tract infection.

infection (UTI) and in experimental catheter-associated UTI (CAUTI) (Singh *et al.*, 2007; Nielsen *et al.*, 2012). Monoclonal antibodies raised specifically against the major pilin component EbpC, as well as polyclonal anti-EbpA antibodies, were shown to provide protection against *E. faecalis* CAUTI in mice and infective endocarditis in rats (Flores-Mireles *et al.*, 2014; Pinkston *et al.*, 2014).

Despite the clear role of Ebp in attachment and infection, no studies have yet assessed their contribution to microscale cell aggregation or dissemination of genetic material. We previously noted that attenuation of OG1RF $\Delta$ ebpABC in rat infective endocarditis appeared to be less when using mixed inocula vs mono-inocula, suggesting that piliated cells might entrap non-piliated ones in the vegetation so that more mutant cells were present than when used alone for infection (Nallapareddy *et al.*, 2006). In this study, we employed ebpABC isogenic deletion mutants of *E. faecalis* OG1-derivatives and evaluated the effect of this deletion on the ability of *E. faecalis* to interact with neighbouring cells and to facilitate lateral gene transfer by conjugation.

## METHODS

**Bacterial strains, construction of mutants and growth conditions.** Bacterial strains, mutants and plasmids are listed in Table 1. Unless otherwise specified, *E. faecalis* strain OG1RF (Ebp<sup>+</sup>) (Bourgogne *et al.*, 2008) and its derivative were cultivated in brain heart infusion (BHI) (Becton Dickinson) broth supplemented, when appropriate, with 25 mg fusidic acid l<sup>-1</sup> (Sigma-Aldrich). *E. faecalis* strain OG1SSp (Ebp<sup>+</sup>) (Dunny *et al.*, 1978) and derivatives were cultivated in BHI broth supplemented, when appropriate, with

500 mg spectinomycin l<sup>-1</sup> and 2000 mg streptomycin l<sup>-1</sup>. Liquid cultures were grown statically at 37 °C.

For construction of in-frame ebpABC deletion mutants of *E. faecalis* OG1SSp and SD234 (OG1RF :: gfp) (DebRoy *et al.*, 2012), we used the previously constructed pTEX5606 vector as described by Nallapareddy *et al.* (2011b).

***E. faecalis* cell-to-cell aggregation experiments.** As serum is an environmental condition that elicits pili expression (Nallapareddy *et al.*, 2006, 2011a), *E. faecalis* cells for the cell-aggregation experiments were cultivated at 37 °C in BHI broth supplemented with 40 % horse serum (BHI-S) (Sigma-Aldrich). Cells in mid-exponential phase were mixed 1 : 1, allowed to interact for 2 h at 37 °C and dilutions were then plated for single colonies on BHI agar. Ninety-six apparently single colonies were randomly picked into the wells of 96-well plates containing 200  $\mu$ l BHI broth, grown overnight and then replica plated onto BHI agar supplemented with 25 mg fusidic acid l<sup>-1</sup> and onto BHI agar supplemented with 500 mg spectinomycin l<sup>-1</sup> and 2000 mg streptomycin l<sup>-1</sup> to identify colonies that were actually a mixture of OG1RF and OG1SSp cells. A mixed colony was defined as an apparent single colony able to grow on both selective media, indicating that the 'single' colony arose from a mixture of cells of each phenotype.

In a separate experiment, the presence of mixed colonies was evaluated by combining an equal amount (10<sup>8</sup> c.f.u. ml<sup>-1</sup>) of BHI-S-grown GFP-tagged OG1RF (OG1RF :: gfp) and OG1RF :: gfp $\Delta$ ebpABC cells with either piliated or non-piliated OG1SSp. After 2 h, the mixture was serially diluted, plated on BHI agar and grown overnight. Colonies were imaged using a Gel Doc 2000 System (Bio-Rad) equipped with an UV lamp for GFP detection.

**Conjugation experiments.** For the conjugation assays, donor cells carrying the plasmid pAM $\beta$ 1 (Clewell *et al.*, 1974), a non-aggregation

**Table 1.** Bacterial strains and plasmids used in this study

Strain or plasmid	Description†	Reference(s)
<i>E. faecalis</i>		
OG1RF	Chromosomal Fus <sup>R</sup> Rif <sup>R</sup> ; Ebp <sup>+</sup>	Bourgogne <i>et al.</i> (2008); Murray <i>et al.</i> (1993)
OG1SSp	Chromosomal Spc <sup>R</sup> Str <sup>R</sup> ; Ebp <sup>+</sup>	Dunny <i>et al.</i> (1978)
TX5608	OG1RF $\Delta$ ebpABC; Ebp <sup>-</sup>	Nallapareddy <i>et al.</i> (2011b)
SD234	OG1RF harbouring a chromosomally inserted gfp gene under control of the malM promoter; OG1RF :: gfp	DebRoy <i>et al.</i> (2012)
TX5756	OG1SSp $\Delta$ ebpABC; ebpABC isogenic deletion mutant of OG1SSp; Ebp <sup>-</sup>	This study
TX5761	OG1RF :: gfp $\Delta$ ebpABC; ebpABC deletion mutant of SD234; Ebp <sup>-</sup>	This study
TX5758	OG1RF :: pAM $\beta$ 1; OG1RF harbouring the plasmid pAM $\beta$ 1; Ery <sup>R</sup> Fus <sup>R</sup> Rif <sup>R</sup>	This study
TX5760	OG1RF $\Delta$ ebpABC :: pAM $\beta$ 1; OG1RF $\Delta$ ebpABC harbouring the plasmid pAM $\beta$ 1; Ery <sup>R</sup> Fus <sup>R</sup> Rif <sup>R</sup>	This study
TX5755	OG1SSp :: pAM $\beta$ 1; OG1SSp carrying the plasmid pAM $\beta$ 1; Ery <sup>R</sup> Spc <sup>R</sup> Str <sup>R</sup>	This study
TX5757	OG1SSp $\Delta$ ebpABC :: pAM $\beta$ 1; OG1SSp $\Delta$ ebpABC carrying the plasmid pAM $\beta$ 1; Ery <sup>R</sup> Spc <sup>R</sup> Str <sup>R</sup>	This study
pTEX5606	Vector for ebpABC deletion; carries a mutated pheS* gene that renders cells susceptible to p-chloro-phenylalanine; Ery <sup>R</sup>	Nallapareddy <i>et al.</i> (2011b)
pAM $\beta$ 1	Vector employed for conjugation experiments; Ery <sup>R</sup>	Clewell <i>et al.</i> (1974)

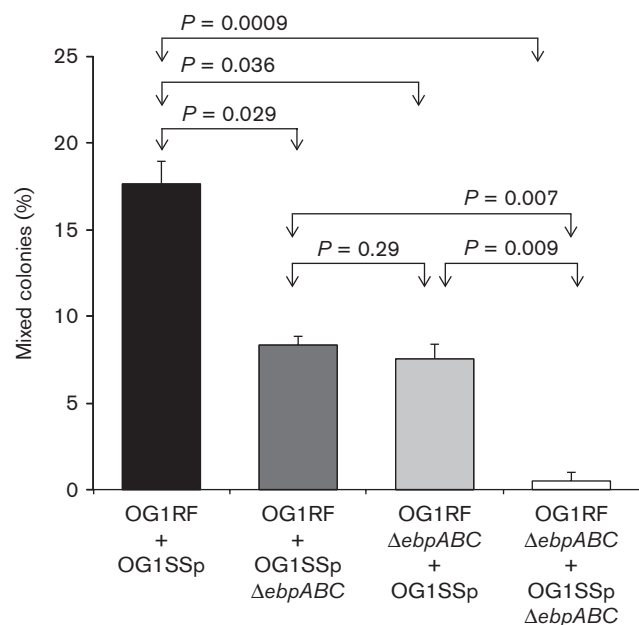
†Ery, Erythromycin; Fus, fusidic acid; Rif, rifampicin, Spc, spectinomycin; Str, streptomycin. Superscript 'R' designates resistance.

substance-producing plasmid, were harvested by centrifugation from exponential phase cultures in BHI-S supplemented with 25 mg erythromycin  $l^{-1}$ . Experiments were done first using OG1RF derivatives as donors and OG1SSp derivatives as recipients. Assays were then repeated using OG1SSp derivatives as donor and OG1RF derivatives as recipients.

After three washes in saline solution to remove the antibiotic, OG1(RF or SSp) : : pAM $\beta$ 1 or OG1(RF or SSp) $\Delta$ ebpABC : : pAM $\beta$ 1 were mixed in a 1 : 10 ratio with either OG1(SSp or RF) or OG1(SSp or RF) $\Delta$ ebpABC and allowed to conjugate in BHI broth for 5 h at 37 °C. The mating mixture was then serially diluted and plated onto selective agar to enumerate transconjugants.

## RESULTS AND DISCUSSION

Previous studies have shown that sortase-assembled pili support bacterial co-aggregation in *Actinobacteria* (Yeung 2000; Turrioni *et al.*, 2013). We therefore evaluated the possible role played by *E. faecalis* Ebp pili in mediating cell-cell interactions. As seen in Fig. 1, when Ebp<sup>+</sup> OG1RF cells were mixed with Ebp<sup>+</sup> OG1SSp cells, 18 % of the resulting apparently 'single' colonies were actually



**Fig. 1.** Contribution of Ebp pili to *E. faecalis* intercellular aggregation leading to mixed colony formation. OG1RF and its Ebp<sup>-</sup> isogenic mutant (TX5608; OG1RF $\Delta$ ebpABC) were tested for the ability to form mixed colonies (co-aggregates) with OG1SSp and a non-piliated OG1SSp derivative (TX5756; OG1SSp $\Delta$ ebpABC). Mixed colonies were identified by replica plating from BHI broth-grown cells inoculated from 'apparent' single colonies onto BHI agar supplemented with 25 mg fusidic acid  $l^{-1}$ , and onto BHI agar supplemented with 2000 mg streptomycin  $l^{-1}$  and 500 mg spectinomycin  $l^{-1}$ . Values represent means  $\pm$  SD of three independent experiments. Statistical analyses were performed by unpaired *t*-test.

'mixed', that is, composed of both OG1RF and OG1SSp cells, while the other colonies were composed of only one of these derivatives. On the other hand, when non-piliated TX5608 (OG1RF $\Delta$ ebpABC) were mixed with non-piliated TX5756 (OG1SSp $\Delta$ ebpABC), only 0.5 % of the resulting single (in appearance) colonies had both RF-resistant cells and SSp-resistant cells ( $P=0.0009$ ), thus indicating a 36-fold reduction for the Ebp<sup>-</sup> Ebp<sup>-</sup> mixture versus the Ebp<sup>+</sup> Ebp<sup>+</sup> mixture. When Ebp<sup>+</sup> cells were combined with cells lacking *ebpABC* (either OG1RF+TX5756 or TX5608+OG1SSp), approximately 8 % of apparently single colonies were in fact a mixture of RF- and SSp-resistant cells ( $P<0.05$  vs Ebp<sup>+</sup> OG1RF+Ebp<sup>+</sup> OG1SSp). Since the *ebpABC* deletion mutants exhibited no differences in growth (data not shown), these results indicate that the lack of pili affects the ability of *E. faecalis* cells to co-aggregate.

Consistent with these data, we observed a similar pattern in the percentage of mixed colonies when combining piliated or non-piliated variants of both OG1SSp and *gfp*-tagged OG1RF cells (Table 2), that is, a higher percentage of colonies in which part of the colony was Gfp<sup>+</sup> and the rest was not (Fig. 2). Hence, these results corroborate the importance of Ebp pili in mediating intercellular aggregation of *E. faecalis*.

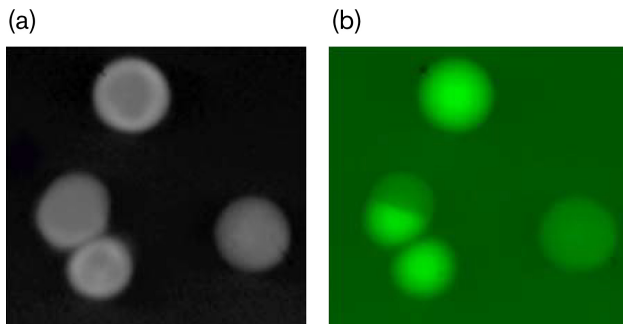
To assess whether co-aggregation mediated by Ebp pili facilitates the transfer of genetic material between cells, we introduced the vector pAM $\beta$ 1, which lacks the aggregation substance that causes cell clumping, into *E. faecalis* OG1RF (resulting in TX5758), and evaluated its transfer to OG1SSp or OG1SSp $\Delta$ ebpABC in 5 h broth mating assays. Transfer of pAM $\beta$ 1 from Ebp<sup>+</sup> OG1RF cells into the Ebp<sup>-</sup> OG1SSp derivative was 170 % less than transfer into Ebp<sup>+</sup> OG1SSp ( $P=0.001$ ) (Fig. 3a). A similar reduction was observed when pAM $\beta$ 1-carrying OG1RF $\Delta$ ebpABC cells (TX5760) were combined with Ebp<sup>+</sup> OG1SSp ( $P=0.03$ ). Most strikingly, absence of pili on the surface of both donor (pAM $\beta$ 1-carrying OG1RF $\Delta$ ebpABC) and recipient (OG1SSp $\Delta$ ebpABC) resulted in a 280 %

**Table 2.** Evaluation of the role of Ebp pili in *E. faecalis* cell-cell adherence by fluorescence imaging of plates containing GFP-tagged and non-fluorescent colonies

GFP-tagged OG1RF (SD234) and OG1RF : : *gfp* $\Delta$ ebpABC (TX5761) cells were mixed with either piliated or non-piliated OG1SSp (TX5756). Values indicate the mean percentage  $\pm$  SD.

Cells mixed	Mixed colonies (%)
OG1RF : : <i>gfp</i> + OG1SSp	12.85 $\pm$ 1.2
OG1RF : : <i>gfp</i> + OG1SSp $\Delta$ ebpABC	7.1 $\pm$ 1.6*
OG1RF : : <i>gfp</i> $\Delta$ ebpABC + OG1SSp	6.8 $\pm$ 1.3*
OG1RF : : <i>gfp</i> $\Delta$ ebpABC + OG1SSp $\Delta$ ebpABC	1.8 $\pm$ 0.9*

\* $P<0.05$  vs OG1RF : : *gfp* + OG1SSp.

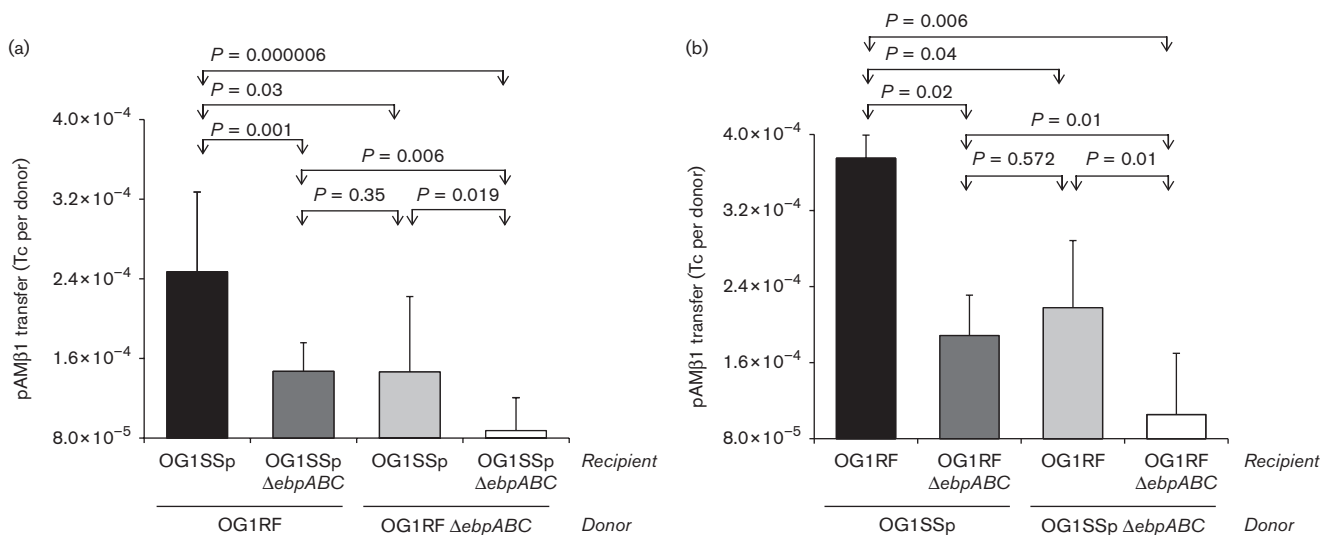


**Fig. 2.** Detection of *E. faecalis* mixed colonies by fluorescence imaging. Colonies were obtained by subculturing from a BHI-S broth culture of the *gfp*-tagged strain (SD234; OG1RF :: *gfp*) grown with a non-*gfp* tagged strain (OG1SSp). Plates were imaged using a Gel Doc 2000 System (Bio-Rad) equipped with an UV lamp for GFP detection. (a) Black and white photo of four colonies and (b) fluorescent picture of the same colonies showing two Gfp<sup>+</sup> colonies, one Gfp<sup>-</sup> colony and one mixed colony with both Gfp<sup>+</sup> and Gfp<sup>-</sup> cells.

reduction in frequency of pAMβ1 transfer compared with the conjugation frequency of the isogenic pilated variants ( $P=0.000006$ ). In addition, we observed similar differences in pAMβ1 transfer frequencies (Fig. 3b) when the donor and recipient were reversed, i.e. when pilated and non-piliated OG1SSp cells were used as donors and OG1RF or OG1RFΔ*ebpABC* as recipients. In particular, a 360 %

increase in conjugation was observed when pilated OG1SSp/RF Ebp<sup>+</sup> Ebp<sup>+</sup> were mixed compared with the OG1SSp/RF Ebp<sup>-</sup> Ebp<sup>-</sup> mixing. On the basis of these results, we conclude that Ebp pili promote plasmid transfer, presumably by facilitating micro-scale cell aggregation, i.e. attachment of cells in close proximity, and by the stabilization of the mating pair.

Previous studies have shown that Ebp pili are major contributors to the ability of *E. faecalis* to adhere to components of the extracellular matrix and to form biofilm, phenotypes linked to various enterococcal infections including endocarditis and UTIs (Sillanpää *et al.*, 2013). When part of a biofilm community, bacteria are less susceptible to the host immune system and to antibiotic treatment; in addition, biofilms are optimal environments for genetic material exchange (Parsek & Singh, 2003). While we previously showed that pili are important for primary attachment to abiotic surfaces (Nallapareddy *et al.*, 2006), our results here imply that a further contribution of pili is the promotion of cell-to-cell adherence at a micro-scale level, so that the pilated cells can attach both pilated and non-piliated ones. Thus, even though *E. faecalis* pili display a bi-phasic expression pattern, with only a portion of the population pilated at any given time (Sillanpää *et al.*, 2013; Pinkston *et al.*, 2014), the presence of pili on at least some cells can still promote accumulation of bacteria at a given site. In such settings, we speculate that the interactions mediated by Ebp pili may represent a driving force for intercellular attachment, colonization of new sites and biofilm formation and for the mobilization of



**Fig. 3.** Transfer frequencies of pAMβ1 in 5 h matings in broth. (a) Pilated or non-piliated OG1RF cells carrying the plasmid pAMβ1 (TX5758 and TX5760, respectively) were mixed with either pilated or non-piliated OG1SSp. (b) Pilated or non-piliated OG1SSp cells carrying the plasmid pAMβ1 (TX5755 and TX5757, respectively) were mixed with either pilated or non-piliated OG1RF. Transfer frequencies are expressed as the number of transconjugants per donor cell (Tc per Donor). Histograms depict the mean and SD of at least three independent experiments. Statistical analyses were performed by unpaired *t*-test.

plasmids carrying selective traits, such as antibiotic resistance or virulence factors, therefore providing *E. faecalis* or other clinically important species with fitness and survival advantages in natural environments. Taking these findings together, the presence of Ebp pili on the surface of *E. faecalis* cells likely has an important impact not only on colonization and adherence but also on bacterial pathogenicity and the spread of antibiotic resistance.

## ACKNOWLEDGEMENTS

We thank Karen Jacques-Palaz and Chungyu Chang for their technical assistance. This work was supported by a grant from the National Institute of Allergy and Infectious Diseases (NIAID) (R01 AI047923) to B.E.M.

## REFERENCES

- Bourgogne, A., Hilsenbeck, S. G., Dunny, G. M. & Murray, B. E. (2006). Comparison of OG1RF and an isogenic *fsrB* deletion mutant by transcriptional analysis: the *Fsr* system of *Enterococcus faecalis* is more than the activator of gelatinase and serine protease. *J Bacteriol* **188**, 2875–2884.
- Bourgogne, A., Singh, K. V., Fox, K. A., Pflughoeft, K. J., Murray, B. E. & Garsin, D. A. (2007). EbpR is important for biofilm formation by activating expression of the endocarditis and biofilm-associated pilus operon (*ebpABC*) of *Enterococcus faecalis* OG1RF. *J Bacteriol* **189**, 6490–6493.
- Bourgogne, A., Garsin, D. A., Qin, X., Singh, K. V., Sillanpää, J., Yerrapragada, S., Ding, Y., Dugan-Rocha, S., Buhay, C. & other authors (2008). Large scale variation in *Enterococcus faecalis* illustrated by the genome analysis of strain OG1RF. *Genome Biol* **9**, R110.
- Clewell, D. B., Yagi, Y., Dunny, G. M. & Schultz, S. K. (1974). Characterization of three plasmid deoxyribonucleic acid molecules in a strain of *Streptococcus faecalis*: identification of a plasmid determining erythromycin resistance. *J Bacteriol* **117**, 283–289.
- DebRoy, S., van der Hoeven, R., Singh, K. V., Gao, P., Harvey, B. R., Murray, B. E. & Garsin, D. A. (2012). Development of a genomic site for gene integration and expression in *Enterococcus faecalis*. *J Microbiol Methods* **90**, 1–8.
- Dunny, G. M., Brown, B. L. & Clewell, D. B. (1978). Induced cell aggregation and mating in *Streptococcus faecalis*: evidence for a bacterial sex pheromone. *Proc Natl Acad Sci U S A* **75**, 3479–3483.
- Flores-Mireles, A. L., Pinkner, J. S., Caparon, M. G. & Hultgren, S. J. (2014). EbpA vaccine antibodies block binding of *Enterococcus faecalis* to fibrinogen to prevent catheter-associated bladder infection in mice. *Sci Transl Med* **6**, 254ra127.
- Gao, P., Pinkston, K. L., Nallapareddy, S. R., van Hoof, A., Murray, B. E. & Harvey, B. R. (2010). *Enterococcus faecalis* *rnjB* is required for pilin gene expression and biofilm formation. *J Bacteriol* **192**, 5489–5498.
- Kemp, K. D., Singh, K. V., Nallapareddy, S. R. & Murray, B. E. (2007). Relative contributions of *Enterococcus faecalis* OG1RF sortase-encoding genes, *srtA* and *bps* (*srtC*), to biofilm formation and a murine model of urinary tract infection. *Infect Immun* **75**, 5399–5404.
- Montealegre, M. C., La Rosa, S. L., Roh, J. H., Harvey, B. R. & Murray, B. E. (2015). The *Enterococcus faecalis* *ebpA* pilus protein: attenuation of expression, biofilm formation, and adherence to fibrinogen start with the rare initiation codon att. *MBio* **6**, e00467–e00415.
- Murray, B. E., Singh, K. V., Ross, R. P., Heath, J. D., Dunny, G. M. & Weinstock, G. M. (1993). Generation of restriction map of *Enterococcus faecalis* OG1 and investigation of growth requirements and regions encoding biosynthetic function. *J Bacteriol* **175**, 5216–5223.
- Nallapareddy, S. R., Singh, K. V., Sillanpää, J., Garsin, D. A., Höök, M., Erlandsen, S. L. & Murray, B. E. (2006). Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *J Clin Invest* **116**, 2799–2807.
- Nallapareddy, S. R., Sillanpää, J., Mitchell, J., Singh, K. V., Chowdhury, S. A., Weinstock, G. M., Sullam, P. M. & Murray, B. E. (2011a). Conservation of Ebp-type pilus genes among Enterococci and demonstration of their role in adherence of *Enterococcus faecalis* to human platelets. *Infect Immun* **79**, 2911–2920.
- Nallapareddy, S. R., Singh, K. V., Sillanpää, J., Zhao, M. & Murray, B. E. (2011b). Relative contributions of Ebp Pili and the collagen adhesin *ace* to host extracellular matrix protein adherence and experimental urinary tract infection by *Enterococcus faecalis* OG1RF. *Infect Immun* **79**, 2901–2910.
- Nielsen, H. V., Guiton, P. S., Kline, K. A., Port, G. C., Pinkner, J. S., Neiers, F., Normark, S., Henriques-Normark, B., Caparon, M. G. & Hultgren, S. J. (2012). The metal ion-dependent adhesion site motif of the *Enterococcus faecalis* EbpA pilin mediates pilus function in catheter-associated urinary tract infection. *MBio* **3**, e00177–e00112.
- Parsek, M. R. & Singh, P. K. (2003). Bacterial biofilms: an emerging link to disease pathogenesis. *Annu Rev Microbiol* **57**, 677–701.
- Pinkston, K. L., Singh, K. V., Gao, P., Wilganowski, N., Robinson, H., Ghosh, S., Azhdarinia, A., Sevick-Muraca, E. M., Murray, B. E. & Harvey, B. R. (2014). Targeting pili in enterococcal pathogenesis. *Infect Immun* **82**, 1540–1547.
- Sillanpää, J., Xu, Y., Nallapareddy, S. R., Murray, B. E. & Höök, M. (2004). A family of putative MSCRAMMs from *Enterococcus faecalis*. *Microbiology* **150**, 2069–2078.
- Sillanpää, J., Chang, C., Singh, K. V., Montealegre, M. C., Nallapareddy, S. R., Harvey, B. R., Ton-That, H. & Murray, B. E. (2013). Contribution of individual Ebp Pilus subunits of *Enterococcus faecalis* OG1RF to pilus biogenesis, biofilm formation and urinary tract infection. *PLoS One* **8**, e68813.
- Singh, K. V., Nallapareddy, S. R. & Murray, B. E. (2007). Importance of the *ebp* (endocarditis- and biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. *J Infect Dis* **195**, 1671–1677.
- Turroni, F., Serafini, F., Foroni, E., Duranti, S., O'Connell Motherway, M., Taverniti, V., Mangifesta, M., Milani, C., Viappiani, A. & other authors (2013). Role of sortase-dependent pili of *Bifidobacterium bifidum* PRL2010 in modulating bacterium-host interactions. *Proc Natl Acad Sci U S A* **110**, 11151–11156.
- Yeung, M. K. (2000). *Actinomyces*: surface macromolecules and bacteria-host interactions. In *Gram-Positive Pathogens*, pp. 583–593. Edited by V. A. Fischetti, R. P. Novick, J. J. Ferretti, D. A. Portnoy & J. I. Rood. Washington, DC: American Society for Microbiology.

Edited by: P. O'Toole