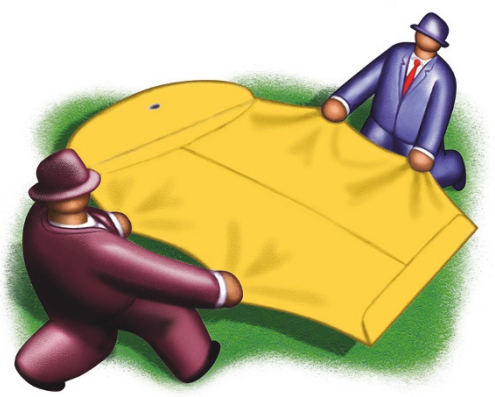
EVOLUTION

Look before you leap



A new study has shown that the genetic background of recipient staphylococcal strains can have an important influence on whether or not the mobile genetic element responsible for methicillin resistance can jump between strains.

Methicillin-resistant *Staphylococcus aureus* (MRSA) are not only the most common cause of nosocomial infections, they are also significant community-acquired pathogens, particularly in children. *S. aureus* resistance to methicillin is conferred by the presence of a modified penicillin-binding transpeptidase, PBP2a, which is encoded by the *mecA* gene. *mecA* is carried on a mobile genetic element known as staphylococcal cassette chromosome *mec* (SCC*mec*), which is integrated into the *S. aureus* chromosome. There are 4 (and perhaps more) main classes of SCC*mec* and, despite the fact that SCC*mec* is mobile, its distribution is narrow and is principally restricted to just 5 clonal complexes worldwide.

What is the role of the genetic background of the staphylococcal host in this restricted clonal distribution? To answer this question, researchers at the University of California, San Francisco constructed two plamids: pYK20, containing the *mecA* gene alone, and pYK644, containing *mecA* and its repressors *mecR1* and *mecI*. Analysis of PBP2a expression revealed that in all 'experienced' staphylococcal host strains — defined as methicillin-sensitive *S. aureus* (MSSA) strains created by the excision of SCC*mec* from the chromosome of MRSA strains — pYK20 was stably maintained. In naive staphylococcal host strains — MSSA strains that have never hosted *mecA* — pYK20 was unstable and PBP2a expression was heterogeneous and varied with the host genotype.

pYK644, by contrast, was stably maintained in naive hosts, implying that the *mecA*-associated regulatory genes can influence the clonal restriction of *mecA* acquisition. In most clinical MRSA isolates, the *mecA* regulatory genes are deleted and *mecA* is regulated by *blaR1* and *blaI*, genes that are homologous to *mecR1*

HIV

Vaginal microflora as HIV inhibitors

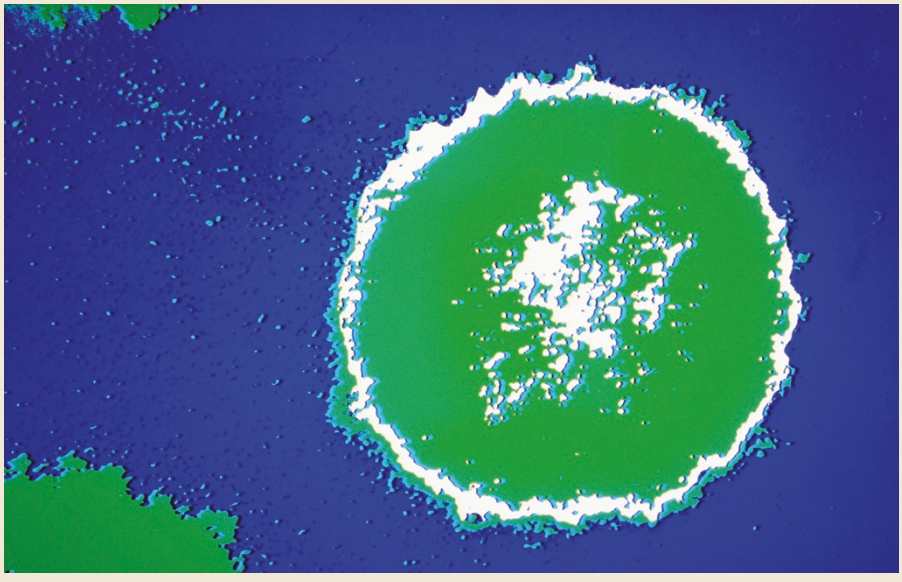
An important proof-of-concept study has shown that is possible to inhibit HIV infectivity by expressing a specific receptor for HIV in a *Lactobacillus* species that is commonly found in the vaginal microflora.

Of the 40 million individuals presently infected with HIV-1, an estimated 80% were infected by heterosexual transmission, with a higher rate of transmission to females than to males. With the continuing absence of an effective vaccine, researchers are turning to novel anti-viral therapies that can empower women to actively protect themselves against infection.

The bacterial microflora present in the vagina of healthy, non-menopausal women is dominated by the genus *Lactobacillus*. Lee and colleagues obtained naturally occurring lactobacilli isolates from vaginal swabs taken from healthy volunteers and, after evaluation for favourable growth and colonization ability, *Lactobacillus jensenii* was selected as the experimental strain.

In this initial study, Lee *et al.* decided to express the HIV-specific CD4 receptor in *L. jensenii*. The coding sequences for the first two extracellular domains of CD4 (2D CD4), which are sufficient for high-affinity binding to the gp120 extracellular glycoprotein of HIV, were modified by PCR to conform to the codon usage in lactobacilli, then cloned into a *Lactobacillus* expression plasmid and protein expression was optimized. A CD4 capture ELISA and HIV-1 gp120-binding assay were used to confirm that the recombinant 2D CD4 was in the correct conformation and was biologically active.

Could the 2D CD4 expressed by *L. jensenii* block the infectivity of HIV? In an HIV entry assay using a reporter virus, 2D CD4 purified from *L. jensenii* and conditioned culture media containing secreted 2D CD4 were both shown to inhibit HIV-1 infection of HeLa cells in a dose-dependent manner. In follow-up assays, Lee *et al.* looked at the effects of co-incubating intact recombinant *L. jensenii* with both a laboratory strain of HIV-1 and a natural strain. The recombinant lactobacilli were shown to inhibit the entry of the laboratory strain by 95% and the natural strain by 55%. As natural strains of HIV-1 are known to be less sensitive to CD4 inhibition, this result is modest yet still statistically significant.



and *mecI*, respectively and which also regulate *blaZ*, the gene encoding β-lactamase. Katayama and colleagues found that the *bla* regulatory regions did have a permissive effect on *mecA* acquisition in naive strains, although this effect was not as strong as that of the *mec* genes.

Horizontal transfer of the *mec* element has long been recognised as a major contributor to the evolution of MRSA strains, yet the clonal restriction of *mecA* distribution has always been puzzling. This work highlights the important contribution of the genetic background of the host staphylococcal strain to this process.

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER Katayama, Y. *et al*. Jumping the barrier to β-lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* **185**, 5465–5472 (2003)

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This *in vitro* demonstration of the inhibition of HIV-1 infectivity by a recombinant constituent of the vaginal microflora is an important proof-of-principle study in the search for novel methods to halt the HIV epidemic. As yet, the engineered lactobacilli are a long way from clinical development. However, Lee and colleagues have already gone on to achieve stable integration of the 2D CD4 coding sequence into the *L. jensenii* chromosome, thereby circumventing the problem that the antibiotic-resistance genes present on the expression plasmid would pose for clinical trials. The fact that in a recent issue of *PNAS* another team report on BMS-378806, a new small-molecule inhibitor that blocks the docking of HIV-1 to CD4, reflects the fact that the efforts of HIV researchers to thwart HIV-1 are not wholly devoted to the search for a vaccine, and all aspects of the infection and transmission cycle are being investigated for potential new targets.

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FUNGAL VIRULENCE

Stuck on you

New research published in Genes and Development from the Cormack lab shows that *Candida glabrata* has at least two groups of adhesin virulence genes located in subtelomeric regions that are regulated by a defined mechanism of transcriptional silencing.

Candida is responsible for 8% of serious nosocomial infections in the United States. In common with other *Candida* species, *C. glabrata* usually populates mucosal surfaces, but can breach the mucosal barrier when host defences are lowered and cause life threatening systemic infections. Unlike the diploid opportunistic pathogen *Candida albicans*, *C. glabrata* is haploid, which makes genetic manipulations far more straightforward. Previous work identified an adhesin protein — Epa1 — which enables *C. glabrata* to stick to epithelial cells. Epa1 is a member of a large class of cell wall proteins that are found in many fungi, including *Saccharomyces* and *Aspergillus* species.

When the *EPA1* gene was deleted from the chromosome, the resulting mutant lost virtually all ability to attach to epithelial cells in the lab. So, *EPA1* was an excellent candidate for a virulence gene. However, when the same mutant was tested for pathogenicity in mouse models of candidiasis it was still virulent. Pathogens like *C. albicans*, *Trypansoma brucei* and *Plasmodium* species commonly encode families of virulence genes. So, one possible solution to this conundrum was that *C. glabrata* might have a family of adhesins. The hunt was on for *EPA1* homologues and now De Las Peñas *et al.* report the characterisation of 5 homologues of *EPA1* in *C. glabrata*.

The 5 *EPA1* homologues are located in two separate subtelomeric clusters. Although *Saccharomyces cerevisiae* and *C. glabrata* have largely similar genetic organisation (with syntenic gene arrangement) the subtelomeric regions, including the *EPA* genes, are completely different. Knocking out both subtelomeric *EPA* clusters reduced virulence of the resultant mutant strain in a mouse model. Genomic sequencing studies have subsequently uncovered additional potential *EPA1* homologues, so the family is growing.

Curiously, De Las Peñas *et al.* found that only *EPA1* was expressed *in vitro*. Not only were the other *EPA* genes repressed, but a marker gene — *URA3* — was repressed when inserted at either subtelomeric locus. So, both subtelomeric *EPA* clusters are regionally silenced. This study also showed that a key component of the transcriptional silencing machinery in *S. cerevisiae* — *SIR3* — regulates transcriptional silencing of the *EPA* genes in *C. glabrata*. Even though the telomere gene complements of these two fungi are different, the gene silencing mechanism is conserved between these species.

The subtelomeric location of the adhesin genes, coupled with a defined silencing mechanism, means that *C. glabrata* is the first pathogen for which the mechanism of transcriptional silencing of virulence factors has been defined. Intriguingly, regulation of subtelomeric silencing in *S. cerevisiae* has been linked to cellular stresses. Since cellular stress is likely to be common during infection, this raises the exciting possibility that *C. glabrata* virulence factors could be switched on and off during infections.

Susan Jones

References and links

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