The *mecA* gene has less nucleotide differences than other MRSA sequences

The *mecA* gene is located on the staphylococcal cassette chromosome (SCC) which is a new class of genetic element that is transferred horizontally (2, 13–16, 21). Only two nucleotide positions vary in the *mecA* gene sequences obtained from MRSA, *Staphylococcus epidermidis* and *Staphylococcus sciuri* strains (Table 1b). The number of differences in the ISR types (31/2645) and the seven housekeeping genes (43/3818) is 15 times greater than in the *mecA* gene sequence (2/2002). Recombination or gene conversion between *rrn* operons on a genome may play a role in homogenizing the sequences of ISR types (5, 17). Similarly, it has been shown that recombination is responsible for SNPs in the seven housekeeping genes of MRSA analysed by MLST (4). If this is the case, the introduction of mutations by recombination may be many times more frequent than during or after the horizontal transfer of SCC*mec*. While it is probable that the introduction of *mecA* into *S. aureus* is a relatively recent event, it is possible that the event is more ancient and its introduction is more likely due to increased transmission in hospitals and by a higher exposure to antibiotics than occurs in nature. However, the low rate of mutation in *mecA* (compared to ISRs and housekeeping genes) must raise the question as to whether there are different mechanisms of mutation in different parts of the bacterial genome.

Measurement of genome evolution in MRSA

It has been proposed that the value and stability of typing and taxonomy of bacteria are highly dependent on mechanisms of genome evolution, with different regions of the bacterial chromosome undergoing different rates of genome evolution (8). The data presented here suggest that at least three mechanisms of genome evolution in MRSA exist including (i) recombination and/or gene conversion of the *rrn* operon resulting in sequence homogeneity in some regions and rearrangements, insertions or deletions in others (5, 17), (ii) recombination of essential housekeeping genes (4) and (iii) horizontal transfer (2, 13, 15, 16). Based on sequence data currently available, the *mecA* gene is a poor candidate for typing because the sequence is highly stable between *S. aureus*, *S. epidermidis* and *S. sciuri*. A more variable region such as the ISR, which may undergo frequent rearrangements, may be more suitable. Some of these questions may be resolved by performing experiments (in *S. aureus*) similar to those performed in *Escherichia coli* to measure the mutation rates within different

genes (18) as a function of bacterial generation times. Analysis of SNPs in different regions of the *S. aureus* genome suggest that different evolutionary mechanisms apply to different regions of the genome and further studies to elucidate these mechanisms may shed light on the origin of MRSA.

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A second type III secretion system in *Burkholderia pseudomallei*: who is the real culprit?

Burkholderia pseudomallei is a Gram-negative motile bacillus that is the causative agent of melioidosis, a severe emerging infection that is endemic in South-East Asia and Northern Australia. Antibiotic therapy of melioidosis is long and difficult, because of the resistance of the bacterium to many antibiotics and a tendency to relapse after recovery from clinical disease (3, 4). Although some potential virulence factors have been suggested, the pathogenesis of the disease is poorly understood (1). Here we report on the presence of a second, *Salmonella* SPI-1-like, type III secretion gene cluster in *B. pseudomallei*.

In more than a dozen major Gram-negative bacterial pathogens of animals and plants, virulence is largely dependent on type III secretion (TTS) systems. The TTS, triggered by a close contact of the bacterium with eukaryotic host cells, involves the assembly of a dedicated secretion/translocation apparatus enabling the injection of pathogenicity (effector) proteins directly into the host cells. The system components are encoded by a set of approximately 20 genes which are usually clustered in the bacterial genome, forming so-called pathogenicity islands (PIs). A major difference between animal and plant pathogens is that the latter interact with the cell cytoplasm by piercing from outside the 200 nm thick plant cell wall, while animal pathogens have to deal with only about 5 nm thick cell membranes. Accordingly, the protein composition and structure of what is often called the 'translocator' seem to be quite different in these two pathogen groups.

Fig. 1. Gene organization of TTS-associated clusters of *B. pseudomallei* and comparison with SPI-1 of *S. typhimurium* and the HRP cluster of *R. solanacearum*. ORFs are represented as arrows according to the pattern code of predicted proteins as indicated. Information on the HRP-like locus of *B. pseudomallei* and the HRP cluster of *R. solanacearum* are available from GenBank (accession nos AF074878 and RSO245811, respectively). ORFs in the new SPI-1-like locus of *B. pseudomallei* are annotated as follows: conserved secretion apparatus components are named Sct (7) or Sct2 when the gene already exists within the HRP-like locus; putative homologues of *Salmonella* Sip, Sic and Sop proteins are designated Bip, Bic and Bop, respectively; other ORFs are annotated by the name of their putative homologue, with a Bpm suffix (e.g. *prgH*_{Bpm}). All putative homologues within the new SPI-1-like locus are from *Salmonella*, with the exception of BpH3 (*Bordetella pertussis*), GacA and PA4094 (*Pseudomonas aeruginosa*), and IcsB (*Shigella flexneri*).

In addition, the effector proteins are species-specific, thus contributing to differences in pathogenicity phenotypes (2, 7).

It has been reported that *B. pseudomallei* contains a cluster of five genes with high similarity to genes of a HRP (hypersensitive response and pathogenicity) locus in the plant pathogen *Ralstonia solanacearum* (10). The same authors recently expanded this locus, identifying several ORFs showing significant similarity with TTS-associated genes of *R. solanacearum*, *Xanthomonas campestris* or *Pseudomonas syringae* (Fig. 1), thus completing the picture of a plant pathogen-like TTS gene cluster in *B. pseudomallei*. Although the role of this locus is unknown, an involvement in either symbiotic or pathogenic bacterium-plant interactions can be speculated. Indeed, the predominant environment

for *B. pseudomallei* is rice fields and its relationship with plants and the rhizosphere has already been suggested (5).

B. pseudomallei is clearly a human pathogen, and it is unexpected that a plant pathogen-like TTS system would be used for human-cell intoxication. We searched the *B. pseudomallei* genome using the BLASTX program with PcrD, a highly conserved component of the secretion apparatus of the human pathogen *Pseudomonas aeruginosa*, as the query sequence. This search, performed on the assembly contigs generated through the *B. pseudomallei* genome sequencing project (www.sanger.ac.uk/Projects/B_pseudomallei), led to the identification of a second cluster of about 25 genes with similarities to TTS systems. The new locus resembles most the TTS-associated SPI-1 of the human/

animal pathogen *Salmonella typhimurium*, in terms of both gene organization (Fig. 1) and sequence similarity (Table 1). Although the G+C content of this 'SPI-1-like' locus is close to that of the core genome of *B. pseudomallei* (about 68 mol%), the presence of sequences similar to transposases at one boundary suggests a possibility of horizontal transfer.

The predicted proteins encoded at the *B. pseudomallei* SPI-1-like locus include the conserved secretion apparatus components [annotated here as Scts, as proposed by Hueck (7)] but also putative homologues of the translocators SipC and SipD and of the effectors SipB and SopE (6). By analogy with their function in *Salmonella* infection, the two latter proteins might be involved in the *B. pseudomallei*-induced apoptosis, cell fusion and actin-associated membrane protrusion, the phenotypes recently reported (8). No putative homologues of other SPI-1 TTS effectors SipA, SopA, SopB, SptP and AvrA (6) were found in the *B. pseudomallei* genome. It is possible that genes encoding additional TTS-delivered effectors are scattered throughout the *B. pseudomallei* genome and await molecular characterization.

B. pseudomallei is the first bacterial pathogen apparently armed with one TTS system dedicated to infecting animal cells and another one to interact with plants. This represents a novel level of diversity and complexity in the bacterial world, and challenges us to elucidate the respective origins and roles of these two TTS systems.

In several animal pathogens, mutants deficient in TTS have been shown to be avirulent or attenuated in their ability to provoke disease in animal models, thus making TTS-secreted proteins attractive targets for antimicrobial therapy and vaccine development (9).

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Table 1. Relatedness of *B. pseudomallei* predicted proteins to proteins encoded within the SPI-1 locus of *S. typhimurium* and to *B. pseudomallei* putative proteins from the HRP-like cluster, and the relatedness of *B. pseudomallei* HRP-like putative proteins to proteins encoded by the HRP cluster of *R. solanacearum*

Relatedness is measured as percentage of identical amino acid matches (percentage of similar amino acid matches).

<i>S. typhimurium</i> SPI-1 cluster	<i>B. pseudomallei</i> SPI-1-like cluster	<i>B. pseudomallei</i> HRP-like cluster	<i>R. solanacearum</i> HRP cluster
InvF	33 (48)	InvF _{Bpm}	
InvG	38 (52)	SctC2	26 (43)
InvE	30 (44)	SctW	SctC
InvA	55 (72)	SctV2	36 (56)
InvB	26 (44)	InvB _{Bpm}	SctV
InvC	54 (66)	SctN2	45 (62)
SpaO	24 (37)	SctQ2	21 (33)
SpaP	58 (75)	SctR2	35 (60)
SpaQ	54 (77)	SctS2	33 (57)
SpaR	38 (55)	SctT2	24 (36)
SpaS	43 (61)	SctU2	25 (44)
SicA	58 (72)	BicA	SctU
SipB	31 (46)	BipB	
SipC	16 (30)	BipC	
SipD	22 (36)	BipD	
PrgH	20 (33)	PrgH _{Bpm}	
PrgI	52 (62)	SctF	
PrgJ	28 (44)	SctI	
PrgK	39 (55)	SctJ2	24 (41)
		SctJ	48 (62)
			HrcC
			HrcV
			HrcN
			HrcQ
			HrcR
			HrcS
			HrcT
			HrcU
			HrcJ

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Lipoxygenase in bacteria: a horizontal transfer event?

Lipoxygenase (LOX; EC 1.13.11.12) is a non-haem iron-containing dioxygenase that catalyses the addition of molecular oxygen to polyunsaturated fatty acids with a (Z,Z)-1,4-pentadiene system to give an unsaturated fatty

acid hydroperoxide. The oxygen can be added to either end of the pentadiene system (regio-specificity).

In plants, linoleic and linolenic acids are the most abundant fatty acids and the principal substrates of LOX. Its products have several functions which include mediators of the stress response and products with bactericidal activity (2). In animals the predominant substrate of LOX is arachidonic acid. Its products include bioregulators, mainly prostaglandins, thromboxanes and leukotrienes, with a role in the maintenance of the homeostasis of the animal cell (4).

Until now, LOX had been found in plants, fungi and animals, but it was not known to be present in yeast and bacteria. A careful search of reported LOX sequences showed two bacterial LOXs, one from *Pseudomonas aeruginosa* (accession no. AE004547) annotated as a putative LOX, and the other from *Sorangium cellulosum* (accession no. AX024393). The sequences of *S. cellulosum* and *P. aeruginosa* were obtained with the BLAST search program of the bacterial database (<http://www.ncbi.nlm.nih.gov/BLAST/>), using as template the *Arabidopsis thaliana* LOX2 (1). The identification of a LOX gene in *P. aeruginosa* may not be surprising, since LOX activity has been reported in the periplasmic

space of *Pseudomonas* (3). Besides, for *S. cellulosum*, an L-dopa decarboxylase has been reported that was thought to be present only in eukaryotes (6) and LOX seems to be another example of a eukaryote-related gene present in this bacterium.

Although LOX activity has not been demonstrated for the specific sequences of *S. cellulosum* and *P. aeruginosa*, results from primary amino acid sequence analysis showed that the residues involved in the iron binding of soybean L-1 [His499, His504, His690, Asn694 and Ile839 (5)], are conserved in an equivalent position in *S. cellulosum* and *P. aeruginosa* LOXs, suggesting that the active site of bacterial and plant LOX is similar (Fig. 1a). The comparison of the *S. cellulosum* sequence with other LOXs reveals 39.7% and 42.3% similarity with soybean L-3 and human LOX5, respectively. *P. aeruginosa* LOX has 36.3%, 36.3% and 36.5% similarity with potato H3 LOX, human LOX5 and *S. cellulosum* LOX, respectively.

A phylogenetic tree was constructed from the aligned protein sequences of LOX from different origins: 19 plant LOXs with 13-, 13/9-, and 9-LOX proven activities, three human LOXs with 5-, 12-, and 15-LOX activity (accession numbers P09917, O75342, O15296, respectively); a red alga *Porphyra purpurea* LOX (accession number U08842); and *P. aeruginosa* and *S. cellulosum* LOXs. The alignment used for the phylogenetic analysis was generated with the CLUSTALW program (<http://www.ebi.ac.uk/clustalw/>). The tree was constructed with the PAUP search program (Wisconsin Package Version 10.1, Genetics Computer Group, Madison, WI), and drawn with the TREEVIEW program. This tree is the best tree by parsimony and it was also the shortest distance tree. Results showed that plant LOXs can be divided into five groups representing four different plant families: Solanaceae, Fabaceae, Curcubitaceae, Poaceae and, in a different group, chloroplastic LOXs from several plant families. There is not phylogenetic relationship between the plant LOXs based on regiospecificity. Bacterial and red algal LOXs were grouped with human LOXs. This result indicates that bacterial and red algal LOXs appear to be more closely related to human than to plant LOXs (Fig. 1b). Two methods (parsimony and distance) agree on the tree topology with moderate to high bootstrap support from 1000 replicas. The presence of LOX gene in *S. cellulosum* and *P. aeruginosa* may be interpreted as separate events of horizontal transfer. It is not an artefact of the phylogenetic method, because different methods agree, and there is statistical support. Furthermore, the BLAST search did not report another LOX sequence from other reported bacterial genomes. A horizontal gene transfer has been also suggested for the L-dopa decarboxylase gene of *S. cellulosum* (6).

(a)

S. cellulosum 26
 LOX5 Human 26
P. aeruginosa 27
 L-1 *G. Max* 107

S. cellulosum 44
 LOX5 Human 110
P. aeruginosa 134
 L-1 *G. Max* 214

S. cellulosum 96
 LOX5 Human 208
P. aeruginosa 209
 L-1 *G. Max* 321

S. cellulosum 193
 LOX5 Human 304
P. aeruginosa 307
 L-1 *G. Max* 427

S. cellulosum 295
 LOX5 Human 402
P. aeruginosa 411
 L-1 *G. Max* 533

S. cellulosum 400
 LOX5 Human 509
P. aeruginosa 509
 L-1 *G. Max* 639

S. cellulosum 502
 LOX5 Human 594
P. aeruginosa 598
 L-1 *G. Max* 740

S. cellulosum 582
 LOX5 Human 674
P. aeruginosa 685
 L-1 *G. Max* 839

(b)

Fig. 1. (a) Alignment of *Glycine max* (*G. max*), *S. cellulosum*, *P. aeruginosa* and human LOX5. Numbers in the right margin refer to nucleotide residues. The asterisks indicate residues involved in iron-atom binding at the active site of LOX (5). (b) Phylogenetic tree of LOX amino acid sequences including 19 plant LOXs, human 5-, 12- and 15-LOXs, red alga, and bacterial LOXs. This tree is the best tree by parsimony and it was also the shortest distance tree. The numbers indicate the bootstrap values (parsimony/distance) from 1000 replicas.

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