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Wall teichoic acid structure governs horizontal gene transfer between major bacterial pathogens

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Mobile genetic elements (MGEs) encoding virulence and resistance genes are widespread in bacterial pathogens, but it has remained unclear how they occasionally jump to new host species. *Staphylococcus aureus* clones exchange MGEs such as *S. aureus* pathogenicity islands (SaPIs) with high frequency via helper phages. Here we report that the *S. aureus* ST395 lineage is refractory to horizontal gene transfer (HGT) with typical *S. aureus* but exchanges SaPIs with other species and genera including *Staphylococcus epidermidis* and *Listeria monocytogenes*. ST395 produces an unusual wall teichoic acid (WTA) resembling that of its HGT partner species. Notably, distantly related bacterial species and genera undergo efficient HGT with typical *S. aureus* upon ectopic expression of *S. aureus* WTA. Combined with genomic analyses, these results indicate that a 'glycocode' of WTA structures and WTA-binding helper phages permits HGT even across long phylogenetic distances thereby shaping the evolution of Gram-positive pathogens.

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Major parts of bacterial genomes consist of genetic material originating from other organisms. Many of these elements can be mobilized and exchanged by horizontal gene transfer (HGT) thereby shaping bacterial genome plasticity and permitting rapid adaptation to changing environmental challenges[1]. HGT of mobile genetic elements (MGEs) usually occurs at high frequency only among closely related bacterial clones because the transfer mechanisms, phage-mediated transduction or plasmid conjugation, rely on specific recognition of cognate recipient strains[1,2]. However, HGT also occurs between members of different species or even genera albeit with lower frequency. Such rare events are responsible for the import of new genes into the species' genetic pool along with the emergence of new phenotypic properties; they are particularly important for evolution of new bacterial pathogen lineages with new virulence and antibiotic resistance traits.

The major human pathogen *Staphylococcus aureus* represents a paradigm for studying the roles of 'short-distance' HGT between strains of the same species and 'long-distance' HGT with other species or genera. MGEs and non-mobile genomic islands constitute *ca.* 22% of the *S. aureus* genomes and govern the virulence and colonization capacities, host-specificity and antibiotic resistance of the various clonal complexes[3,4]. Methicillin-resistant *S. aureus* carrying staphylococcal cassette chromosomes with *mecA* genes represent the most frequent cause of severe community- or healthcare-associated infections in many developing and developed countries[5,6]. While conjugation and uptake of naked DNA by natural transformation seem to occur

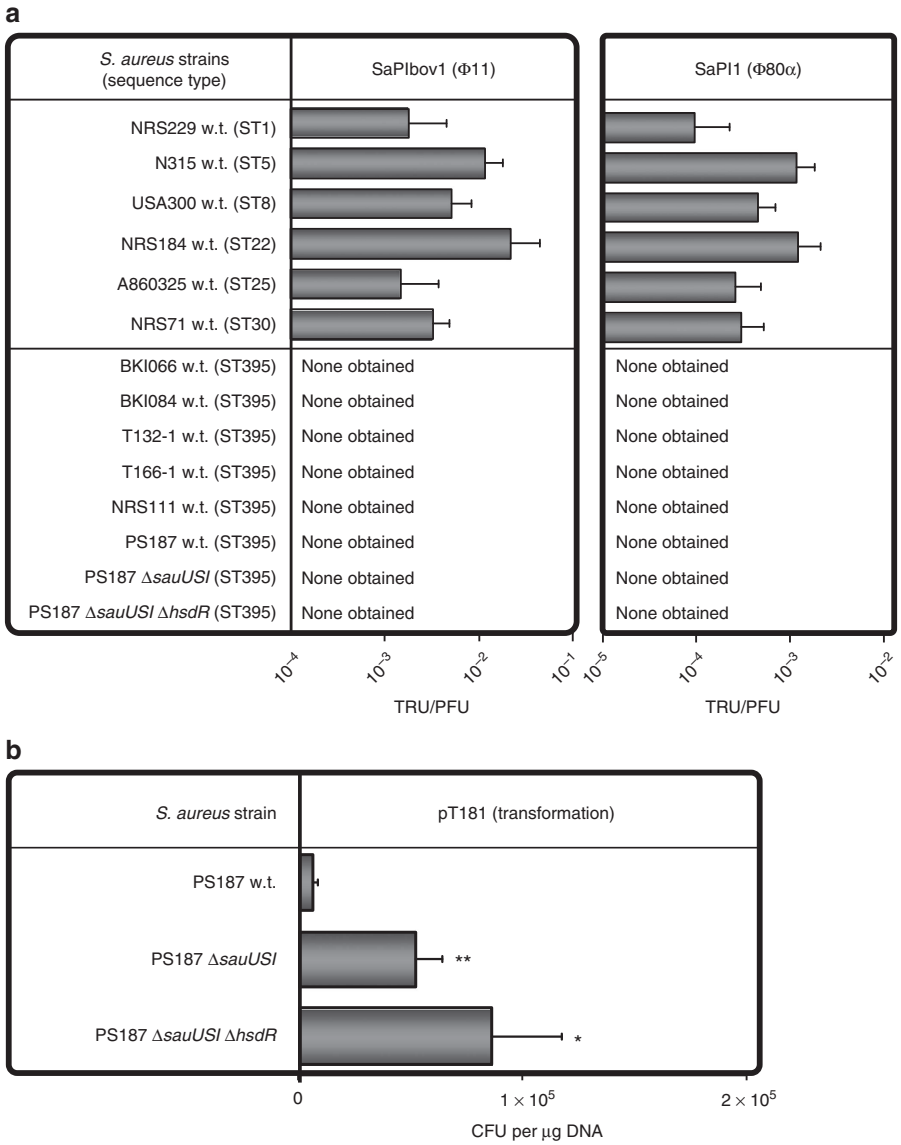

Figure 1 | ST395 is resistant to Φ11 and Φ80α-mediated HGT of SaPIs. (**a**) Various *S. aureus* sequence types and ST395 mutants lacking restriction modifications systems SauUSI or SauUSI plus HsdR were analysed for capacities to acquire SaPIbov1 or SaPI1 via helper phages Φ11 or Φ80α, respectively. SaPI donor strains were JP1794 (SaPIbov1) and JP3602 (SaPI1). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on *S. aureus* RN4220 w.t.) given as means ($n = 3$) ± s.d. No TRU were observed in controls lacking phages or SaPI particles. (**b**) *S. aureus* PS187 w.t. and mutants lacking restriction modification systems were analysed for capacities to acquire the tetracycline resistance plasmid pT181 via electroporation. Values represent the CFU per microgram DNA and given as means ($n = 3$) ± s.d. CFU, colony forming units. No CFU were observed in controls lacking donor DNA. Δ*hsdR*, no type I restriction modification system. Δ*sauUSI*, no type IV restriction modification system, lacks SAOUHSC_02790 homologue of NCTC8325. Statistically significant differences compared with wild type (w.t.) calculated by the unpaired two-tailed Student's *t*-test are indicated: NS, not significant, $P > 0.05$; *$P < 0.01$ to < 0.05; **$P < 0.001$ to 0.01; ***$P < 0.001$.

rarely[4,7], staphylococcal HGT of MGEs is generally believed to depend largely on transducing helper phages[4]. Certain temperate phages of serogroup B such as Φ11 or Φ80α have been shown to be capable of transducing DNA between *S. aureus* clones and to employ the *N*-acetyl-D-glucosamine (GlcNAc) residues on wall teichoic acid (WTA), a surface-exposed glycopolymer, as receptor[4,8]. WTA is produced by most Gram-positive bacteria and usually has species- or strain-specific structure[9]. *S. aureus* produces a WTA polymer composed of ca. 40 ribitol-phosphate (RboP) repeating units modified with α- and/or β-linked GlcNAc and D-alanine[9,10] while the various coagulase-negative staphylococcal species (CoNS) produce WTA with glycerophosphate (GroP) or hexose-containing, complex repeating units modified with different types of sugars[11].

S. aureus pathogenicity islands (SaPIs) are exchanged among *S. aureus* lineages with high frequency by SaPI particles consisting of SaPI genomes and structural proteins from helper phages[12,13]. While such 'short-distance' HGT events occur with high frequency, antibiotic resistance-mediating MGEs have been acquired only occasionally from other bacterial species. Of note, β-lactam antibiotic resistance genes from CoNS have frequently been imported into *S. aureus*[14] whereas enterococcal vancomycin resistance genes have emerged in staphylococci only in a few exceptional cases[15] suggesting that there are mechanisms involved that favour or disfavour specific 'long-distance' HGT events. Restriction modification systems[16–18] and rarely occurring clustered, regularly interspaced, short palindromic repeat (CRISPR) sequences[19] have been shown to interfere with HGT efficiency in staphylococci but the major determinants permitting HGT with other bacterial species and genera have remained unknown.

We demonstrate here that the variable structure of glycosylated WTA constitutes a 'glycocode' that is sensed by transducing phages thereby defining the routes of HGT. Similar WTA structures enable DNA exchange via helper bacteriophages even across the boundaries of species or genera, whereas *S. aureus* clones producing altered WTA become separated from the species' genetic pool and may initiate new routes of HGT with other bacterial species and genera that share related WTA. Thus, related WTA structures are sufficient to initiate HGT even across long phylogenic distances.

Results

ST395 cannot undergo HGT with other *S. aureus* lineages. The various *S. aureus* clonal complexes differ largely in their epidemic potential and number of MGEs[4]. We compared several *S. aureus* lineages for capability to acquire SaPI1 or SaPIbov1 originating from sequence types ST8 and ST151, respectively[13]. Derivatives of these SaPIs with antibiotic resistance gene markers[20] were transferred from *S. aureus* ST8 to a variety of potential recipient strains using helper phages Φ11 (for SaPIbov1) or Φ80α (for SaPI1). The majority of the sequence types acquired SaPIs albeit with varying efficiency, probably as a consequence of different restriction modification systems[16–18] (Fig. 1a). In contrast, several independent clones of the ST395 lineage from various parts of the world including isolates from the lung or blood stream infections and nasal swabs (Supplementary Table S1)[21–23] were completely resistant to HGT of SaPIs (Fig. 1a). Restriction modification systems were obviously not responsible for HGT resistance of ST395 because consecutive inactivation of the genes for type I (Δ*hsdR*) and type IV (Δ*sauUSI*) restriction systems in the ST395 strain PS187 did not enable transfer of SaPIs (Fig. 1a), whereas it considerably increased the rates of plasmid electroporation (Fig. 1b).

Φ187 mediates HGT between ST395 and other bacterial species. While strain PS187 was resistant to infection by Φ80α or Φ11 (Supplementary Fig. S1a), it has previously been shown to be susceptible to phage Φ187 (refs 21,24). Interestingly, most ST395

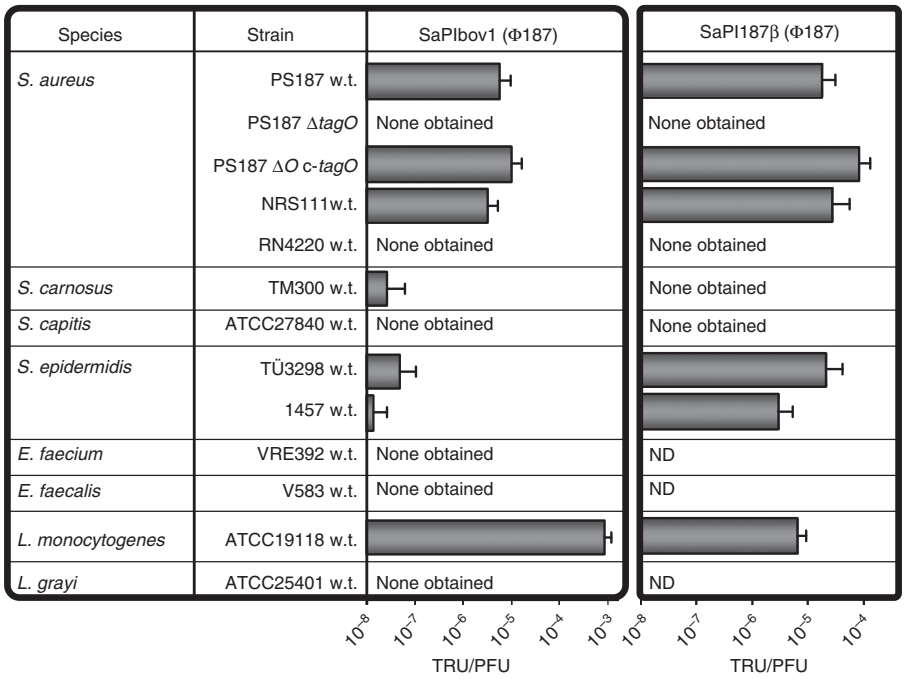

Figure 2 | Φ187 mediates HGT of SaPIs among ST395 and to other species and genera. *S. aureus* ST395 strains with or without WTA or other Gram-positive bacteria were analysed for capacities to acquire SaPIbov1 or SaPI187β via helper phage Φ187. SaPI donor strains were VW1 (SaPIbov1) and VW7 (SaPI187β). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on *S. aureus* PS187 w.t.) given as means ($n = 3$) ± s.d. No TRU were observed in controls lacking phages or SaPI particles. Δ*tagO*, no WTA. c-*tagO*, Δ*tagO* complemented with *tagO*. ND, not determined due to marker resistance or spontaneous occurring clones.

but none of the other *S. aureus* sequence types could be infected by Φ187 (Supplementary Figs S1a and S2a). When Φ187 was analysed for its capacity to transfer MGEs, it was found to facilitate indeed the exchange of SaPI187β (found in the PS187 genome, see below) and SaPIbov1 between different ST395 isolates but not to other *S. aureus* sequence types (Fig. 2). Surprisingly, it also mediated HGT of SaPIbov1 and SaPI187β from ST395 to the CoNS species *Staphylococcus epidermidis* and *Staphylococcus carnosus* and even to *Listeria monocytogenes* serotype 4e (Fig. 2). Thus, ST395 can participate in HGT with other species and genera while it is separated from phage-dependent HGT with typical *S. aureus*.

ST395 has a unique WTA gene cluster and WTA structure. PS187 was sequenced to obtain a prototype ST395 genome. The draft sequence was assembled into 16 large contigs plus two plasmids encompassing 2,529 open reading frames along with 43 and 10 coding sequences for transfer RNAs and ribosomal ribonucleic acid RNAs, respectively. PS187 was found to be a true *S. aureus* but to branch deeply in the *S. aureus* lineage (Fig. 3a). It did not encode CRISPR determinants indicating that the unusual HGT behaviour of ST395 is probably not mediated by 'adaptive immunity' to foreign DNA[19]. Among several unusual MGEs (see below) PS187 contained a novel genomic element, which encompassed transposon-related sequences plus four genes with similarity to WTA-biosynthetic genes (*tagV, tagN, tagD, tagF*) (Fig. 3b). Of note, the new element replaced the 11-kb *tarIJLFS* cluster for biosynthesis of GlcNAc-modified RboP (RboP-GlcNAc) WTA[10], which is found in all other so far known *S. aureus* genomes. Different ST395 clones were found to be related because they exhibited similar DNA fragment patterns in pulsed field gel electrophoreses albeit with some variation in the size of certain DNA bands (Supplementary Fig. S2b). Moreover, all of the 10 available ST395 isolates were tested positive for the *tagN* gene encoded on the new genetic element indicating that the new WTA gene cluster is probably a common feature in the ST395 lineage (Supplementary Fig. S2c). WTA was isolated from PS187 and NMR-based structural elucidation demonstrated that PS187

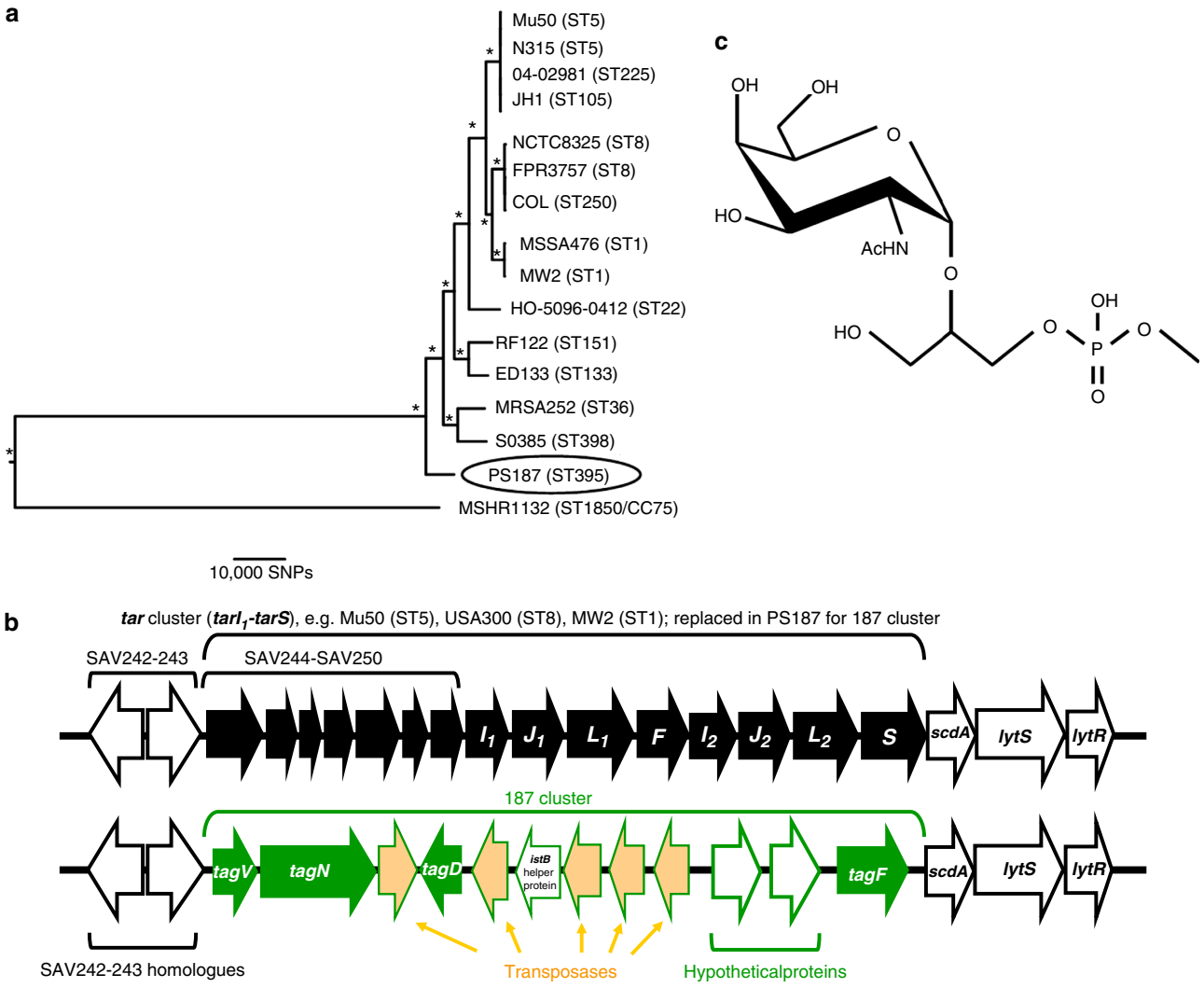

Figure 3 | ST395 branches deeply from other *S. aureus* lineages and bears a novel WTA type and WTA gene cluster. (**a**) Phylogenetic relationships of *S. aureus* sequence types based on DNA sequences from 1,147 orthologous genes. The genome of *S. epidermidis* RP62A was used for rooting the tree. Asterisks indicate 100% branch support in both, the maximum-likelihood tree and the Bayesian maximum clade credibility tree. (**b**) ST395 bears a novel WTA-biosynthetic gene cluster. Genetic organization of the RboP-GlcNAc WTA-biosynthetic *tar* cluster found in all *S. aureus* genomes (upper cluster) that is replaced by a new gene cluster containing putative WTA-biosynthetic genes (green) in ST395 strain PS187 (lower cluster). Gene locus numbers are indicated. Protein sequence alignments of the characteristic ST395 WTA proteins with homologues from CoNS can be found in Supplementary Fig. S6. (**c**) The chemical structure of the WTA repeating unit of *S. aureus* strain PS187 WTA (GroP-α-D-Gal*p*NAc).

produces a unique WTA type with an *N*-acetyl-D-galactosamine (GalNAc) modified GroP backbone (GroP-GalNAc) (Fig. 3c; Supplementary Fig. S3a and Supplementary Table S2), which was in agreement with an earlier analysis of PS187-related strains[11].

Some unusual MGEs from ST395 may be originating from CoNS. Because of its inability to exchange DNA with typical *S. aureus*, we assumed that ST395 may be genetically isolated. ST395 clones have been occasionally described as human commensals and invasive pathogens[22,23] and a recent study has found that ST395 accounts for 5 and 2% of *S. aureus* nasal and blood culture isolates, respectively, in Northeastern Germany[23]. However, the actual numbers may be higher because ST395 isolates are usually methicillin susceptible and such clones are hardly collected and typed. Interestingly, early reports from the 1960s have pointed to a canine reservoir of PS187-related *S. aureus*[25]. In accord with this notion, we found the sequences of some of the host range-determining proteins of PS187 (IsdB and VwBP)[26] to differ from typical human strains whereas the presence of the strictly human-specific *chp, scn* and *sak* genes[26] suggests that PS187 is at least in part adapted to the human host.

Most MGEs of PS187 were distinct in synteny and composition from those found in other *S. aureus* genomes and some were even more related to MGEs found in CoNS. The genomic islands vSAα and vSAβ found in all previously sequenced *S. aureus* genomes were also present in PS187 but lacked typical enterotoxin or lantibiotic gene clusters, respectively (Fig. 4a and Supplementary Fig. S4). Two novel PS187 SaPIs named SaPI187α and SaPI187β

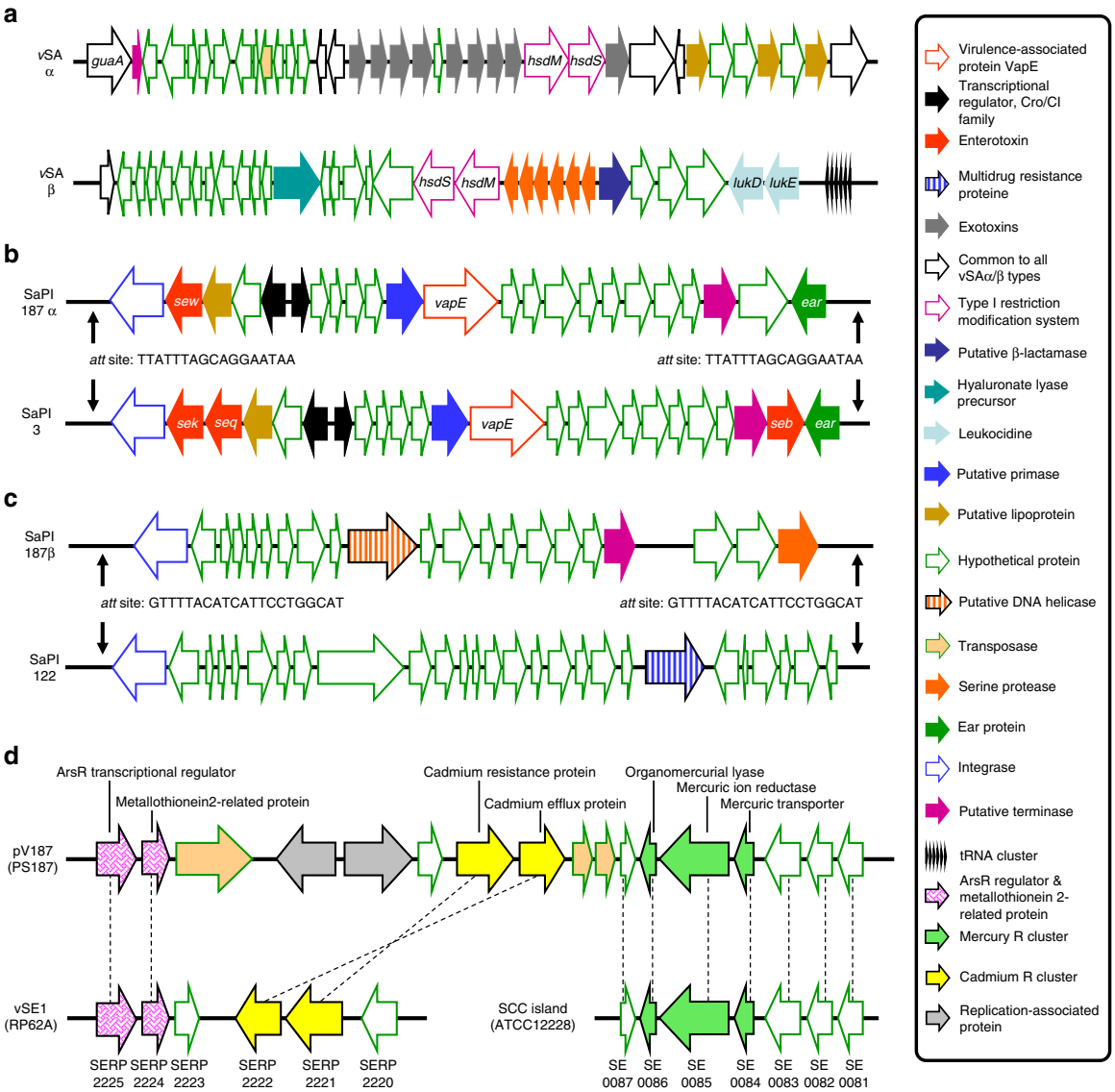

Figure 4 | Characteristic MGEs of *S. aureus* PS187. (**a**) Genetic organization of the two genomic islands found in PS187. The organization of PS187 vSAα is similar to that of other *S. aureus* genomes. The vSAβ of PS187 lacks typical enterotoxin or lantibiotic clusters found in all other vSAβ types sequenced so far. (**b**) Comparison of SaPI187α from PS187 and SaPI3 from typical *S. aureus* genomes. SaPI187α shares the *att* site with SaPI1, SaPI3 and SaPI5 and was found to be similarly organized as SaPI3 from *S. aureus* Col. Note the presence of a novel enterotoxin in SaPI187α designated as enterotoxin SeW. (**c**) Comparison of SaPI187β from PS187 and SaPI122 from *S. aureus* RF122. Although SaPI187β shares the *att* site with SaPI122 the SaPI122-characteristic gene encoding a multidrug resistance protein is absent in SaPI187β. Instead SaPI187β encodes a new serine protease. (**d**) Comparative map of mercury and cadmium resistance operons on a large plasmid from PS187 and corresponding genes from the *S. epidermidis* ATCC12228 SCC composite island (right) and the *S. epidermidis* RP62A integrated plasmid vSE1 (left). Dotted lines indicate sequence identities above >92% (DNA level) and 93% (protein level). Gene locus numbers are listed and ORFs are coloured according to functional categories as indicated.

shared attachment (*att*) sites and some similarity with SaPI3 and SaPI122, respectively[13], but differed with regard to enterotoxin, multidrug resistance transporter and serine protease genes (Fig. 4b,c and Supplementary Fig. S4). PS187 was found to encode two cryptic prophages named ΦPS187a and ΦPS187b (Supplementary Fig. S4), which were similar to previously described *S. aureus* phages Φ77 (ref. 8) and Φ187 (refs 21,24) and were integrated in the genes for sphingomyelinase (*hlb*) and the giant surface protein Ebh, respectively. However, both prophages were defective as no infective phages could be obtained from PS187 upon treatment with the prophage-inducing agent mitomycin C (Supplementary Fig. S5). Φ77 was found to have entirely different host binding specificities from Φ187 with efficient adsorption to *S. aureus* with RboP-GlcNAc WTA and inefficient binding to bacteria with other WTA types such as ST395 (Supplementary Fig. S1b) suggesting that the defective Φ77-related prophage of PS187 may have originated from an ancient HGT event before the new WTA-biosynthetic genes had been acquired.

Notably, some of the PS187 MGEs shared higher similarity with genes from CoNS than from other *S. aureus*. This was found for mercury and cadmium resistance operons on a large plasmid, which were most similar to corresponding genes from an *S. epidermidis* SCC island[27] and the chromosomally integrated *S. epidermidis* plasmid vSe1[28] (Fig. 4d), respectively, and for the new WTA-biosynthetic genes, whose products exhibited highest similarity with proteins from *Staphylococcus pseudintermedius* (TagV), *S. carnosus* (TagN), *Staphylococcus lugdunensis* (TagD), and *Staphylococcus simiae* (TagF) (Supplementary Fig. S6). Thus, the genome sequence confirms that ST395 clones are separated from frequent HGT with typical *S. aureus* but have access to MGEs from CoNS.

SaPI particles adopt receptor requirements of helper phages. The unusual WTA structure of PS187 resembled that of the CoNS strains accepting SaPI DNA from ST395 via Φ187 (Table 1) suggesting that similar WTA structure may be a crucial determinant for the initiation of phage-dependent gene transfer even between distantly related bacteria. However, while *S. aureus* Φ80α or Φ11 phage particles are known to require a defined WTA structure for adsorption to host bacteria[8], the receptor requirements of SaPI particles, which have a much broader host range than helper phages[29], has remained unknown. Using defined mutants of the ST8 strains RN4220 (refs 8,10) and Newman with altered teichoic acids, the susceptibilities to phage Φ80α or Φ11 particle binding and infection were compared with their capacities to acquire SaPI DNA from SaPI particles derived from the same helper phages. Infection by both, phage particles and SaPI particles, was dependent on the presence of WTA and WTA glycosylation (Fig. 5a; Supplementary Fig. S7) whereas lipoteichoic acid was dispensable (Fig. 5a) indicating that SaPI particles adopt the receptor requirements of the corresponding helper phage. In accord with this finding, inactivation of *tagO* encoding the first enzyme of the WTA-biosynthetic pathway rendered strain PS187 resistant to Φ187 infection because of impaired adsorption (Supplementary Fig. 1a,b) and to Φ187-dependent SaPI transfer (Fig. 2) indicating that Φ187 and Φ187-derived SaPI particles require GroP WTA for binding to host bacteria.

WTA structure governs the capacity to undergo HGT. In order to elucidate if it is indeed the similarity of WTA structures that determines if two bacterial strains can exchange DNA via helper phages, even if they are not closely related, we cloned the minimal set of genes (*tarFIJLS*) from *S. aureus* RN4220 (ST8) required for biosynthesis of RboP-GlcNAc WTA[10] and introduced the resulting plasmid into bacteria that were not susceptible to HGT with typical RboP-WTA expressing *S. aureus*. The plasmid-transformed strain PS187-H was indeed found to produce two types of WTA as NMR analysis revealed the presence of both, RboP-GlcNAc WTA and GroP-GalNAc WTA (Supplementary Fig. S3b and Supplementary Table S3) indicating that two different WTA-biosynthetic machineries can be simultaneously functional in bacterial cells. Growth or morphology of these hybrid strains did not show obvious alterations (Supplementary Fig. S8a,b). Indeed, upon expression of RboP-GlcNAc WTA ST395 strains PS187 and NRS111 became susceptible to Φ80α or Φ11-dependent HGT of SaPI1 and SaPIbov1, respectively (Fig. 5b) or of the tetracycline resistance plasmid pT181 (Supplementary Fig. S9) from typical *S. aureus*. Moreover, distantly related bacteria such as *S. epidermidis*, *S. carnosus, S. capitis,* and even *L. monocytogenes* serotype 4e, which are usually resistant to Φ80α or Φ11-dependent HGT, acquired the capacity to take up SaPIs from typical *S. aureus* upon expression of *tarFIJLS* at similarly high rates as RboP-GlcNAc WTA-producing *S. aureus* (Fig. 5b). Of note, successful SaPI transfer correlated with increased phage adsorption upon RboP-GlcNAc WTA expression (Supplementary Fig. S1c). These results demonstrate that related WTA structures can be sufficient for allowing MGE exchange via helper phages even across long phylogenetic distances.

Table 1 | Correlation of WTA structure and capacity to mediate HGT of SaPIs.

Species/Strain	WTA type*	Glycosylation*	Reference	Transduction from ST8 via Φ11/80α	Transduction from ST395 via Φ187
S. aureus RN4220 (ST8)	**RboP**	**GlcNAc (α), GlcNAc (β)**	38	+	−
S. aureus PS187 (ST395)	**GroP**	**GalNAc (α)**	This study	−	+
S. epidermidis 1457†	**GroP**	GlcNAc (α); Glc	11	−	+
S. carnosus TM300	**GroP**	Glc, **(GalNAc)**	42	−	+
E. faecium VRE392†	GalNAc-GroP	absent	30	−	−
E. faecalis V583‡	GalNAc-RboP, WTA1 Glc-GalNAc-RboP, WTA2	Rha (α), WTA1 Glc (α), WTA2	31	−	−
L. monocytogenes ATCC19118†, Serotype 4e	GlcNAc-RboP	**Gal**	43	−	+
L. grayi ATCC25401§	**RboP**	**GlcNAc**	43	+	−

*Bold printed WTA structure correlates with phage and SaPI particle receptor specificity.
†WTA structure published for another isolate is likely to be the same in the strain used here.
‡Expresses two different WTAs as indicated.
§WTA glycosylation is likely to be GlcNAc because of the presence of close homologues of the *S. aureus* WTA glycosyltransferase genes *tarM* and *tarS* in the *L. grayi* DSM20601 genome sequence.

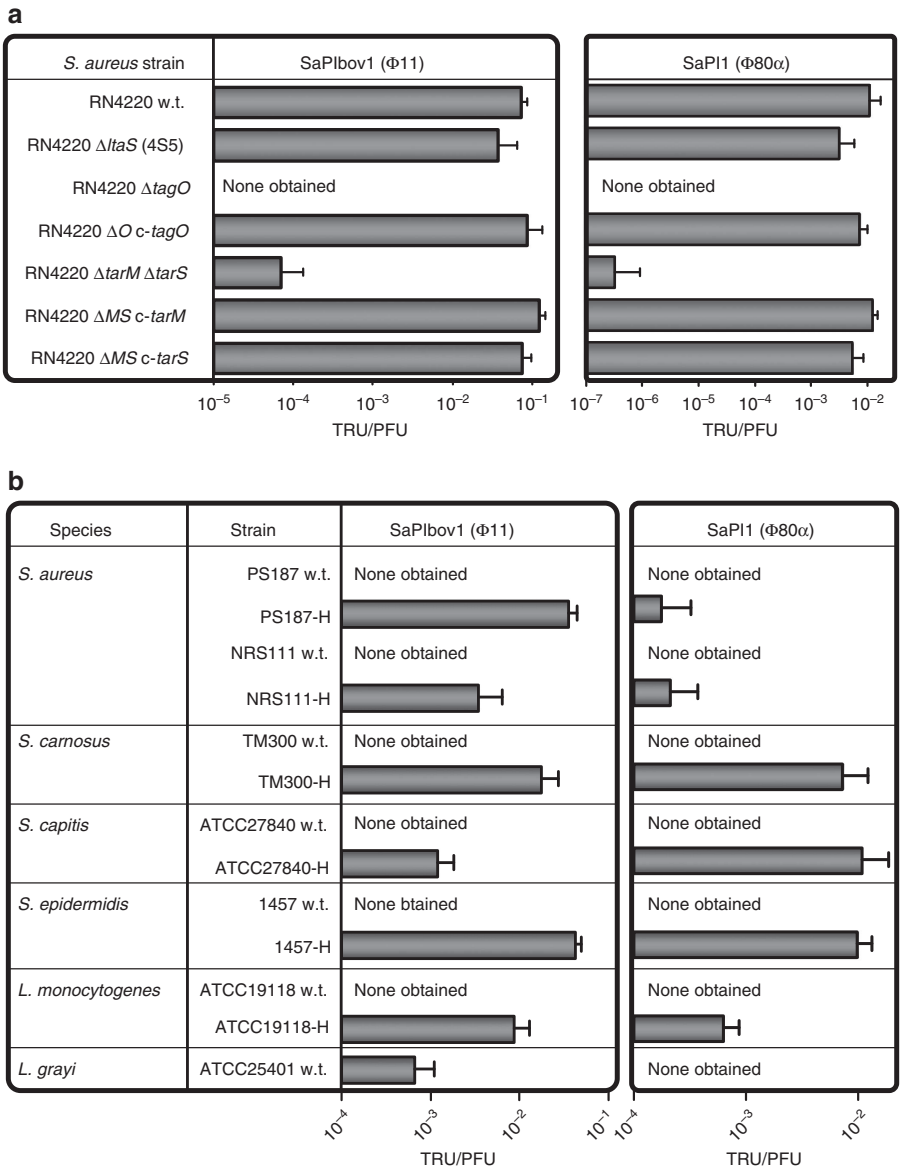

Figure 5 | WTA structure determines the capacity of SaPIs to traverse even long phylogenetic distances. *S. aureus* RN4220 (ST8) strains with, with altered or without WTA (**a**) or *S. aureus* PS187 (ST395) or other Gram-positive bacterial species expressing genes for biosynthesis of RboP-GlcNAc WTA (**b**) were analysed for capacities to acquire SaPIbov1 or SaPI1 via helper phages Φ11 or Φ80α, respectively. SaPI donor strains were JP1794 (SaPIbov1) and JP3602 (SaPI1). Values represent the ratio of transduction units (TRU; transductants per ml phage lysate) to plaque-forming units (PFU; plaques per ml phage lysate on *S. aureus* RN4220 w.t.) given as means ($n = 3$) ± s.d. No TRU were observed in strains expressing WTA other than RboP-GlcNAc. Δ*tagO*, no WTA; Δ*ltaS* (4S5), no lipoteichoic acid; c-*tagO*, Δ*tagO* complemented with *tagO*; Δ*tarM* Δ*tarS*, no WTA glycosylation; c-*tarM* and c-*tarS*, Δ*tarM* Δ*tarS* complemented either with *tarM* or *tarS*. WTA hybrid strains expressing additional RboP-GlcNAc WTA are indicated with H.

In accord with this finding, a systematic analysis with several Gram-positive bacteria revealed a strong correlation between WTA structure and the capacity to exchange resistance and virulence genes with either typical *S. aureus* via Φ11 or Φ80α (for example, *Listeria grayi*) or with ST395 via Φ187 (for example, *S. epidermidis* and other CoNS) (Table 1). The susceptibility of *L. monocytogenes* serotype 4e to Φ187-mediated HGT was reflected by efficient binding of Φ187 (Supplementary Fig. S1b). It may be due to the decoration of WTA with galactose, which may facilitate binding of Φ187-derived SaPI particles in a similar way as GalNAc. Of note, vancomycin-resistant *Enterococcus faecium* or *Enterococcus faecalis* could not undergo HGT with any *S. aureus* probably because of the very complex enterococcal WTA structures[30,31].

Discussion

HGT between *S. aureus* and other bacterial species and genera contributes substantially to the evolution of new epidemic clones, but it has remained unclear if it occurs accidentally or follows certain rules. While restriction modification and CRISPR systems have been shown to limit HGT efficiency[16–19], no data on the criteria that need to be fulfilled by the HGT partners to initiate phage-dependent MGE exchange have been available. Our studies with naturally occurring and engineered bacterial strains with atypical WTA demonstrate that related WTA structures of MGE donor and recipient are sufficient to permit HGT even across long phylogenetic distances. While helper phage particles are known to have quite narrow host ranges for parasitic reproduction[29], we found that their receptor specificities govern the capacity to

transmit MGEs to a broad range of recipient strains expressing cognate surface ligands. On the other hand, changes in helper phage receptor structures can enable binding of new types of helper phages and redirect the routes of HGT, which may facilitate the development of new clonal lineages and species.

The structurally diverse WTA molecules represent a species or lineage-specific signature at the surface of Gram-positive bacteria. WTA has several important roles for bacterial physiology such as the control of autolytic and peptidoglycan-biosynthetic enzymes[9], but all these functions could be achieved without extensive variation of its structure. Our data indicate that WTA structure constitutes a species- or lineage-defining 'glycocode' governing the bacterial access to a common genetic pool via WTA-specific helper phages. A thorough investigation of WTA structures and cognate phage receptor specificities will enable to assess how likely the transfer of new antibiotic resistance genes across the boundaries of species and genera will be in the future. Antibiotic stress is known to activate prophages thereby probably contributing to phage-mediated HGT[32]. In contrast, recently developed compounds blocking the biosynthesis of WTA[33] may help to reduce the frequency of phage-dependent HGT for example, in chronic polymicrobial infections.

Methods

Bacterial strains and growth media. The various bacterial strains listed in Supplementary Table S1 were grown in BM broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose) or Luria Bertani Broth supplemented with appropriate antibiotics at a concentration of 10 μg ml^{-1} (chloramphenicol), 3 μg ml^{-1} (tetracycline) or 100 μg ml^{-1} (ampicillin). In order to assess the growth and microscopic properties of mutant strains, phenotypic characterization of WTA hybrid strains overnight cultures were diluted to OD_{578} 0.1 in BM and grown at 37 °C on a shaker. Samples for microscopy were analysed after 3-h growth using a Leica DMRE microscope and Leica HCX 100 × objective. Bacterial growth was monitored for 8 h.

Molecular genetic methods. For the construction of marker-less Δ*tagO,* Δ*sauUSI* and Δ*sauUSI* Δ*hsdR* mutants in *S. aureus* PS187, the pKOR1 shuttle vector was used according to standard procedures[34]. For knockout plasmid construction, primers listed in Supplementary Table S4 were used. To label SaPI187β with an antibiotic resistance marker, the *ermB* gene was integrated into SaPI187β using the pKOR1 system. Knock-in primers are listed in Supplementary Table S4.

For amplification of the genomic region coding for *tarF, tarI*$_2$*, tarJ*$_2$*, tarL*$_2$ and *tarS* from genomic DNA (RN4220 w.t.) primers, Tar-up and Tar-dn were used. The PCR product was cloned in the *E. coli*/*S. aureus* shuttle vector pRB474[35] at the BamHI/EcoRI restriction sites resulting in plasmid pRB474-*tarFI*$_2$*J*$_2$*L*$_2$*S*. The plasmid was isolated from *E. coli* TOP10 or *E. coli* DC10B and used to transform target strains resulting in hybrid WTA-producing strains *S. aureus* PS187-H and NRS111-H, *S. carnosus* TM300-H, *S. capitis* ATCC27840-H and *S. epidermidis* 1457-H. *L. monocytogenes* ATCC19118 was transformed by electroporation[36] resulting in strain *L. monocytogenes* ATCC19118-H.

For plasmid transformation, 50 μl competent cells were mixed with 500 ng pT181 plasmid DNA, incubated at room temperature for 30 min, transferred into a 1-mm gap electroporation cuvette and pulsed at 1,000 volts. Immediately after electroporation, 950 μl BM medium was added followed by 70-min incubation at 37 °C. Cells were finally plated onto selective media and transformants were counted to calculate electroporation efficiency.

Pulsed field gel electrophoresis typing was performed according to Goerke *et al.*[37] *tagN* genotyping of available ST395 isolates was performed using primers N-up and N-dn (Supplementary Table S4).

Experiments with phages and SaPI particles. For rapid determination of phage susceptibility, the soft agar spot assay was performed according to Xia *et al.*[38] A phage panel encompassing broad host range phage ΦK (ref. 38), serogroup L phage Φ187 (ref. 39), two serogroup B phages Φ11 (ref. 39) and Φ80α[40] and serogroup F phage Φ77 (ref. 8) (Supplementary Table S5) was used. The various phages were propagated on *S. aureus* RN4220 (ΦK, Φ11, Φ80α, Φ77) or PS187 (Φ187). Mitomycin C induction experiments confirmed that these two propagation strains released no phage particles from endogenous prophages thereby ensuring that no other than the propagated phages were present in the obtained lysates. In order to determine phage adsorption rates, the multiplicity of infection was set to 0.005 (for Φ187) or 0.1 (for Φ80α and Φ77). Adsorption was calculated by determining the PFU of the unbound phage in the supernatant and subtracting it from the total number of input PFU. Adsorption efficiency was indicated relative to the adsorption on the parental wild-type strain (RN4220 or PS187), which was set to 100%. Prophages or SaPI particles were induced with 1 μg ml^{-1} mitomycin C.

For phage-mediated SaPI and plasmid transfer, recipient strains were grown overnight and used for HGT experiments. Approximately 8.0×10^7 bacteria were mixed with 100 μl of SaPI lysates produced from *S. aureus* strains JP1794 or JP3602 bearing the resistance marker-labelled SaPIbov1 ($\sim 1.0 \times 10^6$ PFU ml^{-1}) or SaPI1 ($\sim 3.0 \times 10^7$ PFU ml^{-1}), respectively (Supplementary Table S1), incubated for 15 min at 37 °C, diluted, and plated on BM agar supplemented with appropriate antibiotics.

For Φ187-mediated SaPIbov1 transfer, the PS187-H strain producing RboP and GroP WTA was transduced by Φ11 with SaPIbov1. The resulting strain VW1 was infected with Φ187 and 100 μl of the resulting lysate ($\sim 1.3 \times 10^{10}$ PFU ml^{-1}) was used to infect recipient strains as mentioned above. Note that for Φ187-mediated SaPIbov1 transduction of *Listeria* strains, the used lysate contained only $\sim 5.0 \times 10^5$ PFU ml^{-1}. For Φ187-mediated SaPI187β transfer, SaPI187β was labelled with the *ermB* resistance marker in *S. aureus* PS187 wild type resulting in strain VW7, which was subsequently infected with Φ187 and 100 μl of the obtained lysate ($\sim 3.0 \times 10^7$ PFU ml^{-1}) was used to infect recipient strains as mentioned above. To exclude that transductants resulted from spontaneous uptake of nicked DNA, SaPI-containing lysates were treated with 20 U DNase I for 1 h at 37 °C and used for SaPI transfer experiments. For plasmid transduction, pT181-bearing RN4220 was infected with Φ80α and the lysate was used to infect recipient strains. Approximately 8.0×10^7 bacteria were resuspended in phage buffer (100 mM $MgSO_4$, 100 mM $CaCl_2$, 1 M Tris–HCl, pH 7.8, 0.59% NaCl, 0.1% gelatine) and mixed with 100 μl lysate ($\sim 1.1 \times 10^8$ PFU ml^{-1}), incubated for 10 min at 37 °C, mixed with soft agar and poured onto BM agar plates containing 12.5 μg ml^{-1} tetracycline. Transductants were counted after overnight incubation (SaPIs) or after 48 h at 37 °C (pT181 and *Listeria* assays) and transduction efficiency was calculated. SaPI transfer was confirmed by molecular typing of resistance marker.

WTA extraction and purification. WTA was isolated as described previously with minor modifications[41]. Briefly, overnight cultures were washed and disrupted in a cell disrupter (Euler). Cell lysates were incubated at 37 °C overnight in the presence of DNase and RNase. SDS was added to a final concentration of 2% followed by ultrasonication for 15 min. Cell walls were washed several times to remove SDS. To release WTA from cell walls samples were treated with 5% trichloroacetic acid for 4 h at 65 °C. Peptidoglycan debris was separated via centrifugation (10 min, 14,000*g*). Determination of inorganic phosphate as described previously[41] was used for WTA quantification. These crude WTA extracts were further purified as described previously with minor modifications[38]. Briefly, the pH of the crude extract was adjusted to 5.5 with NaOH and dialyzed against water with a Spectra/Por3 dialysis membrane (MWCO of 3.5 kDa; VWR International GmbH, Darmstadt). Samples were concentrated in a SpeedVac concentrator at 45 °C to 5 ml and applied to DEAE-Sephadex A25 matrix according to Xia *et al.*[38] For elution from DEAE-Sephadex A25 matrix 20 mM Tris–HCl pH 7.2 0.35 M NaCl was used. The eluate was dialyzed again against water and concentrated to 1 ml. The purified WTA samples were stored at 20 °C for further analysis. All general analytical chemistry and NMR spectroscopy methods for WTA characterization are described in detail in the supplementary section.

Genome sequencing and analyses. *S. aureus* PS187 was sequenced initially by Roche 454 pyrosequencing (Roche GS-FLX system), then resequenced by using Illumina technology (Illumina HiSeq2000) at a 150-fold coverage. Details are described in supplementary section.

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Author contributions

V.W., C.L., P. S.C., G.X., M.S. and M.M. performed the experiments and analysed the data; B.M.B. and J.R.P. provided essential materials; V.W., J.R.P., U.N., O.H., T.D., A.P. and G.X. conceived the study; V.W., A.P. and G.X. wrote the manuscript.

Additional information

Accession codes: The Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number ARPA00000000 (BioProject PRJNA197438). The version described in this paper is the first version, ARPA01000000.

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