Molecular Microbiology (2003) **49**(6), 1577–1593 doi:10.1046/j.1365-2958.2003.03671.x

Genome-based analysis of virulence genes in a non-biofilm-forming *Staphylococcus epidermidis* strain (ATCC 12228)

Yue-Qing Zhang,[1†] Shuang-Xi Ren,[2†] Hua-Lin Li,[1†] Yong-Xiang Wang,[1] Gang Fu,[2] Jian Yang,[5] Zhi-Qiang Qin,[1] You-Gang Miao,[4] Wen-Yi Wang,[1] Run-Sheng Chen,[5] Yan Shen,[3] Zhu Chen,[2] Zheng-Hong Yuan,[1] Guo-Ping Zhao,[2,4] Di Qu,[1] Antoine Danchin[6] and Yu-Mei Wen[1]*

[1]*Laboratory of Medical Molecular Virology, Shanghai Medical College, Fudan University, 138 Yi Xue Yuan Road, Shanghai, 200032, China.*

[2]*Chinese National Human Genome Center at Shanghai (CHGCS), 250 Bi Bo Road, Zhang Jiang High Tech Park, Shanghai 201203, China.*

[3]*Chinese National Human Genome Center at Beijing, 707 North Yongchang Road, Yi Zhuang High Tech Park, Beijing, 100170, China.*

[4]*Center of Bioinformatics, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 319 Yue Yang Road, Shanghai 200031, China.*

[5]*Laboratory of Bioinformatics, Institute of Biophysics, Academia Sinica, 15 Da Tuen Road, Beijing,100101, China.*

[6]*HKU-Pasteur Research Center, Dexter HC Man building, 8 Sassoon Road, Poklulam, Hong Kong.*

Summary

***Staphylococcus epidermidis* strains are diverse in their pathogenicity; some are invasive and cause serious nosocomial infections, whereas others are non-pathogenic commensal organisms. To analyse the implications of different virulence factors in *Staphylococcus epidermidis* infections, the complete genome of *Staphylococcus epidermidis* strain ATCC 12228, a non-biofilm forming, non-infection associated strain used for detection of residual antibiotics in food products, was sequenced. This strain showed low virulence by mouse and rat experimental infections. The genome consists of a single 2499 279 bp chromosome and six plasmids. The chromosomal G + C content is 32.1% and 2419 protein coding sequences (CDS) are predicted, among which 230 are putative novel genes. Compared to the virulence factors in *Staphylococcus aureus,* aside from δ-haemolysin and β-haemolysin, other toxin genes were not found. In contrast, the majority of adhesin genes are intact in ATCC 12228. Most strikingly, the *ica* operon coding for the enzymes synthesizing interbacterial cellular polysaccharide is missing in ATCC 12228 and rearrangements of adjacent genes are shown. No *mec* genes, IS256, IS257, were found in ATCC 12228. It is suggested that the absence of the *ica* operon is a genetic marker in commensal *Staphylococcus epidermidis* strains which are less likely to become invasive.**

Introduction

In the Staphylococcus genus, at least 30 species have been identified. Among them *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) are the two main species of clinical importance. *Staphylococcus aureus* is a major human pathogen that causes infections ranging from cutaneous infections and food poisoning to life-threatening septicaemia. In contrast, *S. epidermidis* is a normal inhabitant of human skin and mucous membranes that rarely causes pyogenic infections in healthy individuals. However, during the past two decades *S. epidermidis* has emerged as one of the major pathogens in nosocomial infections. Infections caused by this organism generally occur in newborns, elderly individuals, immunocompromised patients and especially in patients with indwelling catheters and other implanted devices(Mack, 1999; von Eiff *et al*., 1999). As catheters and implanted devices are increasingly used in medical practice, infection due to *S. epidermidis* is now regarded as among the top five microbial causes of nosocomial infections (Rupp and Archer, 1994; Thylefors *et al*., 1998).

The essential pathogenesis of foreign-body-associated *S. epidermidis* infection is to colonize on a polymer surface by the formation of multilayered cell clusters, which are embedded in an amorphous extracellular material to form biofilm (Peters *et al*., 1982; von Eiff *et al*. 2002). Biofilm formation is a complex multistep process and has

Accepted 10 June, 2003. *For correspondence E-mail ymwen@shmu.edu.cn; Tel. (+ 86) 21 64165193; Fax (+86) 21 64174578. †These authors contributed equally to the work presented in this paper (Y.Q.-Z. did the shot gun cloning and sequencing, S.X.-R. did the annotation, H.L.-L. did the virulence study and annotation).

been subdivided into the stages of attachment, adhesion and aggregation (Gristina, 1987). Based on recent molecular studies, formation of *S. epidermidis* biofilm is proposed to occur in a two-step manner (Mack *et al*., 1994; Heilmann *et al*., 1996; Rupp *et al*., 2001). Initial adhesion to the polymer surface that may be unmodified or coated with host extracellular matrix is mediated by polysaccharide adhesin (PS/A) (Muller *et al*., 1993; Tojo *et al*., 1988), the bacterial surface-associated proteins SSP-1, SSP −2 (Veenstra *et al*., 1996), Autolysin E (Heilmann *et al*., 1997) and probably Bhp, a protein similar to Bap in *S. aureus* (Cucarella *et al*., 2001). The second step involves the accumulation of multilayered bacterial cell clusters. The production of polysaccharide intercellular adhesin (PIA) encoded by the *ica* locus in *S. epidermidis* is crucial in this step (Heilmann *et al*., 1996; McKenney *et al*., 1998). Proteins also seem to be essential for the accumulation and biofilm formation. A 140 kDa extracellular protein AAP (accumulation-associated protein) has a role in anchoring PIA to the bacterial cell surface, and thus is also implicated in cell accumulation(Hussain *et al*., 1997). In a recent review on the pathogenesis of infections due to coagulase-negative staphylococci, a number of surface structures and their putative functions in pathogenesis have been presented (von Eiff *et al*., 2002). The importance and implications of *S. epidermidis* biofilm have also been reviewed (O'Gara and Humphreys, 2001).

Staphylococcus epidermidis strains differ significantly in their virulence and their capacity to form biofilm. Several genes in *S. epidermidis* have been used to differentiate normal habitat contaminants from pathogenic strains in clinical isolates (Frebourg *et al*., 2000; Gelosia *et al*., 2001). Genomic diversity analysis by pulsed-field gel electrophoresis (PFGE) among coagulase-negative staphylococci in nosocomial infections have also been carried out (Lang *et al*., 1999). As bacterial virulence can be regulated by environmental signals via gene regulators (Cramton *et al*., 2001), and phase variation of virulence in *S. epidermidis* can occur by insertion or excision of insertion elements (IS) (Frebourg *et al*., 2000; Gelosia *et al*., 2001), merely detecting virulence-associated genes has a number of limitations. Besides, regulatory genes and virulence factor genes shared by *S. epidermidis* and *S. aureus* have been reported in recently isolated strains of *S. epidermidis* (Frebourg *et al*., 2000; Gelosia *et al*., 2001). Whether these genes were recently acquired by lateral transfer from *S. aureus* to *S. epidermidis* or *vice versa* remains to be determined. It is thus necessary to sequence the complete genome of a naturally occurring 'naive' commensal *S. epidermidis* strain for analysis of virulence genes, putative virulence genes, potential virulence genes and their regulators. These studies can help to reveal the mechanisms how commensal *S. epidermidis* strains can evolve into invasive virulent strains. We report here the genome sequences of *S. epidermidis* strain ATCC 12228 (deposited by FDA, USA, also known as FDA strain PCI 1200, or WHO 12), a non-biofilm forming, non-infection associated strain which has been universally used in the last century for detection of residual antibiotics, e.g. gentamicin, neomycin, novobiocin, in food products.

Results and discussion

General features of ATCC 12228

To assess the virulence of strain ATCC 12228, the foreign body-infection mouse model (Rupp *et al*., 1999a) and the intravascular catheter-associated infection rat model (Rupp *et al*., 1999b) were used. A biofilm-forming strain of *S. epidermidis* (97–337)(Table S1; *Supplementary material* Fig. S1), recently isolated from the blood of a 70-year-old patient who died of nosocomial *S. epidermidis* septicaemia was used for comparison. In strain 97–337, the major genes coding for adhesion and biofilm forming (*atlE, ica ADBC, fbn*) are present. In the mouse foreign body-infection model at 10^7 CFU inoculum level, a mean of $2.63 \times 10^4 \pm 2.3 \times 10^4$ CFU was recovered from the explanted catheters of mice infected with ATCC 12228, whereas a mean of $2.70 \times 10^5 \pm 1.8 \times 10^5$ CFU was recovered from those of mice infected with strain 97–337 ($P = 0.01$). Subcutaneous abscess was not detected either in animals infected with 97–337 or in animals infected with ATCC 12228. However, mild dilation of subcutaneous blood vessels was shown around the implanted catheters in 97–337-infected mice. In the rat central venous catheter-associated infection model, the inoculum was 1×10^4 CFU per animal. The mean of CFU recovered from the catheters of animals infected with strain 97–337 was 3.4×10^6, higher than that recovered from the catheters of animals infected with ATCC 12228 (1.2×10^5), but this difference was of no statistical significance. Bacteraemia was detected in 1/7 of ATCC 12228 infected rats, and in 4/9 of rats infected with strain 97–337. Based on these studies, compared to the clinical isolate 97–337, ATCC 12228 strain showed the trend of lower virulence.

The genome of ATCC 12228 consists of a 2 499 279 bp chromosome and six plasmids. The chromosomal G + C ratio is 32.1%, and there are 2419 protein coding sequences (CDS), excluding unknown genes which are less than 30 codons in length. Among these, 230 CDS do not match with any coding sequences deposited in the NCBI non-redundancy protein database, and thus may be novel genes with unknown functions. The origins of the replication and termination regions of the chromosome of ATCC 12228 are identified by comparison with *S. aureus* strains. In Fig. 1A and B, the annotation of the organization of the whole genome of ATCC 12228 is shown both in a circular representation and in a linear representation,

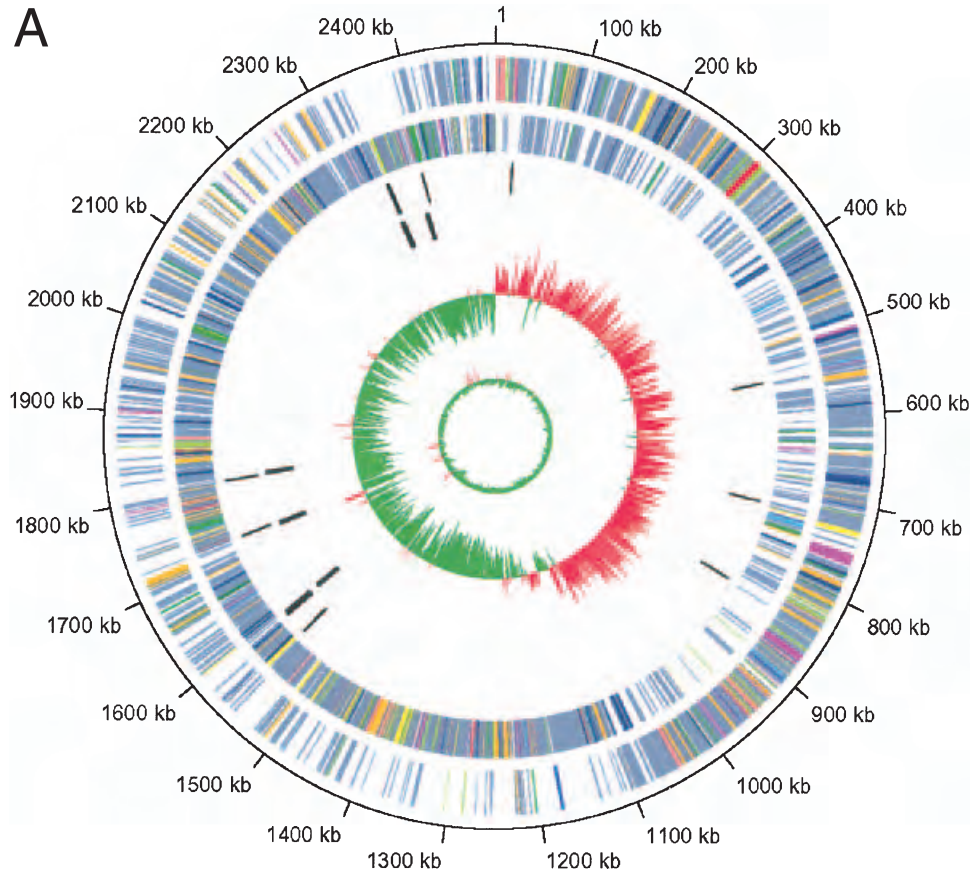
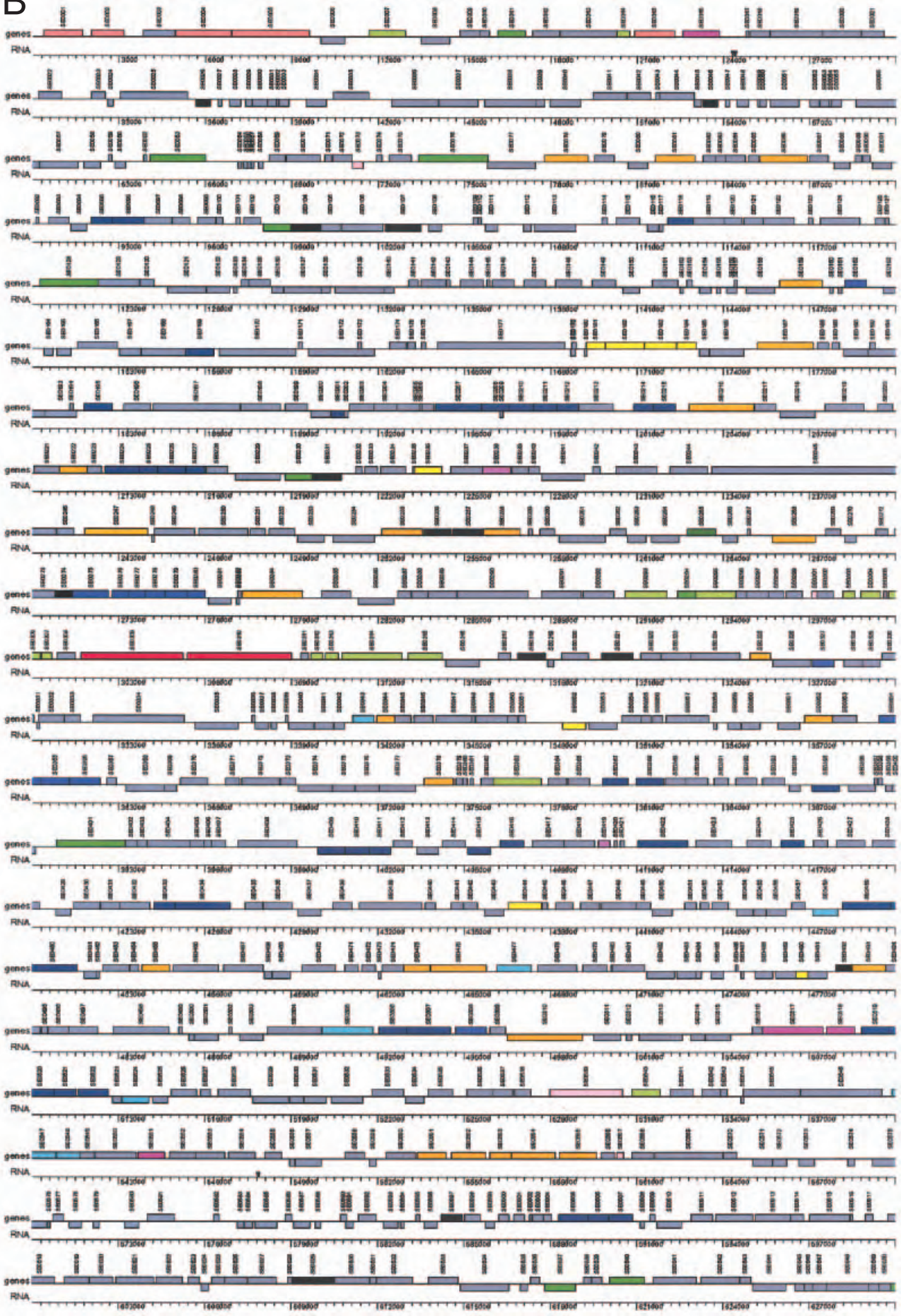


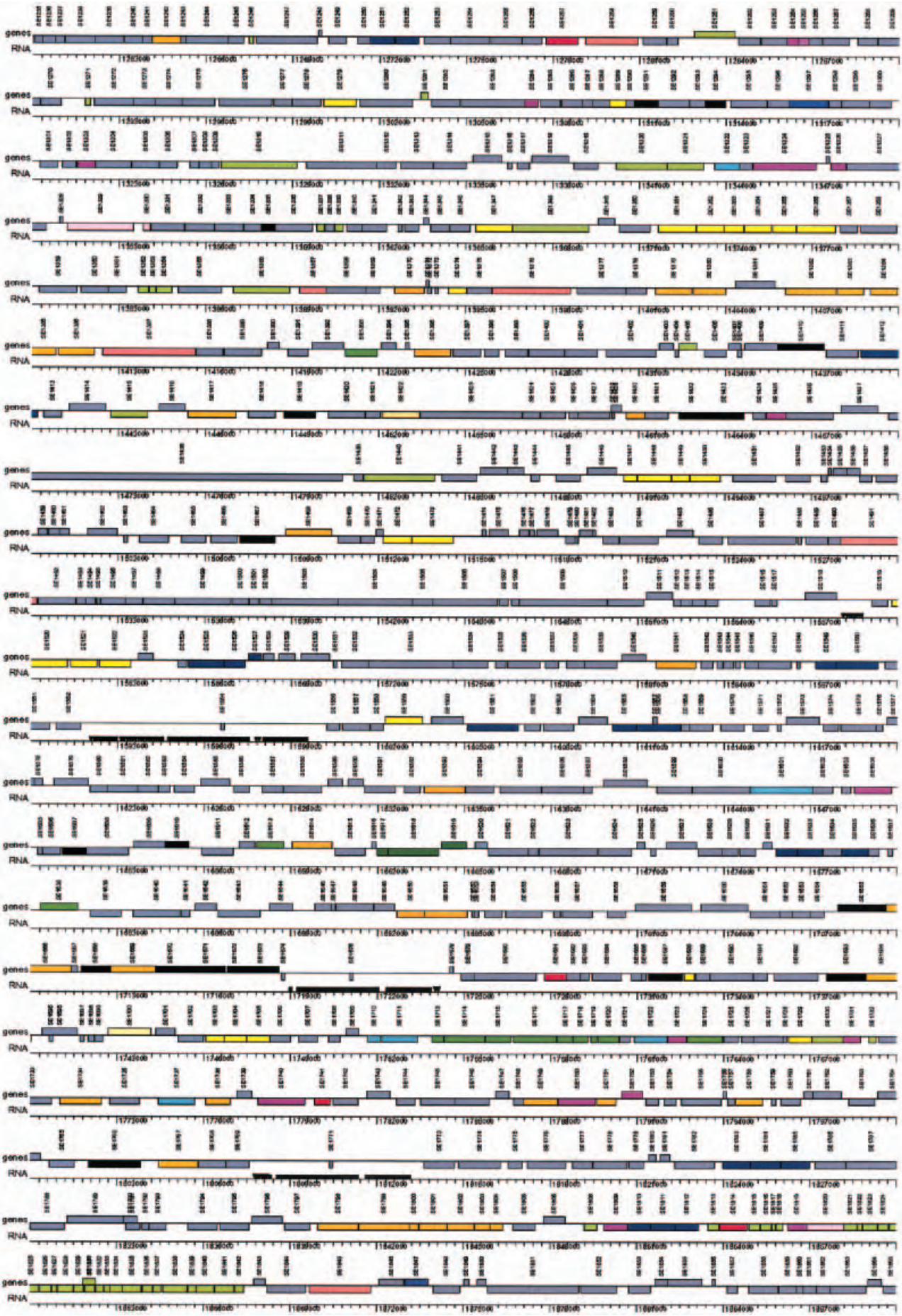
Fig. 1. A. Circular representation of *Staphylococcus epidermidis* ATCC 12228 chromosome. The outer scale is shown in kilobases. Circles range from 1 (outer circle) to 6 (inner circle). Circle 1, genes on forward strand; Circle 2, genes on reverse strand; Circle 3, tRNA genes; Circle 4: rRNA genes; Circle 5: GC bias ((G-C)/(G + C)); red indicates values >0; green < (0); Circle 6: G + C content. All genes are colour-coded according to functions: orange for carbohydrate metabolism, blue for energy metabolism, magenta for nucleotide metabolism, khaki for amino acid metabolism, orchid for metabolism of complex lipids, yellow for metabolism of cofactors and vitamins, dark green for biosynthesis of secondary metabolites, brown for biodegradation of xenobiotics, red for transcription, greenish yellow for translation, pink for sorting and degradation, salmon for replication and repair, navy for membrane transport, slate grey for signal transduction, dim grey for unassigned genes, black for tRNA and rRNA.
B. Linear representation of *Staphylococcus epidermidis* ATCC 12228 chromosome illustrating the location of each predicted protein-coding region and RNA in the genome. Panel 'gene': predicted coding regions on the plus strand (the upper line) and the minus strand (the lower line). The roles of predicted coding regions are classified and shown in colours, according to the colour code of Fig. 1A. Panel 'RNA': rRNAs are in black bars and tRNAs are in black triangles.

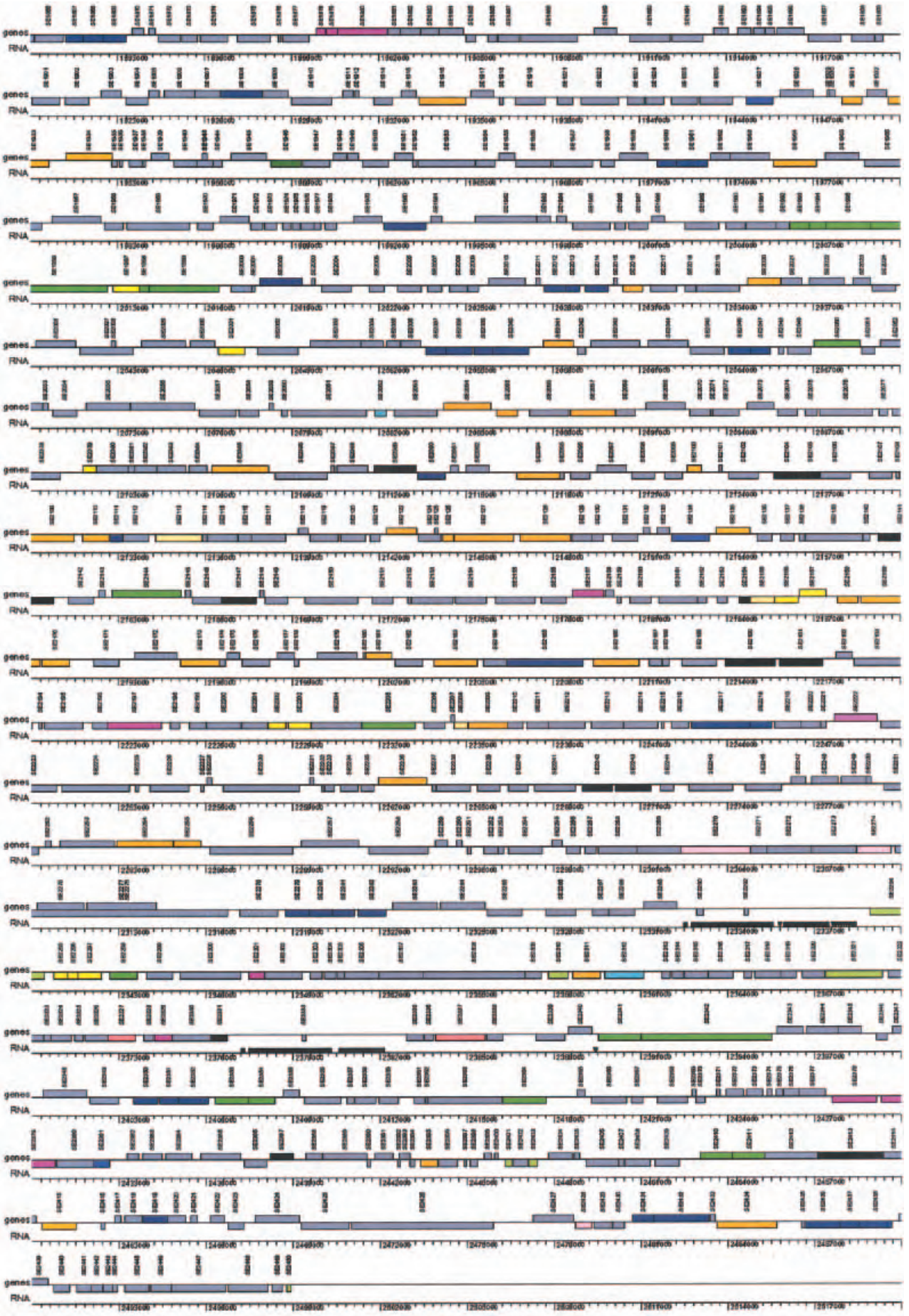
with the latter illustrating the RNAs and predicted proteins. Under the license for using unfinished genomic sequence data from TIGR, we are not permitted to compare the whole genome of ATCC 12228 with that of *S. epidermidis* strain RP62A, therefore we only compared the genomic organization between ATCC 12228 and *S. aureus* strain N315 (Kuroda *et al*., 2001). There are 2056 CDS in ATCC 12228 (84.99%) similar to those in N315, but the majority of genes coding for toxins in N315 are not present in ATCC 12228. In Fig. 2, the general genomic organization of ATCC 12228 and N315 is compared. Pathogenicity islands such as SaPIn 1, 2 and 3, several adhesin genes, osmoprotection genes, cell wall synthesis genes and certain antibiotic resistance genes reported in N315 are not found in ATCC 12228. Interestingly, based at the genomic level, the location of some genes or gene fragments shared by ATCC 12228 and N315 have crossed from close to the 3′-end to the 5′- end of the genome (Fig. 2). The G + C content of the plasmids in ATCC 12228 is 33.41%, similar to that of the chromosome. When mobile elements were compared, the types of insertion sequences (IS) and transposases of *S. epidermidis* differed markedly from those of *S. aureus* strains, e.g. N315 has eight copies of *IS1181* in its chromosome, but none was found in ATCC 12228. On the other hand, four copies of *IS605/IS200*-like transposase are present in ATCC 12228, but none was found in N315. In contrast to strain RP62A, *IS256* or *IS257* are not detected in the genome of ATCC 12228. The genes of hypothetical proteins or conserved hypothetical proteins are distributed throughout the chromosome and tend to cluster into blocks, among which, some of these are flanked by integrases or

B




genes
RNA


genes
RNA


genes
RNA

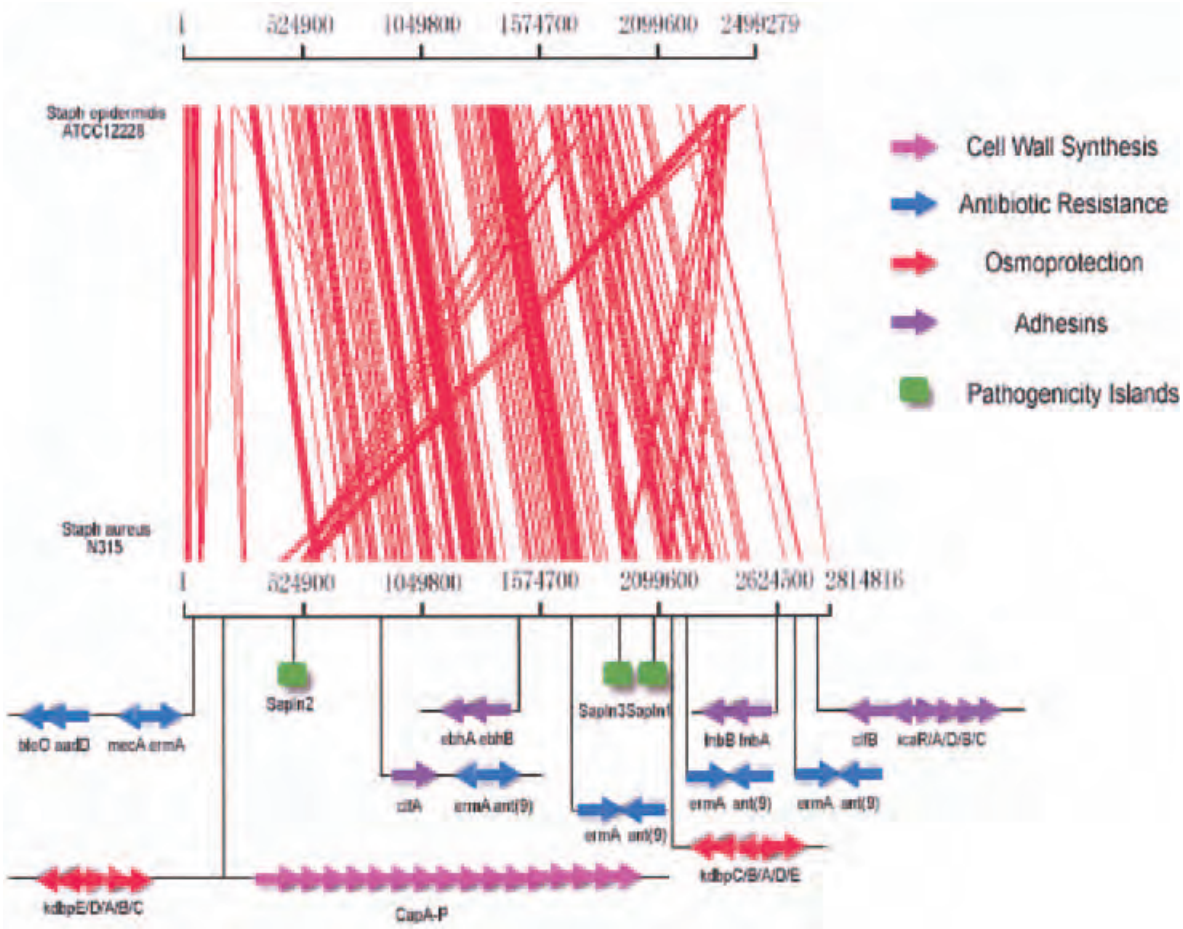


Fig. 2. Genome comparison between ATCC 12228 and N315. The genomic representation of ATCC 12228 and N315 is shown. Part of the genes related to pathogenicity and antibiotic resistance genes present in N315 but missing in ATCC 12228 are shown. The lines between the genomic diagram of the two strains show the crossing of the genes or gene fragments from the 5′end to the 3′end.

truncated transposases (SE1006-1012, SE1468-1471, SE2339-2344). Many of the genes which differ from those in *S. aureus* are bacteriophage genes, and several of them deal with integration and DNA manipulation. This implies that *S. epidermidis* and *S. aureus* do not have the same lysogenic phages. A comparison of the general features of ATCC 12228 genome with those of N315 strain is summarized in Table 1A.

Pathogenicity factors

Genes coding for pathogenicity factors in the genome of *S. aureus* have been categorized into four groups, namely, the exotoxins, exoenzymes, adhesins and others (Kuroda *et al*., 2001). Therefore, we grouped the pathogenic factors of ATCC 12228 as reported and a comparison between ATCC 12228 and N315 is shown in Table 1B.

(1) Exotoxins. Aside from δ-haemolysin and β-haemolysin genes, other potent exotoxin genes, such as the α-haemolysin, leucocidin, enterotoxins, toxic-shock toxins present in *S. aureus* are not found in ATCC 12228. Results are in accordance with the clinical manifestations that no toxic shock syndromes were observed in *S. epidermids* infections, indicating that exotoxins are not important in the pathogenesis of *S. epidermids* infections. The δ-haemolysin gene (*hld*) encodes a short and heat-labile delta-toxin which can be secreted from the bacteria and show lytic activity against many types of membranes (McKevitt *et al*., 1990). In the ATCC 12228 genome, *hld* is located near the 5′-end of RNAIII in the *agr* locus (Tegmark *et al*., 1998) and RNAIII from *S. epidermidis* can regulate *agr*-dependent virulence genes in *S. aureus* strains (Otto *et al*., 1999). Furthermore, *Staphylococcus epidermidis* can generate auto-inducing peptides (also called pheromones) which inhibit the *agr* response of other staphylococcus members (Otto, 2001; Otto *et al*., 2001). The fact that ATCC 12228 retains the *agr* locus and δ-haemolysin gene shows their importance for the survival of this non-pathogenic strain. Studies showed that the β-

Table 1. Genetic comparison between *S. epidermidis* ATCC 12228 and *S. aureus* N315.

A. General features

	ATCC 12228	N315
Chromosome		
Length of sequence (bp)	2499 279	2 814 816
G + C content	32.10%	32.80%
Protein coding regions	2419	2595
Ribosomal RNAs		
16S	5	5
23S	5	5
5S	6	6
Transfer RNAs	60	62
Insertion sequences		
IS 1181	0	8
IS 431	0	2
IS1272	30 (including truncated or frame shifted)	1
IS4-like	14 (truncated included)	3
IS431R	3	1
IS605/IS200-like	4	0
IS657-like	4	1
IS663-like	3	1
Plasmids		
Length of sequences (bp)	P1: 4439 P2: 4679 P3: 8007 P4: 17261 P5: 24370 P6: 6585	24 653
	33.41%	28.70%

B. Genes coding for virulence factors

	ATCC 12228	N315
Pathogenicity islands	0	3
a-Haemolysin	–	+
b-Haemolysin	+	Truncated
g-Haemolysin	–	+
d-Haemolysin	+	+
Enterotoxins	–	+
Leukotoxins	–	+
Toxic shock syndrome toxin -1	–	+
Intercellular adhesion proteins	–	+
Clumping factors(Cl*fA,ClfB*)	–	+
Fibronectin binding proteins	+	+
Adhesin EbhA, EbhB	–	+
Fibrinogen binding proteins	+	+
Elastin binding proteins	+	+
Autolysin E	+	+
Lipase	+	+
Serine protease	+	+
Protease ClpX	+	+
Thermonuclease	+	+
Exoculease	+	+
Lyophospholipase	+	+
Staphylokinase	–	+
Hyaluronidase	–	+
Staphylocoagulase	–	+

C. Antibiotic resistance genes

	ATCC 12228	N315
Bleomycin resistant protein	–	+
Penicillin binding proteins	–	+
Beta-lactamase	+	+
Bifunctional AAC-APH	–	+
Aminoglycoside phosphotransferase(ant)	–	+
Tetracycline resistant protein	+	+

D. Others

	ATCC 12228	N315
Cell wall synthesis (cap)	–	+
Osmoprotection	–	+

haemolysins from *S. aureus* and *S. intermedius* are similar but not identical (Dziewanowska *et al*., 1996). Structure and functional studies on β-haemolysin suggest that the mode of action of this toxin may mimic the *B. cereus* sphingomyelinase (Bohach *et al*., 2000). By BLAST analysis, the amino acid identity of β-haemolysins between *S. aureus* and ATCC 12228 is 94% (314/334 amino acids). Whether there are functional differences in the β-haemolysins between these two staphylococcal species needs to be pursued.

(2) Exoenzymes. The secreting exoenzymes that degrade or digest lipids are all present in ATCC 12228. The *geh1* gene which encodes a lipase playing important roles in *S. epidermidis* skin colonization is intact, despite its immediate flanking genes coding for the extracellular polysaccharide adhesion synthesizing enzyme operon (*icaR* and *ica ADBC*) being completely absent (see below). Other genes coding for proteolytic enzymes, such as the extracellular metallosprotease (SE2219) with elastase activity (Teufel and Gotz, 1993), serine protease (V8 protease) (SE1543), which catalyses the processing of the epidermin precursor peptide (Geissler *et al*., 1996) and genes coding for nucleases, e.g. thermonuclease(SE1004), are also found in ATCC 12228. However, genes coding for more virulent exoenzymes, such as staphylocoagulase, staphylokinase and hyaluronidase, which can interact with host tissues or intertissue components for invasion into deeper tissues are absent. Similar situation is also found in *S. epidermidis* strain RP62A. These findings can explain why *S. epidermidis* strains are common inhabitants of skin or mucous membrane, but usually do not invade deeper tissues.

(3) Adhesins. Lack of potent exotoxins and exoenzymes renders adhesins the most important pathogenic factors for *S. epidermidis*. Polysaccharide adhesin (PS/A) and one or more proteins, including the autolysin E (Mack

et al., 1994; Heilmann *et al*., 1997) are involved in the initial adherence, whereas the accumulation of cells is due to the production of polysaccharide intercellular adhesin (PIA), the synthesizing enzyme of which is encoded by the *ica* operon. Recently, it was reported that this operon also encodes enzymes for synthesizing PS/A and PIA and PS/A are chemically closely related (Heilmann *et al*., 1996; McKenney *et al*., 1998). Comparison of *S. epidermidis* strains from blood cultures and from mucosal origin has shown that *ica* genes are highly associated with biofilm formation (Ziebuhr *et al*., 1997). Therefore the *ica* operon is crucial for biofilm formation and pathogenesis of *S. epidermidis.*

In ATCC 12228, most strikingly, the entire *ica* operon is absent. The *ica* operon is located at the immediate upstream region of the lipase gene *geh1/lip* (SE0281) (Simons *et al*., 1998). However, genome sequencing of ATCC 12228 revealed that no *icaR, icaA, icaC, icaB, icaD* genes were detected in this region, or in other regions of the genome. Furthermore, when compared to N315 and RP62A, the genes adjacent to the putative *ica* operon region in ATCC 12228 showed inversions, insertions and significant rearrangements (Fig. 3). Results indicate that the non-biofilm forming phenotype of ATCC 12228 (*Supplementary material,* Table S1, Fig. S2) is due to a genetic defect which is different from the previously described phase variation mediated by insertion or excision of mobile elements in other *S. epidermidis* strains(Ziebuhr *et al*., 1999). Another important gene cluster missing in ATCC 12228 is *capA-P.* The capsular polyssacharides encoded by *cap* found in *S. aureus* are important virulence factors. In fact, these polysaccharides have been studied in animals as a candidate for anti-staphylococcal infection vaccine (Fattom *et al*., 1996).

Other adhesin genes described in *S. epidermidis* clinical isolates, such as the *atlE* gene and the *fbe* gene (Nilsson *et al*., 1998) are present in ATCC 12228. Recently, an extracellular-matrix-binding protein Embp has been identified as the fibronectin binding protein of *Staphylococcus epidermidis* in strain NCTC 11047 (Williams *et al*., 2002), and this protein is also found in *Staphylococcus aureus* (Hussain *et al*., 2001). In ATCC 12228 there is a CDS (SE1128) in which a part of the predicted amino acid (30%) is 100% identity with Embp. In *S. aureus* the two fibrinogen binding MSCRAMM (microbial surface component recognizing adhesive matrix molecules) ClfA and ClfB (McDevitt *et al*., 1994; Ni Eidhin *et al*., 1998) have an A domain and a R domain. The R domain is necessary for the presentation of the proteins at the bacterial cell surfaces. It consists of Serine-aspartic acid (SD) dipeptide repeats and followed by a short cell wall-associated domain, a transmembrane domain and a cytoplasmic domain. McCrea *et al*. (2000) found three genes encoding SD repeats namely *sdrF, sdrG* and *sdrH*. SdrG (Fbe) is a fibrinogen binding protein (Davis *et al*., 2001; Hartford *et al*., 2001). In ATCC 12228, the *sdrG* (Nilsson *et al*.,

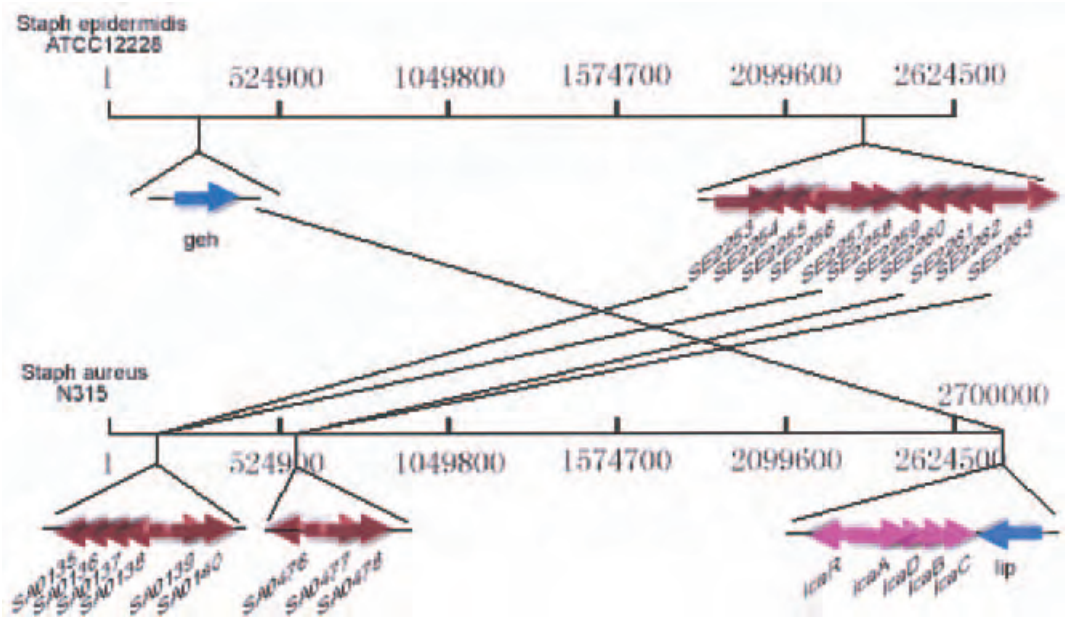


Fig. 3. Illustrative diagram of *ica* cluster deletion and rearrangements in ATCC 12228. The chromosomal fragments covering the reported *ica* cluster and adjacent genes of ATCC12228 and strain N315 are presented. As shown in the diagram, in ATCC 12228 the fragments starting from SE0270 to *his1,* SE 0278 to SE 0280 and the *lip/geh1* gene in strain 12228 are all reversed. Strikingly, the *ica* cluster which follows *lip/geh1* gene is completely deleted in SE 12228. SE0282 is a transposase gene, and SE0283 is homologous to SA 315 (SA0479).
Arrows and arrowheads = open reading frames and their direction of transcription.

1998) *sdrF* and, *sdrH* genes are all present. Presently, the ligands for SdrH and SdrF are unknown (Hook and Foster, 2000). Nine proteins predicted to contain LPXTG motif can be expressed by ATCC 12228 (*Supplementary material,* Table S2). The enzyme sortase cleaves the LPXTG motif for anchoring surface proteins to the cell wall of *S. aureus* (Mazmanian *et al*., 1999). A predicted protein homologue of this enzyme has been detected in ATCC 12228 (SE2076), which indicates that this enzyme is conserved in *S. epidermidis* strains. The finding of sortase in other species of staphylococcus supports the suggestion of using this enzyme as a target for development of new antimicrobial drug (Mazmanian *et al*., 1999). Another possible pathogenic factor is a 67 KDa myosin-cross reactive protein (SE0776) which may initiate immunopathological responses in host tissues. Coupled with this, there is a CDS (SE0997) which codes for a cardiolipin synthetase with unknown function. However, whether these predicted proteins are involved in SE pathogenicity is far from clear. All possible pathogenic factors predicted in ATCC 12228 genome are listed in Table 2.

Despite the presence of these adhesin genes and the conserved regulatory genes, ATCC 12228 is not a biofilm-forming strain and is graded as of low virulence. Genome-based analysis clearly indicates that the absence of *ica* operon, together with the inversion, insertion and rearrangements of adjacent genes, is responsible for non-biofilm formation. Though a few biofilm-positive strains which did not produce detectable PIA have been reported, the mechanism was not due to the absence of the *ica* operon, but was a result of reduced transcription of *ica* gene, or the encoded proteins were not translated (Muller *et al*., 1993; Ziebuhr *et al*., 1997). The mechanism responsible for the absence of the complete *ica* operon in ATCC 12228 is not clear. In a recent analysis on the *ica* operon genotypes in *S. epidermidis* strains, the expression of the *ica* operon and PIA synthesis, as well as other independent determinants of biofilm formation were shown integrated into a complex regulatory network (Rohde *et al*., 2001). Deletion of the *ica* operon could have occurred by an unknown genetic mechanism. It is tempting to speculate that *S. epidermidis* strains without *ica* operon are 'innate' commensal strains, which are less likely, or not likely converted to invasive strains. In contrast, commensal *S. epidermidis* strains which contain *ica* operon can easily be converted to biofilm-forming invasive strains by insertion or excision of insertion sequences in the *ica* operon or by environmental changes (Cramton *et al*., 2001; Rohde *et al*., 2001). In accordance with this speculation is that by screening with *ica* and *mecA* genes,

Table 2. Possible pathogenic factors detected in SE ATCC 12228.

Adhesins	ORF	Gene name	Function
Autolysin	SE1881	atlE	Adhere to polymers (Heilmann *et al*., 1997)
Fibrinogen binding protein	SE0331	sdrG(fbe)	FIbrinogen binding protein (Nilsson *et al*., 1998;Davis *et al*., 2001)
SD-rich cell surface adhesin	SE2395, SE1632	sdrF,sdrH	Not known (McCrea *et al*., 2000)
Extracellular matrix binding protein Embp	SE1128	embp	Fibronectin binding protein (Williams *et al*., 2002)
Elastin-binding protein	SE1169	ebpS	Adhesion on host proteins (Park *et al*., 1996)
Cell accumulation	SE0175	aap	Accumulation associated protein (Accession No: AJ249487)
Fmt	SE0754	fmt	Autolysis and penicillin resistant (Komatsuzawa *et al*.,1997)
Exoenzymes			
Serine protease, V8 protease	SE1543	sspA	Degrade or digest proteins (Rice *et al*., 2001)
Glycerol ester hydrolase	SE2403	geh	Degrade lipids (Simons *et al*., 1998)
Lipase A	SE0424		Possible degrade lipids
Lysophospholipase	SE0980		Possible degrade lipids
Putative esterase lipase^{-1}	SE0389		Possible digest lipids
Putative carboxyl esterase	SE2328		Possible degrade lipids
Metallosprotease	SE2219	SepP1	Elastase (Teufel and Gotz, 1993)
Protease ClpX	SE1349	clpX	Degrade or digest proteins
Thermonuclease	SE1004	nuc	Digest host nucleic acids
Putative 5′-3′exonuclease	SE1130		Possible degrade host nucleic acid
Exonuclesae	SE1028, SE1029		Possible degrade host nucleic acid
Toxins			
Delta-haemolysin	SE1634	hld	Destruction of blood and tissue cells (McKevitt *et al*.,1990)
Beta-haemolysin	SE0008		Phospholipase C synthesis (Katerov *et al*., 1994)
Others			
67 KDa myosin cross-reactive protein	SE0776		Cross-reactive with host cardiac myosin (Dale andBeachey, 1985)
Putative lipoprotein similar to streptococcal PsaA	SE0405		Putative adhesin essential for virulence (Berry andPaton, 1996)
Chitinase B	SE0760		Invasion of skin
Putative protein similar to attachment and virulence	SE1951		Possible host cell attachment
Similar to streptococcal haemagglutinin	SE2249		Unknown
Lactococcal lipoprotein	SE2320		Possible host cell attachment

significantly more virulent isolates than contaminating isolates were detected (Ziebuhr *et al*., 1997; Frebourg *et al*., 2000). By serial culture passages, some *S. epidermidis* strains showed biofilm phase variation. However, in some biofilm-negative *S. epidermidis* strains no phase variation could be induced (Ziebuhr *et al*., 1997).

Resistance to drugs and chemicals

In biofilm, bacteria are hundreds or a thousand times more resistant to antibiotics and can evade host defences (Ceri *et al*., 1999; Khoury *et al*., 1992). The continuous release of single-cell *S. epidermidis* from the biofilm into the blood stream causes persistent infections. In many cases, *S. epidermidis* infections cannot be cured unless the intravascular or implanted devices are removed. Further complicating the matter, strains of *S. epidermidis* are usually characterized as resistant to multiple drugs(Lyytikainen *et al*., 1996), and transfer of drug resistance has been reported among bacterial species in multispecies biofilms (Hausner and Wuertz, 1999; Lamont and Bryers, 2001).

In vitro antibiotic resistance assay for ATCC 12228 was carried out following National Committee for Clinical Laboratory Standards (NCCLS) and was separately done in two laboratories (Hong Kong Chinese University and Ruijing Hospital, Shanghai). According to the interpretive standard, only resistance to methicillin and tetracycline were shown in ATCC 12228, whereas this strain was susceptible to fusidic acid, vancomycin, oxacillin, erythromycin, rifamycin, chloramphenicol and fluorquinolone. Compared to strain N315, there are less antibiotic resistance genes in the genome of ATCC 12228 (Table 1C). Only the tetracycline resistance gene and the *bla* operon (SE1606-1608) coding for beta-lactamase (*blaZ*) were detected. There are fewer antibiotic resistance genes in ATCC 12228 than in RP62A. The *mecA* gene and the gene coding for the bifunctional aminoglycoside modifying enzyme (AacA-AphD), the gene coding for 3′5′-aminoglycoside phosphotransferase (ANT), and *erm*A present in RP62A were not detected in ATCC 12228. As *mecA* is not found in ATCC 12228, its resistance to methicillin is not due to the expression of penicillin binding protein 2a (PBP 2a), but is most likely due to over production of β-lactamase or PBPs, or to a reduction of their binding affinity (Tomasz *et al*., 1989). There is a putative gene coding for fosfomycin resistance in ATCC 12228 (SE0231). It is also worth mentioning that genes coding for the osmoprotection (*kdbpE/D/A/B/C*) found in *S. aureus* N315 and *S. epidermidis* RP62A are not seen in ATCC 12228, which suggests that these genes might function against drugs which block the synthesis of bacterial cell wall, and may be indirectly associated with drug resistance.

There are several copies of genes coding for hypothetical proteins similar to multidrug resistance proteins in the chromosome. These are the SMR-type efflux transporters (SE1877, SE1878) and the UVRA-like protein (SE0706). In contrast to the observation that most of the drug resistance genes of *S. aureus* are located on plasmids or mobile genetic elements (Kuroda *et al*., 2001), aside from the *tet* gene, drug resistance genes are located on the chromosome of ATCC 12228. The conservation of multidrug resistance genes in bacteria shows that these genes may have other important biological functions besides drug resistance. The majority of antibiotic resistance genes in the chromosome of ATCC 12228 are not flanked with mobile elements, which suggests that these genes were not acquired by lateral gene transfer. Because there are much less antibiotic resistance genes in this 'innate' commensal ATCC 12228, it seems unlikely that this strain can serve as a reservoir of antibiotic resistance genes.

In addition to the antibiotic resistance genes, there are many copies of genes associated with resistance to metal ions or chemicals. These genes code for arsenic resistance protein, arsenic efflux proteins, arsenate reductase, mercury resistance protein, tellurite resistance protein, copper transporting ATP-binding proteins and cadmiumbinding proteins. Hypothetical proteins not associated with resistance but involved in utilization and transport of heavy metals are present in the genome, such as proteins similar to Mn^{2+}-transport protein, Zn-binding lipoprotein, nickel transport-ATP-binding protein, aluminium transport-ATP-binding protein, molybdoprotein biosynthesis proteins and molybdenum transport-ATP-binding protein. Similar to the antibiotic resistance genes, the genes coding for heavy metal resistance proteins are not flanked by genetic mobile elements. The abundant presence of these genes in SE 12228 is not surprising, as commensals need to utilize various sources for their survival in the environment.

Regulatory genes

ATCC 12228 does not have more divergent types of regulatory genes than those present in *S. aureus*. Regulatory genes identified in ATCC 12228 are similar to those reported from clinical isolates of *S. epidermidis* and *S. aureus* strains. Regulators involved in pathogenesis including the sigma B operon (SE1668-SE1671), autolysin (*atl*) transcriptional regulator (SE0749), the staphylococcal accessory regulators (*sarA*, SE0390, *sarR*, SE1868), the *agr* locus (SE1635-SE1638) and the RNAIII regulator are all detected. This indicates that these conserved regulators are important in controlling the expression of *S. epidermidis* proteins. Conserved regulatory proteins for iron uptake and a transcriptional regulator of the Fur family (Xiong *et al*., 2000) in ATCC 12228 indicates that similar to certain pathogens, *S. epidermidis*

needs iron for their survival. Other regulators detected in the chromosome of ATCC 12228 are transposon regulatory proteins, regulatory proteins for antibiotic resistance and a number of regulators which participate in the respiratory chain and metabolic pathways of *S. epidermidis*.

In summary, sequencing and analysis of the genome of a naturally occurring non-biofilm forming, non-infection associated strain ATCC 12228 revealed the absence of the *ica* operon in this strain. In addition, inversion and rearrangements of adjacent genes have also been shown. Results suggest that there could be two groups of commensal SE strains. One group of *S. epidermidis* strains do not contain the *ica* operon and are 'innate' commensal *S. epidermidis* strains which are unlikely to become invasive. The other group of *S. epidermidis* strains do have the *ica* operon and can become invasive. Because ATCC 12228 is not a clinical isolate, the 230 putative new genes coding for proteins may not be related to pathogenesis, but may have functions important for the survival of *S. epidermidis* in hosts or in the environment. The conserved regulatory genes in ATCC 12228 indicate they are important for the regulation of adhesion and exoenzyme expression for survival when changes occur in the host or in the environment.

In this manuscript we have concentrated on analysing genes associated with virulence and pathogenesis. Further studies to compare the genomes of ATCC 12228 with *S. epidermidis* strains isolated from other sources, or with other coagulase-negative staphylococci will help to elucidate the mechanisms of how commensal inhabitants can be converted to invasive pathogens.

Experimental procedures

Identification of S. epidermidis *strains 12228 and 97–337*

Staphylococcus epidermidis strains were separately identified by Gram staining, API STAPH system (Biomerieux, France) and ID 32 STAPH identification systems in two independent laboratories. For strain 97–337, PCR amplification followed by sequencing of the 16 s rRNA was used for confirmation. Polymerase chain reaction and sequencing were carried out to confirm the presence of *ica* genes, *atlE* gene and *fbe* gene.

Assay for virulence in animals

Mouse foreign body infection model: according to the method reported by Rupp *et al*. (1999a), two sets of experiments were done on separate days. Briefly, in experiment 1, 16 Balb/c, 6-week-old all males were divided into two groups and 1 cm segment of 18-gauge Vialon intravenous catheter was implanted subcutaneously. *Staphylococcus epidermidis* was injected into the catheter bed. One group was inoculated with 10^7 colony forming units (CFU) inoculum of ATCC 12228, whereas the other group was inoculated with the same number of CFU of 97–337. On day 7, animals were sacrificed and the catheters aseptically removed, vortexed in wash fluid and bacteria recovered from the catheters were identified based on colony morphology, Gram-staining and coagulase assay. By appropriate dilution, *S. epidermidis* in the wash fluid were cultured on agar plates and the number of colonies counted. Subcutaneous abscess was observed visually. In the second experiment, 22 mice were used and repeated as above. The number of bacteria from the catheter adherence study was counted as CFU/catheter, and data were log normalized before analysis. All statistical tests were performed with SPPSS11.0 (Chicago, Illinois).

Rat central venous catheter-associated infection model: according to the method reported by Rupp *et al*. (1999b) with some modification, 16 Wistar rats were divided into two groups. A catheter was inserted into the right external jugular vein of each animal and the catheter was advanced to reach the superior vena cava. The proximal part of the catheter was tunnelled subcutaneously to exit from the midcapsular space. A knitted jacket was used to hold the catheter in place. Twenty-fours hours after the placement of catheters, 1×10^4 CFU of bacteria per rat were inoculated into the catheter via the exit. One group of rats was inoculated with ATCC 12228, and the other group was inoculated with 97–337. The catheters were flushed with heparin solution every day. Animals were sacrificed on day 8, and blood collected from the catheters as well as from the peripheral blood of animals were separately cultured to monitor the incidence of bacteraemia. The mean CFU recovered from the catheters of animals were converted to logrithm and compared between the two groups of animals. Statistical analysis was done using the same method as that used in the mouse model study. Bacteraemia was recorded by the number of animals with positive blood culture/total number of animals studied.

Antibiotic resistance assay

Antibiotic resistance assay of ATCC 12228 followed the National Committee for Clinical Laboratory Standards (NCCLS). Bacteria was first assayed for resistance to methicillin and because this strain was resistant to methicillin, assay was done in MRSA trays by standard MIC tests against fusidic acid, vancomycin, teicoplanin, erythromycin, tetracycline, oxacillin, rifampicin and linezolld. Results were interpreted according to the standard provided.

Cloning and sequencing of the genome of SE strain 12228

One single colony of ATCC 12228 was picked, passaged and further picked and passaged for three times on agar plates, followed by incubating in 100 ml of Luria–Bertani culture medium at 37°CÊ with vigorous shaking for 48 h. Bacterial cells were harvested by centrifugation (3000 rpm at 4°CÊ for 10 min), and embedded in low melting point agarose. Total genomic DNA was treated by *in situ* decomposition using proteinase K and SDS. Before the construction of genomic library, the plugs containing total genomic DNA were subjected to electrophoresis at low voltage (15 V) at 4°CÊ for 14 h. Two genomic libraries, both cloned in pUC18 vector,

were constructed. The small-insert plasmid library (1.5–3.0kb) was generated by random ultrasonic shearing of genomic DNA followed by ligation to pUC18/SmaI/BAP vector (Stratagene). The large-insert plasmid library (8.0–10.0kb) was generated by partial digestion with Sau3AI and ligation to pUC18/BamHI/CIP vector (Stratagene). In the random sequencing phase, 10.5-fold sequence coverage was achieved with 41640 nucleotide sequences from the small-insert library (average read length, 624 bp). Another onefold sequence coverage was achieved with 4195 nucleotide sequences from the large-insert library (average read length, 604 bp). All sequences were assembled into 106 contigs by using the PHRED/PHRAP/CONSED software package (Ewing *et al*., 1998). The chromosome draft composed of 100 contigs, and the other six contigs composed of complete sequences of six plasmids (lengths: 4439 bp, 4679 bp, 6585 bp, 8007 bp, 17261 bp, 24370 bp). Seventy sequence gaps were closed by primer walking on the corresponding bridge of plasmids. Thirty physical gaps were closed by combinational PCR followed by sequencing of the PCR products. Weak regions on chromosome were solved by direct resequencing or primer resequencing methods. The complete chromosome sequence was 2499 279 base pairs with an average error rate of 0.506/10kb.

Blast and protein prediction

ORFs were determined using Glimmer 2.0 (Delcher *et al*., 1999) and the results were checked manually. A few ORFs were found by hand correction as guided by BLASTN and BLASTX results. Annotation was carried out mostly by comparison with sequences in the public database, using BLASTN, BLASTX (Altschul *et al*., 1997) and tRNAscan-SE (Lowe and Eddy, 1997). CDSs were also analysed using hidden Markov models constructed for a number of conserved protein families (PFAM) using HMMER. TopPred (Claros and von Heijne, 1994) was used to identify potential membrane-spanning domains (MSDs) in proteins. The presence of signal peptides and the probable position of cleavage sites in secreted proteins were detected using Signal-P (Nielsen *et al*., 1999). Lipoproteins were identified by scanning for a lipobox. For comparison, preliminary sequence data of the *ica* operon of *Staphylococcus epidermidis* strain RP62A was obtained from The Institute for Genomic Research website at http://www.tigr.org.

Genome sequence

The complete genome of ATCC 12228 chromosome and six plasmids have been deposited in International Nucleotide Sequence Database (INSD, GenBank) (accession number: AE015929 for the genome sequence and AE015930 to AE015935 for the six plasmids).

Acknowledgements

Preliminary sequence data of the *ica operon* of *Staphylococcus epidermidis* strain RP62A was obtained from The Institute for Genomic Research website at http://www.tigr.org for comparison. We thank Prof. Colin R. Howard (Royal Veterinary College, London University) for help in editing this manuscript. We also thank Drs Fu Wang and Ying-Yuan Zhang (Hua Shan Hospital, Fudan University, Shanghai) for providing the *Staphylococcus epidermidis* strain 97–337. We thank Prof. Zhang Yun-Yi (School of Pharmacy, Fudan University) for demonstration of the technologies used in the rat model study. The drawing of the illustrative diagrams by Mr Chen Zhe is highly appreciated. This work was supported partly by the Chinese National Natural Science Foundation Grant (No.30170845), China State High Technology Foundation Grant (No. 2001AA223013), Shanghai Municipal Science and Technology Grant (No. 02DJ14002). China Xinjiang Youhow Company partly provided funding for the sequencing of this strain, but has no scientific contribution in this work.

Supplementary material

The following material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/mmi/mmi3671/mmi3671sm.htm

Fig. S1. Scanning electron microscopy of the biofilm formed by ATCC 12228.

Fig. S2. Scanning electron microscopy of the biofilm formed by *S. epidermidis* 97-337.

Table S1. Results of *in vitro* quantitative biofilm formation.

Table S2. CDS with LPXTG motifs predicted in *S. epidermidis* ATCC 12228.

References

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25:** 3389–3402.

Berry, A.M., and Paton, J.C. (1996) Sequence heterogeneity of PsaA, a 37-kilodalton putative adhesin essential for virulence of *Streptococcus pneumoniae*. *Infect Immun* **64:** 5255–5262.

Bohach, G.A., and Foster, T.J. (2000) *Staphylococcus Aureus Exotoxins in Gram-Positive Pathogens.* Fischetti, V.A., Novick, R.P., Ferretti, J.J., Portnoy, D.A., and Rood, J.I. (eds). Washington DC: American Society for Microbiology Press, pp. 368–369.

Ceri, H., Olson, M.E., Stremick, C., Read, R.R., Morck, D., and Buret, A. (1999) The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J Clin Microbiol* **37:** 1771–1776.

Christensen, G.D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barrett, F.F., Melton, D.M., and Beachey, E.H. (1985) Adherence of coagulase-negative staphylococci to plastic tissue culture plates: a quantitative model for the adherence of staphylococci to medical devices. *J Clin Microbiol* **22:** 996–1006.

Claros, M.G., and von Heijne, G. (1994) TopPred II: an improved software for membrane protein structure predictions. *Comput Appl Biosci* **10:** 685–686.

Cramton, S.E., Ulrich, M., Gotz, F., and Doring, G. (2001) Anaerobic conditions induce expression of polysaccharide intercellular adhesin in *Staphylococcus aureus* and

Staphylococcus epidermidis. *Infect Immun* **69:** 4079–4085.

Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, I., and Penades, J.R. (2001) Bap, a *Staphylococcus aureus* surface protein involved in biofilm formation. *J Bacteriol* **183:** 2888–2896.

Dale, J.B., and Beachey, E.H. (1985) Epitopes of streptococcal M proteins shared with cardiac myosin. *J Exp Med* **162:** 583–591.

Davis, S.L., Gurusiddappa, S., McCrea, K.W., Perkins, S., and Hook, M. (2001) SdrG, a fibrinogen-binding bacterial adhesin of the microbial surface components recognizing adhesive matrix molecules subfamily from *Staphylococcus epidermidis*, targets the thrombin cleavage site in the Bbeta chain. *J Biol Chem* **276:** 27799–27805.

Delcher, A.L., Harmon, D., Kasif, S., White, O., and Salzberg, S.L. (1999) Improved microbial gene identification with GLIMMER. *Nucleic Acids Res* **27:** 4636–4641.

Dziewanowska, K., Edwards, V.M., Deringer, J.R., Bohach, G.A., and Guerra, D.J. (1996) Comparison of the beta-toxins from *Staphylococcus aureus* and *Staphylococcus intermedius*. *Arch Biochem Biophys* **335:** 102–108.

von Eiff, C., Heilmann, C., Herrmann, M., and Peters, G. (1999) Basic aspects of the pathogenesis of staphylococcal polymer-associated infections. *Infection* **27** (Suppl. 1)**:** S7–S10.

von Eiff, C., Peters, G., and Heilmann, C. (2002) Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis* **2:** 677–685.

Ewing, B., Hillier, L., Wendl, M.C., and Green, P. (1998) Base-calling of automated sequencer traces using phred. I. Accuracy assessment. *Genome Res* **8:** 175–185.

Fattom, A.I., Sarwar, J., Ortiz, A., and Naso, R. (1996) A *Staphylococcus aureus* capsular polysaccharide (CP) vaccine and CP-specific antibodies protect mice against bacterial challenge. *Infect Immun* **64:** 1659–1665.

Frebourg, N.B., Lefebvre, S., Baert, S., and Lemeland, J.F. (2000) PCR-Based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J Clin Microbiol* **38:** 877–880.

Geissler, S., Gotz, F., and Kupke, T. (1996) Serine protease EpiP from *Staphylococcus epidermidis* catalyzes the processing of the epidermin precursor peptide. *J Bacteriol* **178:** 284–288.

Gelosia, A., Baldassarri, L., Deighton, M., and van Nguyen, T. (2001) Phenotypic and genotypic markers of *Staphylococcus epidermidis* virulence. *Clin Microbiol Infect* **7:** 193–199.

Gristina, A.G. (1987) Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* **237:** 1588–1595.

Hartford, O., O'Brien, L., Schofield, K., Wells, J., and Foster, T.J. (2001) The Fbe (SdrG) protein of *Staphylococcus epidermidis* HB promotes bacterial adherence to fibrinogen. *Microbiology* **147:** 2545–2552.

Hausner, M., and Wuertz, S. (1999) High rates of conjugation in bacterial biofilms as determined by quantitative *in situ* analysis. *Appl Environ Microbiol* **65:** 3710–3713.

Heilmann, C., Hussain, M., Peters, G., and Gotz, F. (1997) Evidence for autolysin-mediated primary attachment of *Staphylococcus epidermidis* to a polystyrene surface. *Mol Microbiol* **24:** 1013–1024.

Heilmann, C., Schweitzer, O., Gerke, C., Vanittanakom, N., Mack, D., and Gotz, F. (1996) Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol Microbiol* **20:** 1083–1091.

Hook, M., and Foster, T.J. Staphylococcal surface proteins. In *Gram-Positive Pathogens.* Fischetti, V.A., Novick, R.P., Ferretti, J.J., Portnoy, D.A., and Rood, J.I. (eds). Washington, D.C.: American Society for Microbiology Press, pp. 386–389.

Hussain, M., Herrmann, M., von Eiff, C., Perdreau-Remington, F., and Peters, G. (1997) A 140-kilodalton extracellular protein is essential for the accumulation of *Staphylococcus epidermidis* strains on surfaces. *Infect Immun* **65:** 519–524.

Hussain, M., Becker, K., von Eiff, C., Schrenzel, J., Peters, G., and Herrmann, M. (2001) Identification and characterization of a novel 38.5-kilodalton cell surface protein of *Staphylococcus aureus* with extended-spectrum binding activity for extracellular matrix and plasma proteins. *J Bacteriol* **183:** 6778–6786.

Katerov, V.E., Golubkov, V.I., Totolian, A.A., Shalen, K., Ensen, L., Mikula, I., and Smola, I. (1994) [The cloning and expression of the gene for *Staphylococcus aureus* beta-hemolysin.] *Zh Mikrobiol Epidemiol Immunobiol* **28:** 33.

Khoury, A.E., Lam, K., Ellis, B., and Costerton, J.W. (1992) Prevention and control of bacterial infections associated with medical devices. *Asaio J* **38:** M174–M178.

Komatsuzawa, H., Sugai, M., Ohta, K., Fujiwara, T., Nakashima, S., Suzuki, J., *et al.* (1997) Cloning and characterization of the fmt gene which affects the methicillin resistance level and autolysis in the presence of triton X-100 in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* **41:** 2355–2361.

Kuroda, M., Ohta, T., Uchiyama, I., Baba, T., Yuzawa, H., Kobayashi, I., *et al.* (2001) Whole genome sequencing of meticillin-resistant *Staphylococcus aureus*. *Lancet* **357:** 1225–1240.

Lamont, R.J., and Bryers, J.D. (2001) Biofilm-induced gene expression and gene transfer. *Methods Enzymol* **336:** 84–94.

Lang, S., Livesley, M.A., Lambert, P.A., Elliott, J., and Elliott, T.S. (1999) The genomic diversity of coagulase-negative staphylococci associated with nosocomial infections. *J Hosp Infect* **43:** 187–193.

Lowe, T.M., and Eddy, S.R. (1997) tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* **25:** 955–964.

Lyytikainen, O., Vaara, M., Jarviluoma, E., Rosenqvist, K., Tiittanen, L., and Valtonen, V. (1996) Increased resistance among Staphylococcus epidermidis isolates in a large teaching hospital over a 12-year period. *Eur J Clin Microbiol Infect Dis* **15:** 133–138.

Mack, D. (1999) Molecular mechanisms of *Staphylococcus epidermidis* biofilm formation. *J Hosp Infect* **43** (Suppl.): S113–S125.

Mack, D., Nedelmann, M., Krokotsch, A., Schwarzkopf, A., Heesemann, J., and Laufs, R. (1994) Characterization of transposon mutants of biofilm-producing *Staphylococcus epidermidis* impaired in the accumulative phase of biofilm

production: genetic identification of a hexosamine-containing polysaccharide intercellular adhesin. *Infect Immun* **62:** 3244–3253.

Mazmanian, S.K., Liu, G., Ton-That, H., and Schneewind, O. (1999) *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science* **285:** 760–763.

McCrea, K.W., Hartford, O., Davis, S., Eidhin, D.N., Lina, G., Speziale, P., *et al.* (2000) The serine-aspartate repeat (Sdr) protein family in *Staphylococcus epidermidis*. *Microbiology* **146:** 1535–1546.

McDevitt, D., Francois, P., Vaudaux, P., and Foster, T.J. (1994) Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol Microbiol* **11:** 237–248.

McKenney, D., Hubner, J., Muller, E., Wang, Y., Goldmann, D.A., and Pier, G.B. (1998) The ica locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. *Infect Immun* **66:** 4711–4720.

McKevitt, A.I., Bjornson, G.L., Mauracher, C.A., and Scheifele, D.W. (1990) Amino acid sequence of a deltalike toxin from *Staphylococcus epidermidis*. *Infect Immun* **58:** 1473–1475.

Muller, E., Takeda, S., Shiro, H., Goldmann, D., and Pier, G.B. (1993) Occurrence of capsular polysaccharide/adhesin among clinical isolates of coagulase-negative staphylococci. *J Infect Dis* **168:** 1211–1218.

Ni Eidhin, D., Perkins, S., Francois, P., Vaudaux, P., Hook, M., and Foster, T.J. (1998) Clumping factor B (ClfB), a new surface-located fibrinogen-binding adhesin of *Staphylococcus aureus*. *Mol Microbiol* **30:** 245–257.

Nielsen, H., Brunak, S., and von Heijne, G. (1999) Machine learning approaches for the prediction of signal peptides and other protein sorting signals. *Protein Eng* **12:** 3–9.

Nilsson, M., Frykberg, L., Flock, J.I., Pei, L., Lindberg, M., and Guss, B. (1998) A fibrinogen-binding protein of *Staphylococcus epidermidis*. *Infect Immun* **66:** 2666–2673.

O'Gara, J.P., and Humphreys, H. (2001) *Staphylococcus epidermidis* biofilms: importance and implications. *J Med Microbiol* **50:** 582–587.

Otto, M. (2001) *Staphylococcus aureus* and *Staphylococcus epidermidis* peptide pheromones produced by the accessory gene regulator agr system. *Peptides* **22:** 1603–1608.

Otto, M., Echner, H., Voelter, W., and Gotz, F. (2001) Pheromone cross-inhibition between *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Infect Immun* **69:** 1957–1960.

Otto, M., Sussmuth, R., Vuong, C., Jung, G., and Gotz, F. (1999) Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis* agr pheromone and derivatives. *FEBS Lett* **450:** 257–262.

Park, P.W., Rosenbloom, J., Abrams, W.R., and Mecham, R.P. (1996) Molecular cloning and expression of the gene for elastin-binding protein (ebpS) in *Staphylococcus aureus*. *J Biol Chem* **271:** 15803–15809.

Peters, G., Locci, R., and Pulverer, G. (1982) Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J Infect Dis* **146:** 479–482.

Rice, K., Peralta, R., Bast, D., de Azavedo, J., and McGavin, M.J. (2001) Description of staphylococcus serine protease (ssp) operon in *Staphylococcus aureus* and nonpolar inactivation of sspA-encoded serine protease. *Infect Immun* **69:** 159–169.

Rohde, H., Knobloch, J.K., Horstkotte, M.A., and Mack, D. (2001) Correlation of biofilm expression types of *Staphylococcus epidermidis* with polysaccharide intercellular adhesin synthesis: evidence for involvement of icaADBC genotype-independent factors. *Med Microbiol Immunol (Berl)* **190:** 105–112.

Rupp, M.E., and Archer, G.L. (1994) Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* **19:** 231–243; quiz 244–235.

Rupp, M.E., Ulphani, J.S., Fey, P.D., Bartscht, K., and Mack, D. (1999a) Characterization of the importance of polysaccharide intercellular adhesin/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. *Infect Immun* **67:** 2627–2632.

Rupp, M.E., Ulphani, J.S., Fey, P.D., and Mack, D. (1999b) Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. *Infect Immun* **67:** 2656–2659.

Rupp, M.E., Fey, P.D., Heilmann, C., and Gotz, F. (2001) Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide intercellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. *J Infect Dis* **183:** 1038–1042.

Simons, J.W., van Kampen, M.D., Riel, S., Gotz, F., Egmond, M.R., and Verheij, H.M. (1998) Cloning, purification and characterisation of the lipase from *Staphylococcus epidermidis* – comparison of the substrate selectivity with those of other microbial lipases. *Eur J Biochem* **253:** 675–683.

Tegmark, K., Morfeldt, E., and Arvidson, S. (1998) Regulation of agr-dependent virulence genes in *Staphylococcus aureus* by RNAIII from coagulase-negative staphylococci. *J Bacteriol* **180:** 3181–3186.

Teufel, P., and Gotz, F. (1993) Characterization of an extracellular metalloprotease with elastase activity from *Staphylococcus epidermidis*. *J Bacteriol* **175:** 4218–4224.

Thylefors, J.D., Harbarth, S., and Pittet, D. (1998) Increasing bacteremia due to coagulase-negative staphylococci: fiction or reality? *Infect Control Hosp Epidemiol* **19:** 581–589.

Tojo, M., Yamashita, N., Goldmann, D.A., and Pier, G.B. (1988) Isolation and characterization of a capsular polysaccharide adhesin from *Staphylococcus epidermidis*. *J Infect Dis* **157:** 713–722.

Tomasz, A., Drugeon, H.B., de Lencastre, H.M., Jabes, D., McDougall, L., and Bille, J. (1989) New mechanism for methicillin resistance in *Staphylococcus aureus*: clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. *Antimicrob Agents Chemother* **33:** 1869–1874.

Veenstra, G.J., Cremers, F.F., van Dijk, H., and Fleer, A. (1996) Ultrastructural organization and regulation of a biomaterial adhesin of *Staphylococcus epidermidis*. *J Bacteriol* **178:** 537–541.

Williams, R.J., Henderson, B., Sharp, L.J., and Nair, S.P. (2002) Identification of a Fibronectin-binding protein from

Staphylococcus epidermidis. *Infect Immun* **70:** 6805–6810.

Xiong, A., Singh, V.K., Cabrera, G., and Jayaswal, R.K. (2000) Molecular characterization of the ferric-uptake regulator, fur, from *Staphylococcus aureus*. *Microbiology* **146:** 659–668.

Ziebuhr, W., Heilmann, C., Gotz, F., Meyer, P., Wilms, K., Straube, E., and Hacker, J. (1997) Detection of the intercellular adhesion gene cluster (ica) and phase variation in *Staphylococcus epidermidis* blood culture strains and mucosal isolates. *Infect Immun* **65:** 890–896.

Ziebuhr, W., Krimmer, V., Rachid, S., Lossner, I., Gotz, F., and Hacker, J. (1999) A novel mechanism of phase variation of virulence in *Staphylococcus epidermidis*: evidence for control of the polysaccharide intercellular adhesin synthesis by alternating insertion and excision of the insertion sequence element IS256. *Mol Microbiol* **32:** 345–356.