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Conversion of *Staphylococcus epidermidis* Strains from Commensal to Invasive by Expression of the *ica* Locus Encoding Production of Biofilm Exopolysaccharide

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To test if biofilm formation in *Staphylococcus epidermidis* is dependent on the polysaccharide intercellular adhesin, whose biosynthesis is driven by the *ica* locus, a plasmid containing the *ica* locus was transferred to three *ica*-negative strains. Using in vitro biofilm assays and a rat central venous catheter infection model, we confirmed the importance of the *ica* locus for biofilm production and pathogenesis of *S. epidermidis*.

Staphylococcus epidermidis has become one of the most important pathogens of nosocomial infections associated with catheters and other indwelling medical devices (6, 7, 9). Previous studies have tried to determine factors that discriminate between commensal and invasive strains of *S. epidermidis* (5). Elucidation of the major differences between the two types of strains is believed to promote our understanding of *S. epidermidis* pathogenesis. Several studies have suggested that the *ica* locus, which encodes production of the *N*-acetylglucosamine polysaccharide intercellular adhesin (PIA), plays a critical role in distinguishing the two types of strains, indicating an important function of *ica* in invasiveness of *S. epidermidis* (4, 5, 17). Studies using insertional *ica* mutants, controllable expression of *ica*, and heterologous expression in *Staphylococcus carnosus* have further underscored the important role of *ica* in biofilm formation and pathogenesis of *S. epidermidis* (8, 10, 12–15). However, other studies have raised doubt about the critical function of the *ica* locus in causing *S. epidermidis* biofilm-associated infection (3). Therefore, to test the hypothesis that the *ica* locus is a major factor of *S. epidermidis* invasiveness, we attempted to convert commensal, *ica*-negative strains to invasive strains of *S. epidermidis* by *ica* locus expression.

To investigate the impact of introducing the *ica* locus in *ica*-negative *S. epidermidis* strains, a plasmid containing the entire *ica* locus (*icaRADBC*) was constructed and transferred into *ica*-negative strains of *S. epidermidis*. A 4,215-bp fragment encompassing *icaRADBC* was PCR amplified using DNA from strain 97-337 as a template (16). The PCR product was cloned into vector pYJ90 and was confirmed by sequencing (GenBank accession number AY382582). The constructed plasmid was electroporated into three *ica*-negative strains, ATCC 12228, HB, and Tü3298 (1), as described previously to generate the respective isogenic strains ATCC 12228-*ica*, HB-*ica* and Tü3298-*ica*. The biofilm phenotypes of the strains were determined using semiquantitative biofilm assays and scanning electron microscopy (SEM) (2, 17). In the semiquantitative biofilm assay, the strains were allowed to form biofilm for 18 h at 37°C. The biofilm was stained with crystal violet and quantified by measuring the absorbance at 492 nm. ATCC 12228, HB, and Tü3298 were biofilm negative, whereas the strains with the *ica* locus expressed formed biofilm (Fig. 1 and 2). Biofilm formation of HB-*ica* was significantly more pronounced than that of ATCC 12228-*ica* ($P = 0.0064$) and Tü3298-*ica* ($P = 0.0059$). We also determined expression of PIA by immunodot blots using anti-PIA antiserum (Fig. 3). The three wild-type strains lacked PIA expression, whereas the *ica* locus-expressed strains were PIA positive by immunodot blot. In SEM, strains ATCC 12228, HB, and Tü3298 adhered as individual cells on the coverslips, while the *ica* locus-expressed strains formed biofilm, with that of strain HB appearing most dense. Thus, expression of *ica* resulted in conversion of biofilm-negative to biofilm-positive *S. epidermidis* in all three investigated cases, confirming the reported immense importance of *ica* in the accumulation phase of *S. epidermidis* biofilm development (8).

A rat central venous catheter (CVC)-associated infection model (11) was used to evaluate the relative virulence of the

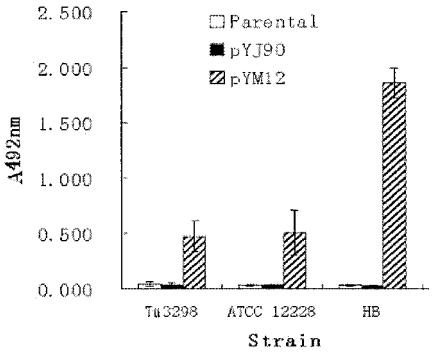

FIG. 1. Semiquantitative biofilm assay of *ica*-negative *S. epidermidis* strains and corresponding *ica* locus-expressed isogenic strains. Plasmid pYM12 (containing *icaRADBC*) or the control plasmid pYJ90 was transformed into three *ica*-negative *S. epidermidis* strains, ATCC 12228, HB, and Tü3298 respectively. Each experiment was repeated eight times. When A_{492} exceeded 0.12, the strain was defined as biofilm positive. The mean of eight experiments ± standard error is shown.

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TABLE 1. Metastatic infections in different organs by *S. epidermidis* strains in the rat CVC-associated infection model

Parameter and organ	Result for strain[a]:					
	ATCC 12228	ATCC 12228-*ica*	HB	HB-*ica*	Tü3298	Tü3298-*ica*
Liver						
No. of rats infected/ total	3/10	7/10	2/6	8/8	6/9	7/9
Median (range) CFU/g tissue	0 (0×10^2–1.3×10^2)	1.7×10^3 (0×10^5–2.7×10^5)	1.7 (0×10^2–1×10^2)	3.8×10^2 (3.3×10^1–6.2×10^3)	3.3×10^2 (0×10^4–4×10^4)	1×10^3 (0×10^4–8.3×10^4)
P value	0.019		0.0078		0.1631	
Kidney						
No. of rats infected/ total	2/10	7/10	2/6	8/8	5/9	6/9
Median (range) CFU/g tissue	0 (0×10^2–2.3×10^2)	2.7×10^2 (0×10^7–1.3×10^7)	2.5×10^1 (0×10^2–8.5×10^2)	2.6×10^3 (3.0×10^2–3.3×10^4)	6.7×10^2 (0×10^5–3.2×10^5)	4.3×10^3 (0×10^6–2.7×10^6)
P value	0.015		0.0066		0.7502	
Heart						
No. of rats infected/ total	3/10	7/10	1/6	6/8	4/9	7/9
Median (range) CFU/g tissue	0 (0×10^2–3.6×10^2)	3.8×10^2 (0×10^4–7.2×10^4)	5 (0×10^1–8.3×10^1)	2.0×10^2 (0×10^3–7.2×10^3)	0 (0×10^3–2.7×10^3)	2.3×10^3 (0×10^4–2.9×10^4)
P value	0.023		0.0645		0.0792	
Blood						
No. of rats infected/ total	4/10	6/10	0/6	7/8	2/9	5/9
Median (range) CFU/g tissue	0 (0×10^2–1.3×10^2)	1.5×10^2 (0×10^2–4.9×10^2)	0 (0–0)	8.6×10^2 (0×10^3–1.75×10^3)	0 (0×10^2–6.0×10^2)	10 (0×10^3–8.0×10^3)
P value	0.123		0.0037		0.2649	
Infection						
Infection rate (%)	30.0 ± 8.2	67.5 ± 5.0	20.8 ± 16.0	90.6 ± 20.0	47.2 ± 19.0	69.4 ± 10.6
P value	<0.0001		<0.0001		0.056	

[a] For statistical significance (Wilcoxon test), results represent animals challenged with *S. epidermidis* ATCC 12228, HB, or Tü3298 versus those challenged with *S. epidermidis* ATCC 12228-*ica,* HB-*ica,* or Tü3298-*ica.*

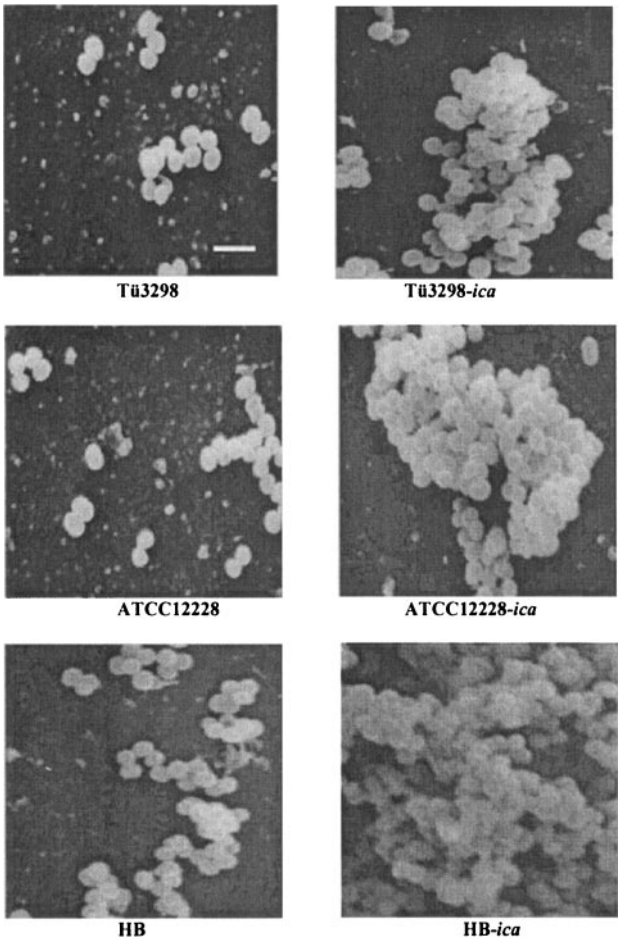

FIG. 2. Scanning electron microscopy of *S. epidermidis* biofilm. Bacteria adhered to the cover slides were fixed, treated, and observed by SEM. The bar represents a 1.5-μm scale.

parental strains ATCC 12228, HB, and Tü3298 and their isogenic strains, respectively. Briefly, the neck of the rat was dissected and a Silastic catheter was inserted in the right external jugular vein and advanced into the superior vena cava. A definite quantity of bacteria (about 10^5 CFU) was injected into the catheters after 24 h following CVC placement. The catheters were flushed daily with a heparin solution, and the animals were sacrificed at day 8. The comparison of overall infection rates, defined as recovery of the bacteria from the blood, liver, kidney, and heart at sacrifice, showed that more rats developed CVC-associated infection when they were challenged with ATCC 12228-*ica* and HB-*ica* than when challenged with the parental strains ATCC 12228 and HB (chi-square test, both $P < 0.0001$). For all organ systems, there were more animals with metastasis disease in the group challenged with strains with *ica*. In addition, for almost all tested organ systems, the number of bacteria recovered per gram of tissue was greater in the animals challenged with ATCC 12228-*ica* and HB-*ica* than in those challenged with their parental strains. According to Wilcoxon's test, these differences were statistically significant. There were differences in the infection rate and the number of bacteria recovered per gram of tissue between Tü3298 and Tü3298-*ica*. However, the differences were not significant. Table 1 summarizes the results from defining the burden of metastasis disease in animals challenged with either parental strains or their isogenic *ica* locus-expressed strains. Notably, strains with higher production of PIA also caused more pronounced virulence in the infection model, underlining the importance of PIA in biofilm-associated *S. epidermidis* infection. In conclusion, our results demonstrate that presence of *ica* significantly increases the virulence of *S. epidermidis*, confirming previous work by Rupp et al., who compared wild-type to *ica* mutant strains (12–14). As in our work, *ica* was present in multiple copies on a plasmid: the differences seen might be more pronounced than in those studies. Further, the differences in the amount of biofilm formation that we observed in the *ica* locus-expressed strains suggest that factors other than *ica* contribute to biofilm formation in *S. epidermidis* strains.

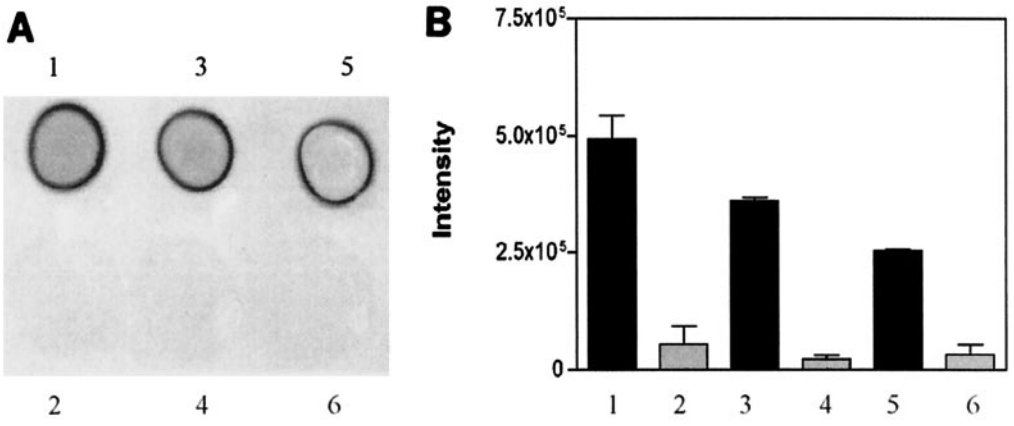

FIG. 3. PIA production in *ica*-negative *S. epidermidis* wild-type and corresponding *ica* locus-expressed strains. PIA was extracted from 24-h cultures by boiling with 0.5% EDTA for 5 min. Three-μl samples of the extract were spotted on nitrocellulose membrane, and PIA production was assayed by immunodot blot and evaluated by densitometry as described (15). The experiment was performed twice. (A) Representative immunodot blot. (B) Bars show the mean ± standard error: 1, Tü3298-*ica*; 2, Tü3298; 3, ATCC 12228-*ica*; 4, ATCC 12228; 5, HB-*ica*; 6, HB.

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