Evidence for low temperature regulation of biofilm formation in Staphylococcus epidermidis

In the intensive-care unit, device-related infections caused by Staphylococcus epidermidis and other coagulase-negative staphylococci represent an increasingly significant clinical problem because they involve the development of intrinsically resistant biofilms by pathogens that are already resistant to a growing number of antimicrobial agents. The high rate of intravascular catheterization and presence of multiple medical devices in intensive-care unit patients further highlights the clinical impact of these infections.

The best-characterized mechanism of S. epidermidis biofilm development is mediated by the icaA/icaBC-encoded polysaccharide intercellular adhesin (PIA) (Heilmann et al., 1996; Mack et al., 1994). The ica gene cluster, which is regulated by IcaR (Conlon et al., 2002a, 2002b; Jefferson et al., 2004), appears to have an important role in the pathogenesis of S. epidermidis infections and is thought to be an important marker discriminating between significant and contaminating isolates (Ziebuhr et al., 1997). Anaerobic growth (Cramton et al., 2001), the presence of sub-inhibitory concentrations of certain antibiotics, high temperature and environmental stresses (Rachid et al., 2000; Knobloch et al., 2001; Conlon et al., 2002a) all result in elevated expression of the ica operon or PIA synthesis in S. epidermidis.

We previously examined the environmental regulation of biofilm production by 21 S. epidermidis isolates associated with device-related infection and 19 contaminating isolates that were not associated with clinically diagnosed infection (Fitzpatrick et al., 2002). This study suggested that the presence of the ica locus alone was not sufficient for biofilm formation and that regulation of biofilm formation under altered growth conditions, which may exist in the in vivo environment, also plays a possible role in the pathogenesis of biomaterial-associated S. epidermidis infections (Fitzpatrick et al., 2002). Given that another important virulence determinant, methicillin resistance, is maximally expressed at 30 °C under laboratory conditions and that a number of studies have demonstrated a relationship between methicillin susceptibility and biofilm expression (Mempel et al., 1994, 1995), we examined the effect of varying incubation temperatures on biofilm development amongst this collection of S. epidermidis isolates.

Biofilm assays were performed as described previously (Conlon et al., 2002b) at 30 °C, 37 °C (control temperature) and 42 °C. In 22 % (9/40) of the strains biofilm formation was induced by altering incubation temperature. Four isolates demonstrated an increase in biofilm formation at 30 °C, four formed more biofilm at 42 °C and one ica-negative isolate formed biofilm at both incubation temperatures. These data, which confirm earlier findings, suggest that elevated temperatures can induce S. epidermidis biofilm development, but also further reveal for the first time that lower temperatures can also induce biofilm formation.

We chose two ica-positive S. epidermidis isolates, which were biofilm-negative at 37 °C, for further analysis. Biofilm development in isolate BH17 could be strongly induced at 30 °C (OD490 1.18 at 30 °C compared to OD490 0.1 at 37 °C) whereas biofilm was strongly induced in BH38 grown at 42 °C (OD490 1.6 at 42 °C compared to OD490 0.1 at 37 °C).

RT-PCR was employed as described previously (Conlon et al., 2002b) to measure the transcriptional activity of the icaA and icaR genes at 30 °C, 42 °C and 37 °C in these isolates (Figs 1 and 2). Consistent with previous findings (Rachid et al., 2000; Conlon et al., 2002a), this revealed that activation of the ica operon was associated with biofilm development at 42 °C in BH38. In contrast, in BH17 the enhanced biofilm formation at 30 °C was not associated with activation of the ica operon suggesting that induction of biofilm formation at 30 °C may be ica-independent. Consistent with this, three of the isolates in which biofilm formation was temperature-regulated lacked the ica gene cluster. Two of these demonstrated increased biofilm-forming capability at 30 °C compared to 37 °C, while the other formed more biofilm at both 30 °C and 42 °C than at 37 °C.

The transcriptional regulation of atlE, which encodes the major autolysin, and aap, which encodes the accumulation-associated protein, were also investigated in isolate BHSE17. Both of these genes have been implicated in S. epidermidis biofilm formation (Hussain et al., 1997; Heilmann et al., 1997). RT-PCR analysis revealed no
temperature-dependent regulation of aap (Fig. 1). However, a small increase in atLE transcription was measured at 30°C (Fig. 1), which may contribute in part to enhanced biofilm formation.

Taken together these findings reveal that lower temperatures can be added to the list of environmental conditions known to influence biofilm formation in S. epidermidis. Given that the normal temperature of skin is approximately 33°C it is tempting to speculate that this temperature may favour adherence and contribute to staphylococcal colonization and persistence. Moreover, our data reveal that of the five isolates identified in this study that were capable of enhanced biofilm formation at 30°C, three were contaminating strains, possibly shed from the skin of the patient or a healthcare professional. Finally, because our evidence suggests that biofilm development at 30°C is not necessarily dependent on the ica locus or ica activation, it seems possible that S. epidermidis colonization and persistence on skin may also involve an uncharacterized, ica-independent adherence mechanism.

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