SHORT ARTICLE

The partitioning of *Staphylococcus epidermidis* in Aqueous two-phase systems

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Summary. The surface properties of two isolates of *Staphylococcus epidermidis* were compared by cell partitioning in aqueous two-phase systems. Strain K805 was isolated from the cerebrospinal fluid of a child with a shunt infection and strain 1105 was obtained from human faeces and not known to have caused infection. Strain K805 was significantly more negatively charged than strain 1105 but there was no significant difference in hydrophobicity when cultures were grown for 18 h. However, prolonged incubation of strain K805 caused the production of extracellular slime and a marked increase in surface hydrophobicity. Both strains showed enhanced growth in biphasic cultures.

Introduction

In recent years the incidence of infections due to coagulase-negative staphylococci has increased (Wade *et al.*, 1982; Hensey *et al.*, 1985). Such infections are generally associated with the insertion of inert materials into normally sterile body compartments and have been seen in patients with ventriculo-atrial or -peritoneal catheters (Callaghan *et al.*, 1961; Shurtleff *et al.*, 1974), vascular prostheses (Liekweg and Greenfield, 1977) or in patients undergoing continuous ambulatory peritoneal dialysis (Chan *et al.*, 1981). A key step in the initiation of these infections is the adherence of bacteria to the inert surface. This in part depends upon the nature of the inert surface because bacteria are able to adhere to different plastics with different degrees of efficiency (Ashkenazi, 1984), but also depends upon bacterial surface properties. By employing partitioning in aqueous two-phase systems of polyethylene glycol (PEG) and dextran, which have been used extensively to delineate surface properties of bacterial cells (Magnusson and Stendahl, 1985), we have examined the hydrophobicity and surface charge of two isolates of *Staphylococcus epidermidis*.

Materials and methods

Bacteria

Both strains of *S. epidermidis* were identified by the API-Staph system (API Products, Basingstoke). Strain K805 was isolated from the CSF of a child with a ventriculo-peritoneal shunt infection, and strain 1105 was isolated from the faeces of a healthy adult.

Bacteria were grown in Nutrient Broth (Oxoid) at 37°C in a shaking incubator for 18 h. Numbers of bacteria were estimated turbidimetrically at 550 nm, having established that optical density was linearly related to bacterial numbers determined with a haemocytometer ($r = 0.95$; $n = 23$; $p < 0.001$).

Slime production was visualised by staining bacteria with alcian blue-carbol fuchsin (McKinney, 1953).

Phase partitioning

Aqueous polymer two-phase systems containing PEG 8000 (Union Carbide) 4.62% w/w and Dextran T500 (Pharmacia) 4.62% w/w in 0.03M Tris-HCl buffer, pH 7.0, were prepared in 10-ml glass centrifuge tubes from autoclaved stock solutions of PEG (40% w/w) and dextran (20% w/w, standardised by polarimetry). To determine bacterial surface negative charge, the positively-charged polymer trimethyl ammonium PEG 8000 (PEG-TMA, Aqueous Affinity, S23200, Arlov, Sweden) at concentrations of 0.625 or 1.25% w/w was included in the phase system, replacing an equivalent amount of the PEG.

Table. Partitioning behaviour of two strains of *S. epidermidis*

Two-phase system	Percentage of bacteria present in PEG-rich top phase*		
	Strain K805	Strain 1105	<i>p</i> value
PEG 8000 and Dextran T500	4.7 ± 4.4 (7)	2.3 ± 3.2 (3)	NS
with PEG-TMA 0.625% w/w	29.5 ± 18.4 (7)	8.2 ± 5.9 (3)	<0.05
with PEG-TMA 1.25% w/w	58.7 ± 15.0 (7)	11.9 ± 2.3 (3)	<0.05
with PEG-PALMITATE 0.5% w/w	20.0 ± 12.8 (7)	14.1 ± 9.5 (3)	NS

* Results are expressed as the mean ± SD for the number of experiments given in parenthesis.

Hydrophobic affinity partitioning was performed by replacing part of the PEG by monopalmitoyl PEG (PEG-palmitate), synthesised by the method of Shanbhag and Johansson (1974).

Partitioning was performed by adding 0.1 ml of a bacterial suspension (containing $c. 10^8$ – 10^9 cells, washed and gently resuspended in Tris buffer) to 3.9 g of the phase system, mixing by gentle shaking, and allowing phase separation to proceed at 0°C for 1 h. The final concentration of both PEG and Dextran was 4.5% w/w. The numbers of bacteria in the PEG-rich top phase and the dextran-rich bottom phase were then estimated turbidimetrically. Results were expressed as the percentage of cells added to the phase system that were present in the top phase. Statistical analyses were performed by two-sample *t*-tests.

Results

Both isolates of *S. epidermidis* partitioned predominantly into the dextran-rich lower phase (table). Inclusion of the positively-charged PEG-TMA at a concentration of 0.625% w/w or 1.25% w/w caused increases in the number of each of the isolates being found in the upper phase. This indicated that both were negatively charged. However, the increase in partitioning into the upper phase was significantly greater for strain K805 (table), indicating that this strain was more negatively charged than strain 1105. When PEG-palmitate 0.5% w/w was included in the phase system, there was an increase in the partitioning of both isolates into the upper phase, showing that both had a degree of surface hydrophobicity, but there was no significant difference between the two isolates. The high concentration of PEG-palmitate required to produce significant partitioning into the top phase (0.5% w/w) indicated a low tendency to hydrophobic interactions.

By allowing strain K805 to remain at 37°C in nutrient broth without shaking for 7 days, slime

production was greatly increased. The surface properties of strain K805 grown in these conditions were compared with those of strain K805 grown for 18 h as described in the *Materials and methods* section, conditions that produced no detectable slime. There was no difference in the partitioning into the upper phase between the slime-producing and non-producing cells in the presence of PEG-TMA 0.625% w/w (29% and 25%, respectively) or PEG-TMA 1.25% w/w (78% and 58%, respectively). However, in the presence of PEG-palmitate a far greater proportion of the slime-producing cells partitioned into the upper phase (70% and 10%, respectively). This indicated that the presence of slime may be associated with an increase in surface hydrophobicity. Strain 1105 did not produce slime on prolonged incubation.

Strains K805 and 1105 were grown for up to 24 h in a shaking incubator in a phase system of PEG 4.5% w/w and dextran 4.5% w/w prepared in nutrient broth. Both strains showed rapid growth and grew to higher densities than bacteria incubated in either nutrient broth alone, or in broth containing only one of the polymers (figure). No difference in growth rate was detected between the pathogenic and non-pathogenic strains. Preliminary comparisons of the surface properties of phase-grown bacteria and normally grown bacteria showed no differences for the pathogenic strain K805 but a decrease in charge and hydrophobicity for phase-grown strain 1105.

Discussion

Our observation that the surface of *S. epidermidis* is negatively charged and is of low hydrophobicity is consistent with that of Colleen and co-workers (1979, 1980). They compared isolates of *S. epidermidis* with *S. saprophyticus* and found that *S.*

Figure. Growth curves of *S. epidermidis*. Strain K805(a) and strain 1105(b) were grown in nutrient broth (□), Dextran T500 4.5% w/w in nutrient broth (○), PEG 4.5% w/w in nutrient broth (×), and in a biphasic system of Dextran 4.5% w/w and PEG 4.5% w/w in nutrient broth (■). Samples were removed at intervals, diluted and absorbance measured at 550 nm.

saprophyticus had a higher net negative charge and showed greater adherence to human uroepithelial cells than *S. epidermidis*. Both species exhibited poor hydrophobicity. We have compared the surface properties of two isolates of *S. epidermidis*. Strain K805 had been isolated from the CSF of a child with a shunt infection and strain 1105 was from human faeces and was not known to have caused infection. Although it is not possible to be certain that strain 1105 could not produce shunt infection, there were clear differences in their surface properties. The "pathogenic" strain K805 was significantly more negatively charged than strain 1105.

Prolonged incubation of strain K805 in nutrient broth without shaking induced the production of large amounts of extracellular slime. This was associated with an increase in surface hydrophobicity. Adherence of *S. epidermidis* to inert surfaces has been associated with the production of extracellular slime (Bayston and Penny, 1972); consequently its physicochemical properties may be important in determining the ability of a bacterium to adhere. An increase in hydrophobicity due to

slime production would tend to favour increased association of a bacterium with an inert surface.

It is known that bacteria can be grown in biphasic polymer systems, a procedure which is used for extractive bioconversion (Mattiasson, 1983). Both isolates of *S. epidermidis* grew better in a biphasic system in nutrient broth than in nutrient broth alone, in PEG 4.5% w/w in nutrient broth, or in dextran 4.5% w/w in nutrient broth. Stimulation of growth may be a consequence of the emulsion formed on shaking. This would allow partitioning of culture components and also provide an extensive liquid-liquid interface. The observation that growth in biphasic culture did not alter the surface properties of the pathogenic strain K805 whereas it did for strain 1105 suggests that biphasic culture may provide information on the role of bacterial surface properties in pathogenesis. Furthermore, growth of bacteria in such biphasic systems may prove useful in examining the attachment to, and growth of, bacteria on surfaces.

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