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Encapsulation, slime production and surface hydrophobicity of coagulase-negative staphylococci

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1. INTRODUCTION

Infections associated with prosthetic implants or medical devices are most commonly caused by coagulase-negative staphylococci (CNS) [1]. The ability of CNS to produce slime has been suggested to play a critical role in the adhesion onto surfaces of implants and medical devices [2] and in the course of medical device-associated infections [3]. *Staphylococcus aureus*, the pathogenic counterpart of CNS, can be encapsulated [4]. Such strains possessed increased virulence in comparison with their non-encapsulated variant strains [5,6]. Characterization of CNS has been advocated [7,8], but no special attention has been given to the presence of a capsule, although encapsulated CNS have been reported [9]. Bacterial adhesion onto surfaces is also regarded to be an important virulence factor [10]. Bacterial interaction with mammalian cells and artificial surfaces is promoted by the hydrophobicity of the bacterial cell surface [11-13].

After characterization of twenty one CNS, the presence of a capsule and the ability to produce extracellular slime were studied. In addition, the surface hydrophobicity of the strains was measured using a xylene-water system [14].

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2. MATERIALS AND METHODS

2.1. Bacterial strains and growth conditions

21 CNS strains isolated from open-heart surgery patients and laboratory staff members were classified according to the scheme of Kloos and Schleifer [8] using the API Staph gallery (API Systems S.A., Montalieu Vercieu, France). 7 strains (NCTC) were a gift of Dr. R.R. Marples, Central Public Health Laboratory, Colindale Avenue, London, England. Strains stored at $-20°C$ in skim milk were grown on sheep blood agar plates (Oxoid, Ltd., U.K.) and maintained on nutrient agar slants (Oxoid) at 4°C for 1 month.

The strains were cultured in Trypticase soy broth (TSB, BBL Microbiology Systems, Cockeysville, MD) for 20 h at 37°C. Late exponential phase cells were obtained by culturing 2 ml of the overnight broth into 100 ml of fresh TSB for 5 h at 37°C in a rotary shaker incubator (New Brunswick Sci. Co., New Brunswick, NJ) at 90 rev./min. The bacteria were harvested by centrifuging ($20000 \times g$, 4°C, 10 min; Beckman Instruments Inc., J2-21, Palo Alto, CA), washed three times with phosphate-buffered saline (PBS; 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 140 mM NaCl, 3 mM KCl, pH 7.2) and resuspended in PBS. To determine the effect of glucose depletion, all strains were grown in glucose-free TSB (GF-TSB, BBL).

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2.2. *Presence of bacterial capsules and production of slime*

Bacteria grown for 20 h at 37°C were stained using the India ink wet-film method to demonstrate the presence of capsules [15]. The ability of the strains to produce slime was tested as follows. Glass culture tubes containing 20-h-old cultures in TSB were emptied and filled with alcian blue (0.1% w/v, ICI, Macclesfield, Cheshire, U.K.) or safranin (0.1% w/v, Brocades, Maarssen, The Netherlands) solutions for 60 s to stain the extracellular material (slime) adherent onto the glass tubes [2].

2.3. *Bacterial cell-surface hydrophobicity*

The bacterial cell-surface hydrophobicity was determined by measuring the affinity of the bacteria towards xylene in a water–xylene two-phase system according to Rosenberg et al. [14]. Washed cells (late exponential growth phase) were suspended in 3 ml of PBS to an absorbance (A) of 1.0 at 540 nm (Beckman Instruments Inc. model 24 spectrophotometer, Fullerton, CA), corresponding with approx. 10^9 colony forming units/ml as determined by colony count. Various volumes of *p*-xylene (Merck, Darmstadt, F.R.G.) ranging from 0.03 to 0.25 ml were added to test tubes containing the bacterial suspensions. The tubes were vortexed for 60 s and, after phase separation, A was measured at 540 nm. Bacterial suspensions without xylene were used to measure the initial A. All strains were tested twice.

By washing, slime-producing cells will lose their extracellular slime layer [15]. Therefore, non-washed cells were also subjected to the xylene-test. However, non-washed cells suspended in PBS may still be contaminated with compounds from their growth medium. To determine the effect of the presence of medium compounds, fresh TSB was added in a final concentration of 1% (v/v) to suspensions of washed bacterial cells in PBS before cells were subjected to the xylene-test.

To study the effect of a proteolytic enzyme on the bacterial cell surface, the bacteria were suspended for 1.5 h at 37°C in 0.2 M citrate buffer (pH 3.0) containing 0.65% (w/v) NaCl (citrate buffered saline) and 0.1% (w/v) pepsin (Sigma Chemical Co., St. Louis, MO). The suspensions were then chilled rapidly, centrifuged (10 000 × *g*, 4°C, 10 min) and the bacteria were washed with ice-cold PBS. Bacterial cells suspended in enzyme-free citrate buffered saline (pH 3.0) were used as controls.

3. RESULTS AND DISCUSSION

Classification of 21 CNS strains revealed that they belonged to 5 different species (Table 1). Capsules were observed in 8 strains of which only one (NCTC100892) was an *S. epidermidis* species. All strains of the 4 other species were encapsulated. However, less dense capsules are very difficult to observe using wet India ink films, because the ink particles may penetrate such capsules. Slime was produced by some of the encapsulated and non-encapsulated strains grown in TSB. In the xylene-test, six of the non-encapsulated strains showed a decrease of their initial A values to 11–21% (Table 1). The decrease of the A values of 4 other non-encapsulated strains was moderate (36–59%) and that of three was negligible. Except for *S. saprophyticus* (SAP1), encapsulated CNS consistently showed a strong decrease in A (4–12%) indicating the prescence of hydrophobic constituents in their capsules, whereas the A values of non-encapsulated CNS varied indicating that their cell-surface character was either hydrophilic or hydrophobic.

According to the results obtained by Christensen et al. [2] slime production substantially decreased if simple-producing CNS were cultured in glucose-free (GF)-TSB. The surface hydrophobicity of washed cells of slime-producing strains grown either in GT-TSB or TSB was similar (Table 2), although the slime production was decreased in cells grown in GF-TSB. However, the cell surface hydrophobicity of washed bacteria can be affected by glucose depletion, as was shown in one non-slime-producing strain (NCTC100835) which was hydrophilic after growth in TSB, but showed an increased hydrophobicity after growth in GF-TSB.

The presence of the M protein in the cell wall of *Streptococcus pyogenes* or the protein A in *S. aureus*

Table 1

Encapsulation, slime production and surface hydrophobicity of 21 strains of coagulase-negative staphylococci identified using API Staph gallery

Species	Assigned code	Encapsulation [a]	Slime production [b]	Absorbance in xylene-test [c] (%)
S. epidermidis	NCTC100835	−	−	100
S. epidermidis	NCTC100623	−	−	96
S. epidermidis	K2	−	−	72
S. epidermidis	NCTC100641	−	−	59
S. epidermidis	SL76	−	+	50
S. epidermidis	G1	−	−	49
S. epidermidis	NCTC100600	−	−	36
S. epidermidis	SEP1	−	−	21
S. epidermidis	NCTC100894	−	−	18
S. epidermidis	PRE69	−	+	15
S. epidermidis	SEP2	−	−	13
S. epidermidis	SL58	−	+	12
S. epidermidis	SL26	−	+	11
S. epidermidis	NCTC100892	+	−	7
S. saprophyticus	SAP1	+	−	100
S. saprophyticus [d]	NCTC100619	+	+	7
S. saprophyticus	A1	+	−	4
S. hominis	Y2	+	−	7
S. hominis	SL33	+	+	7
S. cohnii	K1	+	−	12
S. haemolyticus	A2	+	−	8

[a] Presence (+) or absence (−) of a capsule as demonstrated by the India ink wet-film method [14].
[b] Presence (+) or absence (−) of slime production as demonstrated by staining emptied glass culture tubes using alcian blue or safranin [2].
[c] Percentage of the initial *A* (1.0) of the bacterial suspensions (3 ml) after addition of 0.25 ml xylene [15].
[d] This strain showed aberrant arginin hydrolase and urease test using API Staph gallery.

has been regarded to affect the cell surface hydrophobicity of the bacteria [11]. Protein constituents at the bacterial surface also appeared to affect the hydrophobicity of CNS, since the hydrophobicity of all pepsin-treated bacteria strongly decreased (Table 2). The observation of encapsulated *S. saprophyticus*(SAP1) which is hydrophilic, suggests the absence of such protein-containing components at the capsular surface of this strain. Protein-containing constituents which are pepsin-sensitive appeared to be the major hydrophobic sites in the cell wall surfaces or in the capsules. Such structures might contribute to the hydrophobic bonding of bacteria onto surfaces [12,13].

In order to study the effect of washing on the affinity of bacterial cells towards xylene, *A* values of suspensions of non-washed cells were compared to those of suspensions of normal PBS-washed cells. After addition xylene, the *A* decrease of suspensions of non-washed cells of a slime-producing strain (final value 70% of the initial *A*) was much less than that of suspensions containing washed cells of this strain (final value 14%) (Fig. 1a). Suspensions of non-washed cells of a non-slime producing strain reached an *A* value of 52%, whereas suspensions of washed cells had an *A* of 14% (Fig. 1b). The *A* values of suspensions of washed bacteria from the slime-producing or non-slime-producing strain raised from 14% to 46% after addition of a small volume of TSB. These findings show that the higher *A* values of non-washed bacteria probably were caused by residues

Table 2

Slime production and optical density values of suspensions of seven coagulase-negative staphylococci subjected to the xylene-test. Bacteria were grown in Trypticase soy broth (TSB) or in glucose-free Trypticase soy broth (GF-TSB). Bacteria grown in TSB were washed with PBS and treated with pepsin (0.1% w/v) in citrate buffered saline (pH 3.0).

Staphylococci coded	Culture Medium				Treatment pepsin (0.1%) *A* (%)
	TSB		GF-TSB		
	Slime production [a]	*A* [b] (%)	Slime production	*A* (%)	
SAP1 (encapsulated)	–	100	–	97	100
NCTC100835	–	100	–	56	100
SEP1	–	21	–	13	100
PRE69	+	15	–	15	93
SEP2	–	13	–	13	100
SL26	+	11	–	6	ND [c]
A1 (encapsulated)	–	4	–	4	96

[a] See Table1, footnote b.
[b] See Table 1, footnote c.
[c] not determined.

of broth. However, the final *A* of suspensions of non-washed slime-producing cells was much higher than that of suspensions of non-slime producing cells, indicating that slime was removed by washing.

These results show that the presence of slime strongly affects the hydrophobicity of the bacterial surface.

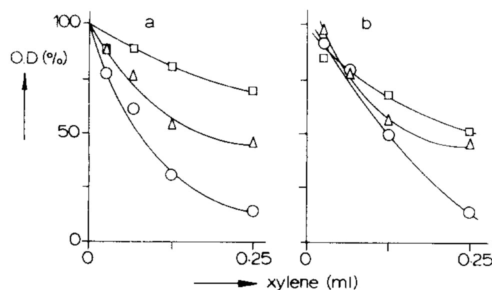

Fig. 1. Absorbance (*A*) of suspensions of a slime-producing *Staphylococcus epidermidis*, PRE 69 (a) and a non-slime-producing *Staphylococcus epidermidis*, SEP 1 (b) in phosphate buffered saline as a function of the volume of xylene added. *A* values obtained from suspensions with washed bacteria (○——○) and non-washed bacteria (□——□). To determine the influence of residues of growth medium in non-washed cell suspensions, *A* values were measured in suspensions with washed cells to which TSB was added to a final concentration of 1% (v/v) (△——△).

The results obtained from the xylene-test indicate that CNS strains showed marked differences in their cell surface hydrophobicity. Data on hydrophobicity of CNS are scarce. Colleen et al. [16] using aqueous polymer two-phase systems found that three *S. epidermidis* and two *S. saprophyticus* strains were rather hydrophilic. The xylene test was used in our study because it is simple to perform and has shown to give useful information [14].

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