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Occurrence of lectins and hydrophobicity of bacteria obtained from biofilm of hospital catheters and water pipes

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J.C. FIORINA, M. WEBER AND J.C. BLOCK. 2000. Bacteria isolated from biofilms of water distribution pipes and colonized catheters from hospitalized patients were studied for their haemagglutination ability, expression of lectins and hydrophobicity. Higher haemagglutination ability of clinical strains for human red blood cells was demonstrated, which could be an expression of their adaptation to the human ecosystem. Environmental strains had higher hydrophobicity, possibly related to adaptation to a low nutritive ecosystem. Expression of lectins was relatively low and comparable in both bacterial populations, but carbohydrate specificities were very different, possibly related to a different implication of these structures in the two ecosystems.

INTRODUCTION

Almost all surfaces in contact with natural water are colonized by bacteria, to form biofilms (Costerton *et al.* 1987). The initial step by which bacteria adhere to a substrate involves a variety of bacterial surface components and may require both non-specific and specific interactions (Busscher and Weerkamp 1987). Non-specific interactions consider bacteria to be negatively charged particles at a specified distance from a surface. Hence, physicochemical interactions between bacteria and the surface can be separated into long-range interactions, which can be calculated according to the DLVO theory, and short-range interactions like hydrophobic interactions (Busscher and Weerkamp 1987; van Loosdrecht *et al.* 1987). Hydrophobic interactions have been extensively used in physicochemical assessment of bacterial adhesion (Doyle and Rosenberg 1995; Rosenberg *et al.* 1996). Cell surface hydrophobicity is a major parameter controlling the adhesion of bacteria to stainless steel (Vanhaecke *et al.* 1990), silicone, polystyrene, glass (van Loosdrecht *et al.* 1987; Millsap *et al.* 1997), plastic catheters (Kristinsson 1989; Martinez-Martinez *et al.* 1991; Galliani *et al.* 1996), extracellular proteins (Zabel *et al.* 1996) and eukaryotic cells (Drumm *et al.* 1989; Wibawan *et al.* 1992; Ding *et al.* 1993; Bartková and Ciznár 1994; Frisk and Lagergård 1998).

Specific interactions occur between the components on the surface of a bacterium, adhesins, and specific molecules on the surface of the substrate (Mouricout 1997; St Geme 1997). Lectins are protein adhesins that bind specifically and reversibly to carbohydrates (Mirelman and Ofek 1986). The affinity of bacterial lectins for cell surfaces or in exocellular polymers is of biological significance in *Ps. aeruginosa* infections (Ramphal *et al.* 1991; Gilboa-Garber *et al.* 1994; Plotkowski *et al.* 1996). Lectins also play a role in the colonization of prosthetic devices by *Ps. aeruginosa* since blocking the lectin with carbohydrates significantly decreases the adherence of the bacteria to the coated surfaces (Jansen *et al.* 1990). Because of their wide range of surface carbohydrates the haemagglutination of human or animal erythrocytes is widely used to screen for lectins on bacteria, whereas inhibition of haemagglutination by carbohydrates indicates the specificity of lectins (Goldhar 1995).

Hydrophobic interactions have been widely studied in environmental bacteriology, while specific interactions have been mostly explored in medical bacteriology. Studies on the involvement of both hydrophobic interactions and lectin production in bacterial adhesion are rare (Ellen *et al.* 1994). There is some evidences of a link between specific and non-specific interactions in bacterial adhesion: haemagglutinin may be responsible for the hydrophobic properties of bacteria (Fish *et al.* 1987); some lectins contain an above-average fraction of hydrophobic amino acids (Yamada *et al.* 1994); lectins may also be produced by bacteria on highly hydrophobic structures (Mirelman and Ofek 1986); hydrophobic interactions are implicated in lectin-carbohydrate binding (Sharon 1993; Weis and Drickamer 1996) and bacterial hydrophobicity depends on the carbohydrate specificity of lectins (Jann *et al.* 1981).

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We have studied hydrophobicity, haemagglutination ability and the production of lectin-like structures to identify specific and non-specific interactions. As environmental conditions partially control bacterial hydrophobicity (Rogers *et al.* 1984; Allison *et al.* 1990; Ljungh and Wadström 1995) as well as bacterial lectin production (Gilboa-Garber 1988; Krogfelt 1995), we have worked on strains of bacteria from two ecosystems to explore the influence of environment on hydrophobicity and lectin production. Bacteria were obtained from the biofilms on domestic water distribution pipes (31 strains), and from colonized catheters in hospitalized patients (28 strains).

The bacteria studied were members of the Pseudomonadineae suborder for both ecosystems, as these bacteria are implicated in catheterization-related septicaemia (Elting and Bodey 1990) and are important in water distribution supplies (Hardalo and Edberg 1997).

MATERIALS AND METHODS

Sampling of bacteria and strains selection

The bacteria obtained from the biofilms on domestic water distribution pipes were named 'environmental' and those from colonized catheter in hospital patients 'clinical'.

Environmental bacteria (31 strains) were obtained from domestic drinking water pipes (16 strains), tank sediments of drinking water distribution system (eight strains) and drinking water contact surfaces (seven strains). Samples were obtained with sterile cotton swabs (Sayag) and plated on R2A-agar (Difco). We tested only quantitatively dominant microflora growing from the samples to enhance the probability of working with bacteria adapted to their environment. Strains were selected from quantitatively dominant microflora after incubation for 24–48 h at 30 °C. A total of 112 morphologically different strains were isolated and identified with Api20NE identification kits (BioMérieux, Marcy-l'Etoile, France). Non glucose-fermenting Gram-negative bacilli with profiles likely to correspond to Pseudomonadineae were selected (Table 1). The identified bacteria were stored at −80 °C in Microbank cryotubes (Pro-Lab Diagnostics, Ontario, Canada).

Clinical bacteria (28 strains) all came from catheters removed from infected patients at the C.H.R.U. Nancy. The end of the catheter was immersed in 1 ml BHI broth (BioMérieux), vortexed for 1 min and then 0·1 ml of broth was plated on Colombia agar supplemented with horse blood 5% (BioMérieux) and incubated for 24 h at 37 °C. Bacteria were identified with Api20NE identification kits and stored at −80 °C (Table 2).

Haemagglutination ability and lectin profiles

Each strain was grown at 30 °C in 200 ml R2A-broth (per litre: 0·5 g yeast extract (Merck), 0·5 g tryptic peptone (Merck), 0·3 g Na pyruvate (Merck), 0·1 g K_2HPO_4 (Prolabo), 0·05 g $MgSO_4,7H_2O$ (Aldrich), 0·01 g $CaCl_2$ (Prolabo) with a sterile air input. The flasks were shaken at 150 rev min^{-1} for 24 h. Cells were collected from the culture medium by centrifugation (10 000 *g*), washed twice in PBS, pH 7·2 and suspended to obtain an absorbance at 600 nm (A_{600}) of 2, corresponding to 2×10^{10} bacteria ml^{-1}.

The haemagglutination capacity of each strain was tested with human group O (Sigma, R0043), human group B (Sigma, R0252) and rabbit (Sigma, R1629) red blood cells (RBC). All tests were performed in duplicate in U microtitre plates (Greiner). Washed bacteria were twofold serially diluted in PBS up to 1/128. The haemagglutination titre was the greatest dilution causing haemagglutination. Suspensions of washed bacteria were adjusted to $A_{600} =$ 1·0. Lectins were detected by the inhibition of haemagglutination following incubation of the suspended bacteria with sterile solutions (50 mmol l^{-1} final concentration) of D-fructose, L-fucose, D-galactose, D-glucose, D-mannose, α-lactose and sucrose. Reproducibility of haemagglutination test on nine strains showed a significant difference (deviation higher than one dilution) between two sets of measurements for only one strain (data not shown).

The influence of proteolysis on haemagglutination was tested by incubating the suspensions of washed bacteria ($A_{600} = 1·0$) with trypsin type II-s (Sigma) at a final concentration of 10 mg ml^{-1} for 30 min at 30 °C and repeating the haemagglutination test. The resulting values of haemagglutination are given as the maximum haemagglutination titre (i.e. maximum haemagglutination titre obtained for the three RBC types tested).

Hydrophobicity measurement

The hydrophobicity of bacteria was measured by their adhesion to octane, as modified by Jorand *et al.* (1994). Each measurement was performed in triplicate on bacteria suspended in PBS. The resulting values are given as the median of the nine possible combinations of triplicates prior to and after adding octane, plus the interquartile value. Negative hydrophobicity values were reset to zero. The reproducibility of hydrophobicity tests on 17 measurements (nine strains) gave a mean difference of 8% in absolute value between two series of measurements (data not shown).

To test the influence of proteolysis and carbohydrates on hydrophobicity, each strain was washed three times with PBS (10 000 *g*, 15 min) for untreated bacteria hydrophobicity measurement. One suspension of bacteria was incu-

Table 1 Haemagglutination titre (HA), red blood cell (RBC) specificity, lectinic specificity and hydrophobicity of environmental strains (H%)

Strain*	Maximum HA titre	RBC specificity	Carbohydrate inhibition	H%	IQV
Sphingomonas paucimobilis	4	B, O, rabbit	–	70	6
†*Chr. luteola*/‡*Sph. paucimobilis*	0	–	–	1	2
§*Com. testosteronii*/*Ps. alcaligenes*	8	B	–	91	2
Stenotrophomonas maltophilia	0	–	–	22	1
Ps. vesicularis	16	O, rabbit	–	8	6
Sphingomonas paucimobilis	2	Rabbit	Galactose	21	2
Sphingomonas paucimobilis	4	B, O, rabbit	–	64	6
Sphingomonas paucimobilis	4	O	–	42	3
Ps. putida	4	O	Galactose, lactose	39	3
	4	Rabbit	Glucose, mannose	39	3
Ps. fluorescens	2	B, O, rabbit	Galactose, lactose	49	10
Ps. vesicularis	0	–	–	28	10
Ps. aeruginosa	4	O, rabbit	–	76	15
Ps. aeruginosa	4	B, O, rabbit	–	4	2
†*Chr. luteola*/‡*Sph. paucimobilis*	2	B, O	–	44	52
†*Chr. luteola*/‡*Sph. paucimobilis*	0	–	–	26	13
Sphingomonas paucimobilis	1	O	–	93	8
§*Com. acidovorans*	8	O	–	8	6
Ps. putida	128	O	Galactose, lactose	17	2
Unidentified strain	0	–	–	0	4
Pseudomonas fluorescens	2	O	–	25	7
Ochrobactrum anthropi	4	O	–	44	5
Ps. putida	8	Rabbit	Galactose, lactose	26	5
Ps. aeruginosa	32	Rabbit	–	60	7
Ps. putida	2	B, rabbit	Galactose, lactose	68	3
Ps. fluorescens	2	B, rabbit	Lactose	70	9
Ps. aureofaciens	32	Rabbit	Lactose	26	11
Ps. vesicularis	0	–	–	51	18
Ps. fluorescens	2	O	Mannose	24	7
Sphingomonas sp.	0	–	–	0	4
Ps. fluorescens	16	Rabbit	–	73	11
Ps. diminuta	128	B	–	56	7

*Putative identification of bacterial species are given as species 1/2.
†*Chr. Chryseomonas*; ‡*Sph. Sphingomonas*, §*Com. Comamonas*.
–, no reaction; IQV, interquartile value.

bated for 30 min at 30 °C with carbohydrate (50 mmol l^{-1} final concentration) that did not inhibit haemagglutination (glucose) or did inhibit haemagglutination. Another suspension ($A_{600} = 1{\cdot}0$) was incubated with 10 mg ml^{-1} trypsin type II-s (Sigma) for 30 min at 30 °C.

Statistical tests

All statistical analysis were computed with StatView (SAS Institute Inc., Cary, NC, USA). Equivalent distribution of tested parameter in populations studied was always postulated as the null hypothesis. Statistical tests were considered significant for *P*-values $< 0{\cdot}05$. The results are given as medians of the distribution. Three tests were used for continuous variables: the Wald–Wolfowitz test was used to compare two independent sets of measurements, the Wilcoxon signed rank test was used to compare two dependent series of measurements, and the Kruskal–Wallis test was used to analyse the distribution of variance. The chi-square of test has been used to test the distribution of discrete variables.

RESULTS

The environmental and clinical strains tested belonged to 14 species (Tables 1 and 2), with a relatively high number

Table 2 Haemagglutination titre (HA), red blood cell (RBC) specificity, lectinic specificity and hydrophobicity of clinical strains (H%)

Strain	Maximum HA titre	RBC specificity	Carbohydrate inhibition	H%	IQV
**Ps. aeruginosa*	128	O	–	0	23
†*Ac. haemolyticus*	32	O, rabbit	–	7	3
Burkholderia cepacia	4	Rabbit	–	38	9
Ps. aeruginosa	128	O	–	82	3
Ps. aeruginosa	32	B, O	–	0	4
Ac. baumanii	8	B, O	–	98	1
Ps. aeruginosa	128	O	Fucose	1	7
Ps. aeruginosa	0	–	–	5	2
Ps. aeruginosa	128	B, O	–	51	11
Stenotrophomonas maltophilia	0	–	–	24	7
Ps. aeruginosa	128	B	Fucose, mannose	46	4
Stenotrophomonas maltophilia	0	–	–	92	2
Ps. aeruginosa	16	B, O, rabbit	–	1	4
Ps. putida	4	B	–	4	2
Ps. aeruginosa	128	O, rabbit	–	0	8
Ps. aeruginosa	64	O	Mannose	28	14
Ps. aeruginosa	64	rabbit	–	0	12
Sphingomonas paucimobilis	2	O	Mannose	0	5
Ps. picketti	0	–	–	23	4
Ps. aeruginosa	64	O, rabbit	–	0	7
Ps. aeruginosa	8	Rabbit	–	36	9
Ps. putida	0	–	–	36	4
Ps. aeruginosa	32	O, rabbit	Mannose	84	7
Ps. aeruginosa	0	–	–	0	10
Ps. aeruginosa	16	O, rabbit	–	51	2
Ps. aeruginosa	16	O, rabbit	–	0	5
Sphingomonas paucimobilis	0	–	–	5	2
Ps. putida	1	Rabbit	–	5	11

**Ps.*, *Pseudomonas*, †*Ac.*, *Acinetobacter*.
–, no reaction; IQV, interquartile value

of Pseudomonadineae (55 of the 59 strains). The genus *Pseudomonas* was particularly well represented in the clinical population (75% of clinical strains *vs* 55% of environmental strains).

A total of 79% of the clinical strains and 77% of the environmental strains caused haemagglutination of red blood cells (Tables 1 and 2). The highest haemagglutination titres (maximum value obtained for one of the red blood cell types tested) varied from 0 to 128 (highest dilution tested). However, the maximum haemagglutination titre for clinical strains was significantly higher than for environmental strains (Wald–Wolfowitz test, $P = 0{\cdot}004$). *Ps. aeruginosa* strains had significantly higher haemagglutination titres than other clinical strains tested (Wald–Wolfowitz test, $P = 0{\cdot}049$). The clinical strains also had a higher affinity for human than for rabbit red blood cells (Wilcoxon rank test, $P = 0{\cdot}014$) and within the human RBC tested, for group O more than for group B (Wilcoxon rank test, $P = 0{\cdot}013$) (Tables 1 and 2). There was no statistical difference among environmental strains for any one type of red blood cell tested.

The prevalence of lectins as measured by the inhibition of haemagglutination by carbohydrates was relatively low, and statistically equivalent in the environmental (29%) and clinical populations (18%) (χ^2 test, $P = 0{\cdot}314$). The environmental strains were mainly galactose and lactose specific, whereas the clinical strains bore only fucose and mannose specificity. The maximum haemagglutination titres in the clinical population were higher for bacteria with lectins than for bacteria without lectin, but this difference was not statistically significant (Wald–Wolfowitz test, $P > 0{\cdot}05$). There was no difference in maximum haemagglutination

titres of environmental bacteria with and without lectins (Wald–Wolfowitz test, $P > 0.05$). Trypsin treatment had no influence on lectinic activity, except in one *Ps. aeruginosa* clinical strain (data not shown).

The hydrophobicity data were widely dispersed for both bacterial populations (0–98%) (Tables 1 and 2). Nevertheless, the hydrophobicity of environmental strain cell surfaces was significantly higher (39·5%) than that of clinical strains (6·3%) (Wald–Wolfowitz test, $P < 0.001$). *Ps. aeruginosa* strains had a lower hydrophobicity (4%) than the other clinical species (21%; Wald–Wolfowitz test, $P < 0.001$).

Treatment of bacterial cells with trypsin or carbohydrates had little or no significant influence on the hydrophobicity of environmental or clinical strains (Table 3). The environmental strains were all significantly more hydrophobic than the clinical strains regardless of treatment (Wald–Wolfowitz test, $P < 0.001$). The hydrophobicity of lectin-bearing bacteria of both populations was not modified by incubation with an inhibitory carbohydrate prior to the hydrophobicity test (Wilcoxon test, $P > 0.05$).

DISCUSSION

The adaptation of bacteria to environmental conditions markedly influences the structure and composition of bacterial cell surfaces (Costerton *et al.* 1985). Biofilm formation is one of the adaptive responses of bacteria to optimize their survival and development (Costerton *et al.* 1987; Lewis 1990; Andrews 1995). We used haemagglutination ability, possession lectins and hydrophobicity as markers of this bacterial adaptation to environmental conditions.

The haemagglutination ability of clinical and environmental bacteria depends on the species (Kapperud and Lassen 1983; Datta-Roy *et al.* 1986; Podschun and Sahly 1991). We found a wide range of haemagglutination titres in both clinical and environmental populations, indicating a variety of bacterial cell surface components, and different behaviours of individual strains. The greater haemagglutination ability of the clinical strains than the environmental strains is due to the high frequency of *Ps. aeruginosa* occurrence, and may give some ecological advantage to these opportunistic micro-organisms for invading host tissues (Prince 1992; Gilboa-Garber *et al.* 1994). Likewise, the greater affinity of clinical strains for human red blood cells than for rabbit red blood cells may have resulted from their adaptation to human ecosystems.

We found that the inhibition of haemagglutination by carbohydrates was not influenced by treating the bacteria with trypsin, except for one strain. This result should be interpreted cautiously, as the sensitivity will depend on the enzyme type and concentration (Goldhar 1994). Surprisingly, the lectin profiles of the clinical and environmental populations were similar. Growing the bacteria on a nutritive medium that modulates lectin production (Gilboa-Garber 1988; Goldhar 1995) may have masked any difference between the two groups. The low prevalence of lectins in the clinical bacteria may also be due to the attachment of the bacteria to inert synthetic material and not to human tissues, even if dialysis with dextrose solutions decreases the adhesion of *Ps. aeruginosa* to catheters (Reid *et al.* 1994). The comparable prevalences of lectins in clinical and environmental populations can also be interpreted as due to the non-negligible role played by lectins in biofilm cohesion, as has been demonstrated for *Vibrio cholerae* (Watnick *et al.* 1999).

However, there is a major difference in the lectin profiles of the two populations; the clinical bacteria have lectins binding to fucose and mannose, while the environmental ones are mainly orientated toward galactose and lactose. Similar differences in the specificity of lectins have been reported by Nagayama *et al.* (1994) for *Vibrio* strains, but the significance is unknown, as is the *in situ* production of such ligands.

Because of the rarity of lectins in both bacterial populations, we could establish no relationship with the haemagglutination titres. However, considering the haemagglutinating ability of bacteria as being equivalent to the presence of lectins may be a source of confusion. While haemagglutination is often due to the presence of lectins, it

Table 3 Median hydrophobicity (with interquartile values) of environmental and clinical strains

Treatment	Environmental strains	Clinical strains
Hydrophobicity (H%) without treatment	39·5 (40·2)	6·3 (41·4)
H% after trypsin treatment	44·6 (40·2); $P = 0.416$*	5·8 (32·8); $P = 0.455$*
H% after incubation with noninhibitor carbohydrate	38·5 (50·5); $P = 0.234$*	9·9 (33·6); $P = 0.048$*
H% after incubation with inhibitor carbohydrate	22·7 (38·0); $P = 0.213$*	16·8 (43·4); $P = 0.273$*

*Compared with strains without treatment in Wilcoxon test.

may also result from other mechanisms, such as hydrophobic interactions (Garber *et al.* 1985).

The greater hydrophobicity of the environmental strains than the clinical strains could be an adaptation to their environment. *Ps. aeruginosa* strains was less hydrophobic than the other clinical or environmental bacteria. Lower hydrophobicity is related to the easier release of bacteria from biofilm (Allison *et al.* 1990; Ascon-Cabrera 1995), rendering bacteria available for further colonization and hence the spread of infection. The action of trypsin on the hydrophobicity of environmental and clinical bacteria was strain dependent. There appears to be no protein structure that is sensitive to trypsin systematically involved in bacterial hydrophobicity, even digestion with another enzyme could give different results (Moser and Schröder 1997).

Although adhesins are frequently located on highly hydrophobic structures and hydrophobic interactions are involved in lectin–carbohydrate binding, we find no relationship between lectins and hydrophobicity. Furthermore, *Ps. aeruginosa* has a greater haemagglutinating ability than other clinical strains, but is less hydrophobic. Incubation with specific and non-specific carbohydrates to block lectins does not influence overall cell hydrophobicity. The lack of difference in cell hydrophobicity after incubation with specific and non-specific carbohydrates indicates that lectin-like structures have little influence on bacterial hydrophobicity, possibly because the low density of lectin-like molecules or the predominant involvement of other structures.

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