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Cell Surface Hydrophobicity of Pigmented and Nonpigmented Clinical *Serratia marcescens* Strains

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The cell surface hydrophobicity of 10 pigmented and 4 nonpigmented clinical *Serratia marcescens* strains was studied, based on the ability of the strains to adhere to hydrocarbons and to polystyrene. The cell surface hydrophobicity depended greatly on growth temperature; all of the strains tested were adherent following growth at 30°C, whereas none was adherent following growth at 38°C. In previous studies, the pigment prodigiosin has been cited as responsible for cell surface hydrophobicity in various *Serratia* strains. However, the observed ability of the nonpigmented strains to adhere to the test hydrocarbons and to polystyrene indicates that *Serratia* strains can possess hydrophobic surface properties in the absence of this pigment. Moreover, strain 1785 cells were adherent whether they were grown at 30 or 36.5°C, even though pigment was not synthesized at the higher temperature. In *Escherichia coli* correlations have been noted between increased cell surface hydrophobicity and the presence of mannose-specific adhesins; no such relationship was found in the *S. marcescens* strains tested. The expression of cell surface hydrophobicity in clinical *S. marcescens* strains at 30°C and the loss of hydrophobicity at host temperatures raise the possibility that infective cells from the environment are initially hydrophobic, but lose this property upon subsequent proliferation within a host.

In recent years, the role of *Serratia marcescens* strains in causing opportunistic infections has attracted increasing attention (26). Although specific adhesins and aggregating factors of clinical *Serratia marcescens* isolates have been investigated (1, 15, 18), attention has not been given to the cell surface hydrophobicity of such strains, the possible role of such hydrophobicity in microbial pathogenesis, and the molecular components responsible. This is somewhat surprising in view of the many ecologically oriented studies on the importance of the cell surface hydrophobicity of *Serratia* cells in enabling them to adhere at interfaces (21, 22, 24, 24a, 28) and to scavenge nutrients there (12).

Cell surface hydrophobicity is currently regarded as an important factor in mediating bacterial adherence to a wide variety of surfaces (2–6, 8–14, 16, 20–25, 27–29). Most studies on hydrophobicity in *Serratia marcescens* have attributed this property to the presence of the hydrophobic pigment prodigiosin (7, 30, 31) on the cell surface (3, 9, 13, 19, 21, 24). In the present study, we examined the hydrophobic surface properties of pigmented and nonpigmented clinical *Serratia marcescens* isolates based on their ability to adhere to hydrocarbons (24) and to polystyrene (21). Cell surface hydrophobicity was assayed following growth at temperatures which either allow or inhibit prodigiosin synthesis (30, 31). Our data indicate that pigmentation alone cannot account for the hydrophobicity of the strains studied and suggest the possible involvement of additional factor(s). No correlation was found between cell surface hydrophobicity and the presence of mannose-specific adhesins, as has been reported in *Escherichia coli* (15, 18).

The finding that all of the strains tested were hydrophobic following growth at 30°C but not following growth at 38°C raises the possibility that modulation of this property may be of some importance in *Serratia marcescens* pathogenesis.

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MATERIALS AND METHODS

Bacterial strains and growth conditions. Clinical *Serratia marcescens* strains were obtained from the Chaim Sheba Medical Center, Tel-Hashomer, Israel (pigmented strains 55, SH-1, 1704, II_9A, BY-1, and I_5 and nonpigmented strains 3255, 577, 2861, and BY-2) or from the Rambam Hospital, Haifa, Israel (pigmented strains 183, 1785, and 1515). *Serratia marcescens* RZ is the strain used in previous investigations (21, 22, 24) and was originally obtained from R. Zack, Tel-Aviv University, Ramat-Aviv, Israel. The bacteria were maintained on brain heart agar (Difco Laboratories, Detroit, Mich.) at 4°C and were transferred every 2 months. For adherence and aggregation experiments, the bacteria from a single colony were spread onto brain heart infusion agar and incubated for 24 h under aerobic conditions at the temperatures indicated below.

Adherence and aggregation experiments. Following growth, the bacteria were scraped from the agar plates, washed twice, suspended in PUM buffer (22.2 g of $K_2HPO_4 \cdot 3H_2O$, 7.26 g of KH_2PO_4, 1.8 g of urea, 0.2 g of $MgSO_4 \cdot 7H_2O$, distilled water to 1,000 ml, pH 7.1) to an optical density at 400 nm of 1.0 to 1.2, as measured with a Kontron 710 spectrophotometer (Uvikon, Zurich, Switzerland), and subsequently tested for adherence to hydrocarbons and polystyrene and for mannose-sensitive aggregation of yeast cells. Adherence to hydrocarbons was tested as described previously (24). Briefly, 0.2 ml of a test hydrocarbon (*n*-hexadecane, *n*-octane, or *p*-xylene) was added to 1.2 ml of an aqueous bacterial suspension in standard acid-washed test tubes, and the mixtures were vortexed uniformly for 120 s. Following phase separation, the bottom aqueous phase was carefully removed and transferred to cuvettes, and the optical density at 400 nm was read. Results were expressed as the percent drop in the turbidity of the lower aqueous phase compared with the turbidity of the original suspension. To test for adherence to polystyrene,

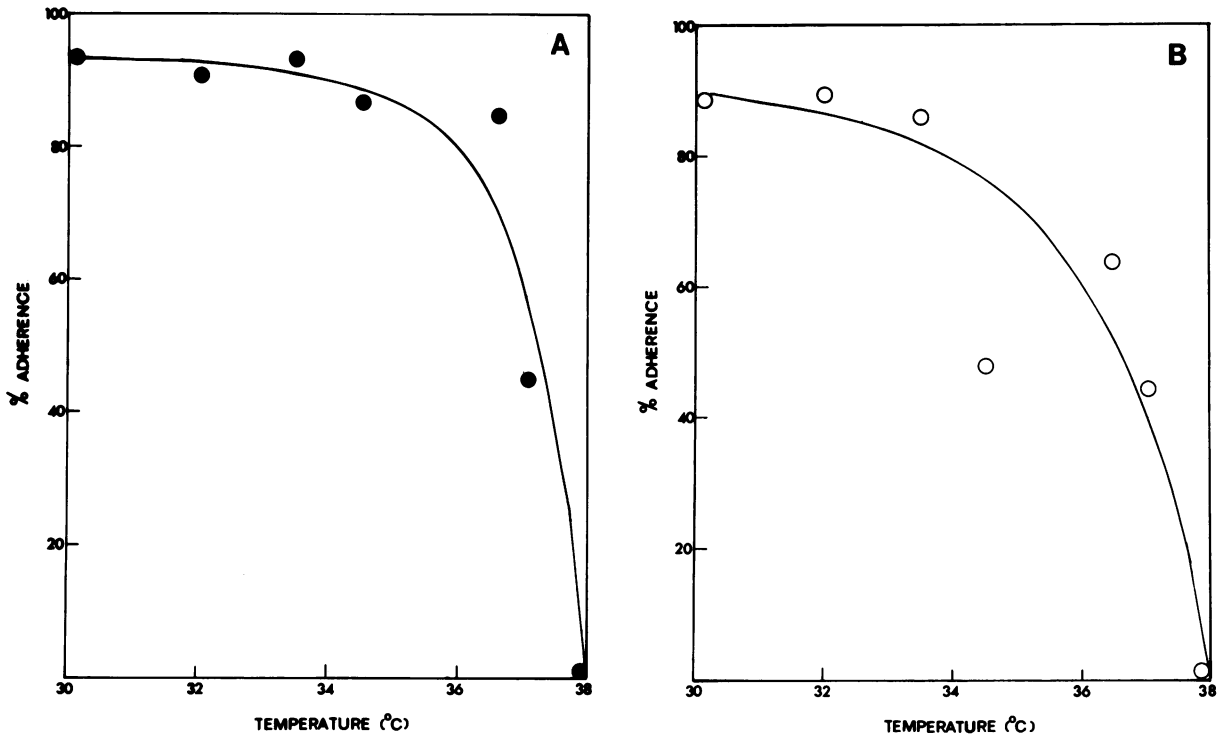

FIG. 1. Effect of growth temperature on adherence to hexadecane. Cells were grown at various temperatures and tested for adherence to 0.2 ml of hexadecane as described in Materials and Methods. The results are presented as the percent adherence of pigmented strain 1785 (A) and nonpigmented strain 3255 (B) to hexadecane as a function of growth temperature. Similar results were obtained for pigmented strain RZ (data not shown).

100-μl portions of a bacterial suspension were placed on polystyrene petri dishes (Miniplast, Ein Shemer, Israel), and following 30 min of incubation at room temperature, each surface was washed vigorously with tap water (21). Adherence was determined visually following staining with Gentian violet (21). The presence of mannose-sensitive adhesins was tested by examining the aggregation of yeast cells, essentially as previously described (15, 16). Dried cells of *Saccharomyces cerevisiae* (Fleischmann's active dry yeast) were suspended in PUM buffer to a concentration of 5 mg/ml. Equal volumes (20 μl) of bacterial and yeast suspensions were mixed on glass slides, and aggregation was scored visually. To ensure that the phenomenon was mannose inhibitable, the assay was simultaneously carried out in the presence of 0.2% (final concentration) D-mannose as a control.

TABLE 1. Adherence characteristics of pigmented *Serratia* strains grown at 30°C

Strain	% Adherence to:[a]			Adherence to polystyrene[b]	Mannose-sensitive aggregation of yeast cells[c]
	n-Hexadecane	*n*-Octane	*p*-Xylene		
183	92	91	90	+	+
55	91	93	88	+	−
1785	94	94	91	+	+
SH-1	78	80	74	+	+
RZ	96	96	95	+	+
1704	84	80	72	+	+
1515	94	85	88	+	+
II_9A	89	90	82	+	−
BY-1	92	90	80	+	−
I_5	84	85	68	+	−
Mean	89	88	83		

[a] Experiments to determine adherence to hydrocarbons were performed as described in Materials and Methods. The results (means of two separate experiments) are expressed as percent decreases in turbidity compared with the initial cell suspension.

[b] Adherence to polystyrene was scored visually following staining with Gentian violet as described in Materials and Methods.

[c] Aggregation of yeast cells was scored visually as described in Materials and Methods.

RESULTS

Figure 1 shows the effect of growth temperature on the adherence of a pigmented strain and a nonpigmented strain to the test hydrocarbon hexadecane. Both strains were highly adherent following growth at 30°C but became nonadherent as the growth temperature approached 38°C. Of

TABLE 2. Adherence characteristics of nonpigmented *Serratia* strains grown at 30°C[a]

Strain	% Adherence to			Adherence to polystyrene	Mannose-sensitive aggregation of yeast cells
	n-Hexadecane	*n*-Octane	*p*-Xylene		
3255	88	90	88	+	−
577	59	62	62	+	+
2861	62	57	55	+	+
BY-2	80	88	76	+	−
Mean	72	74	70		

[a] For experimental conditions see the footnotes to Table 1.

TABLE 3. Adherence characteristics of pigmented *Serratia* strains grown at 38°C[a]

Strain	% Adherence to			Adherence to polystyrene	Mannose-sensitive aggregation of yeast cells
	n-Hexadecane	*n*-Octane	*p*-Xylene		
183	0	0	0	−	+
55	0	0	0	−	−
1785	0	1.1	13	−	+
SH-1	0	0	0	−	+
RZ	2.9	2.2	2.3	−	+
1704	0.8	1.4	8.3	−	+
1515	0	0	0.5	−	+
II_9A	0	0	3.3	−	−
BY-1	3.4	0.3	1.1	−	−
I_5	3.0	3.5	4.0	−	−
Mean	1.0	0.9	3.3		

[a] For experimental conditions (except the growth temperature of cells) see the footnotes to Table 1.

considerable interest was the observation that adherence in the pigmented strain did not correlate directly with pigment synthesis; whereas strain 1785 did not produce pigment when it was grown at temperatures above 36°C, a high proportion (84%) of the cells grown at 36.5°C adhered to hexadecane.

The ability of both pigmented and nonpigmented *Serratia marcescens* strains to adhere to hydrocarbons when they were grown at 30°C is summarized in Tables 1 and 2. The mean level of adherence of the pigmented strains to all three test hydrocarbons (hexadecane, octane, and xylene) (Table 1) was about 15% higher than the level observed for the nonpigmented strains (Table 2). Similarly, all strains grown at 30°C adhered to the hydrophobic solid polystyrene (Tables 1 and 2). In contrast, neither pigmented nor nonpigmented strains adhered to hydrocarbons or to polystyrene when they were grown at 38°C (Tables 3 and 4).

Tables 1 through 4 show the ability of pigmented and nonpigmented isolates to exhibit mannose-sensitive aggregation of yeast cells following growth at 30 or 38°C.

DISCUSSION

The ability of microorganisms to adhere to solid surfaces and to partition at liquid-air and liquid-liquid interfaces is currently regarded as an important factor in bacterial colonization and survival in various habitats (24a). One of the major factors in promoting adherence both in open environments and in hosts is bacterial hydrophobicity. In this context, *Serratia* strains are of considerable interest for the following reasons: (i) they are colonizers of interfaces in the open environment and have also been increasingly implicated in a wide variety of infections in humans (26); and (ii) the avid hydrophobicity observed in pigmented *Serratia* cells has been correlated with their ability to concentrate at air-water interfaces (2–6, 8, 9, 12–14, 28) and water-oil interfaces (22, 24, 24a) and to adhere to solid surfaces (22, 24). Whereas considerable attention has been given to the potential role of *Serratia* hydrophobicity in enabling the colonization of the sea surface by these organisms (2, 6, 9, 14, 28), their dispersion in aerosols and jet bubbles (3–5), and their nutrient scavenging on solid surfaces (12), the possible role of surface hydrophobicity in affecting the infective nature of *Serratia* strains has not been studied.

TABLE 4. Adherence characteristics of nonpigmented *Serratia* strains grown at 38°C[a]

Strain	% Adherence to			Adherence to polystyrene	Mannose-sensitive aggregation of yeast cells
	n-Hexadecane	*n*-Octane	*p*-Xylene		
3255	0	0	0	−	−
577	0	0	5.9	−	+
2861	0	0	2.1	−	+
BY-2	6.2	0	6.4	−	−
Mean	1.6	0	3.6		

[a] For experimental conditions (except the growth temperature) see the footnotes to Table 1.

Our results suggest that most or all clinical *Serratia marcescens* isolates are capable of exhibiting pronounced cell surface hydrophobicity, as determined by adherence to hydrocarbons and polystyrene.

Infectious strains of *Serratia marcescens* may be either pigmented or nonpigmented, with the latter type predominating (7). Since the presence of the pigment prodigiosin has been cited as the factor which is responsible for hydrophobicity in *Serratia* strains (3, 8, 13, 21, 24), we investigated the hydrophobic properties of both pigmented and nonpigmented strains under growth conditions which either allowed or inhibited prodigiosin synthesis. Williams et al. (30, 31) have reported that prodigiosin synthesis occurs at 30°C, but is completely inhibited by growth at higher temperatures. In order to conveniently monitor pigmentation, cells were grown on solid media.

The finding that the mean level of adherence of the pigmented strains was somewhat higher than that of the nonpigmented strains when the organisms were grown at 30°C suggests that the pigment may contribute somewhat to cell surface hydrophobicity. However, the following two lines of evidence indicate that another component(s) is involved: (i) the adherence of all four nonpigmented strains to the test hydrocarbons and to polystyrene following growth at 30°C, and (ii) the observation that strain 1785 remained hydrophobic following growth at 36.5°C, even though pigmentation was no longer visually apparent. These results are consistent with a recent report that a nonpigmented mutant of strain RZ retained partial ability to adhere to hydrocarbons and polystyrene (22).

Preliminary results (data not shown) have shown that a major outer surface protein (molecular weight, approximately 95,000) can be released by shearing (in the presence of 0.2% sodium dodecyl sulfate) from the surfaces of pigmented and nonpigmented strains grown at 30°C, but is absent from the same cells when they are grown at 38°C. The possible contribution of this protein to the cell surface hydrophobicity of pigmented and nonpigmented strains is under investigation.

Several studies on mannose-sensitive adhesins in *E. coli* have suggested that the presence of these adhesins increases cell surface hydrophobicity (11, 27). Since most clinical and other isolates of *Serratia* bear mannose-sensitive adhesins (1, 15, 18), it was of interest to compare cell surface hydrophobicity with mannose-sensitive aggregation of yeast cells. We found no evidence that the ability of clinical *Serratia* isolates to aggregate yeast cells in a mannose-inhibitable fashion is related to the observed hydrophobicity; (i) among the strains that exhibited pronounced cell surface hydrophobicity were strains which did (e.g., strains 183 and

1785 [Table 1]) and did not (e.g., strains 55 and BY-1 [Table 1]) aggregate yeast cells; and (ii) strains which were mannose sensitive following growth at 30°C (Tables 1 and 3) exhibited the same characteristic following growth at 38°C (Tables 2 and 4) despite their complete loss of hydrophobic surface properties following growth at the higher temperature. A separate study has similarly suggested that cell surface hydrophobicity and mannose-specific adherence in *Serratia marcescens* are mutually independent; adherence to hydrocarbons was inhibited by the amphipathic polymer emulsan (20) but not by mannose, whereas adherence of the same bacteria to yeast cells was inhibited by mannose but not by emulsan (M. Rosenberg, A. Gottlieb, and E. Rosenberg, unpublished data).

The finding that all of the clinical isolates tested were hydrophobic following growth at 30°C but lost this property at higher temperatures is of interest. Ofek et al. (17) have implied that pathogenic *Streptococcus pyogenes* cells may regulate their cell surface hydrophobicity, presenting a hydrophobic outer surface in order to promote initial attachment to host tissue, but at some later stage exposing a hydrophilic surface so as to evade phagocytosis (29). Since infections mediated by *Serratia marcescens* often originate in bacteria that are present in the immediate surroundings (26), in which the cells are usually at lower-than-body temperature, the *Serratia marcescens* cells may conceivably be hydrophobic during initial contact with host tissue or with plastic surfaces in contact with a host (10) but not hydrophobic following the onset of infection.

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