Increased Cell Surface Hydrophobicity of a Serratia marcescens NS 38 Mutant Lacking Wetting Activity

RONIT BAR-NESS,1 NAFTALI AVRAHAMY,1 TOHEY MATSUYAMA,2 AND MEL ROSENBERG1,2*

The Maurice and Gabriela Goldschleger School of Dental Medicine,1* and Department of Human Microbiology,2 Sackler Faculty of Medicine, Tel-Aviv University, Ramat-Aviv 69978, Israel, and Department of Bacteriology, Niigata University School of Medicine, 1-757 Asahimachidori, Niigata 951, Japan3

Received 14 December 1987/Accepted 31 May 1988

The cell surface hydrophobicity of Serratia marcescens appears to be an important factor in its adhesion to and colonization of various interfaces. The cell surface components responsible for mediating the hydrophobicity of S. marcescens have not been completely elucidated, but may include prodigiosin and other factors. In the present report we have investigated the potential role of serratamolide, an amphipathic aminolipid present on the surfaces of certain S. marcescens strains, in modulating cell surface hydrophobicity. The hydrophobic properties of a serratamolide-producing strain (NS 38) were compared with those of a serratamolide-deficient mutant (NS 38-9) by monitoring the kinetics of adhesion to hexadecane. Serratamolide production was monitored by thin-layer chromatography and the wetting activity of washed-cell suspensions on polystyrene. Wild-type NS 38 cells were far less hydrophobic than the serratamolide-deficient mutant cells; the removal coefficients were 48 min−1 for the mutant, as compared with only 18 min−1 for the wild type. The data suggest that the presence of serratamolide on S. marcescens cells results in a reduction in hydrophobicity, presumably by blocking hydrophobic sites on the cell surface.

Serratia marcescens is a common environmental microorganism of considerable historical interest (2) which has been increasingly implicated as a primary pathogen in numerous human infections (14, 19). The pronounced hydrophobic surface characteristics of S. marcescens were reported by Mudd and Mudd in 1924 (9) and have been studied in various laboratories during recent years (3, 4, 10, 11, 15). Cell surface hydrophobicity in Serratia strains enables them to concentrate at water surfaces, providing them with the possible advantages of nutrient scavenging (3, 15) and dispersal in jet bubbles (1). Recently, a role for cell surface hydrophobicity in initial adhesion and host colonization by clinical S. marcescens strains was proposed, based on the observation that all 14 clinical isolates examined exhibited hydrophobic surface properties (11).

The molecular moieties which may promote cell surface hydrophobicity in Serratia strains include prodigiosin (1, 3–5, 15) and a 70-kilodalton outer surface protein (R. Bar-Ness and M. Rosenberg, submitted for publication). In a recent report, Matsuyama et al. (7) suggested that serratamolide may also be involved in modulating cell surface hydrophobicity. Serratamolide, an amphipathic aminolipid present on the surfaces of certain Serratia strains, was first characterized by Wasserman et al. (16, 17) and was recently shown to be a potent wetting agent (7). In the work described in this report we have compared the hydrophobic surface properties of a serratamolide-producing S. marcescens strain with those of a mutant deficient in serratamolide production. Hydrophobicity was determined by studying the kinetics of adhesion of hexadecane, a technique which allows highly reproducible, quantitative measurements (6, 13). The data suggest that the presence of serratamolide results in a large reduction in cell surface hydrophobicity.

MATERIALS AND METHODS

Bacterial strains and growth conditions. S. marcescens NS-38 (ATCC 13880) is a pigmented serratamolide-producing strain (7); strain NS 38-9, obtained and characterized by Matsuyama et al. (8), selected for its lack of wetting-agent activity, is deficient in serratamolide production. Bacteria were inoculated as lawns on peptone glycerol (PG) agar (5 g of Bacto-Peptone [Difco Laboratories, Detroit, Mich.], 10 ml of glycerol [Sigma Chemical Co., St. Louis, Mo.], 12 g of agar [Difco], distilled water to 1,000 ml) and grown for 3 days at 30 to 38°C as indicated.

Cell surface hydrophobicity. Cell surface hydrophobicity was determined by measuring the kinetics of adhesion to hexadecane (6), essentially as described by Sharon et al. (13). Bacteria were washed twice and suspended in phosphate buffer (22.2 g of K2HPO4 · 3H2O, 7.26 g of KH2PO4, distilled water to 1,000 ml [pH 7.2]) to an initial absorbance (A0) of 0.95 to 1.05, as measured in a Uvikon 710 spectrophotometer (light path, 1 cm; Kontron, Zurich, Switzerland) at 400 nm. Bacterial suspensions (1.6 ml) were added in quadruplicate to semimicro disposable polystyrene cuvettes (4 ml; Rudolf Brand, Wertheim, Federal Republic of Germany), and hexadecane was then added as indicated. Mixtures were vortexed at room temperature on a Thermolyne Maxi Mix flat-top mixer (Sybron, Dubuque, Iowa) for 5-s intervals. After each mixing period, the phases were allowed to separate and the absorbance of the lower, aqueous phase (A1) was measured. The rate of adhesion, designated the removal rate, R, was calculated as log (A0 / 100A0). Linear regression was used to plot the results.

For endpoint measurement of bacterial adhesion to hexadecane, 25 μl of hexadecane was added to 1.6 ml of bacterial suspension as described above. The mixtures were then vortexed for 30-s periods until no further decrease in turbidity was observed.

Thin-layer chromatography. Bacteria were grown at 30 or 38°C on PG agar for 3 days (7). To examine the wetting agent, we washed wet bacterial mass from the agar surface in

* Corresponding author.
RESULTS

*S. marcescens* NS 38, grown on PG agar at 30°C, produced cell-associated serratamolide (Fig. 1, lane 1). However, when the cells were grown at 38°C serratamolide was no longer detected (lane 2). Wetting-agent-deficient mutant NS 38-9 did not produce serratamolide following growth at either temperature (lanes 3 and 4). These results are in agreement with previous data of Matsuyama et al. (7, 8).

Previous investigations have shown that pigment (prodigiosin) production is temperature dependent (18). Both strains produced pigment (prodigiosin) when grown at 30°C but not at 38°C (Fig. 1).

Figure 2 illustrates the spreading of cell suspensions on polystyrene. Following growth at 30°C, a 10-μl droplet containing a wild-type NS 38 cell suspension (optical density at 400 nm, ca. 7.0) spread over the hydrophobic test surface, an indication of wetting activity (Fig. 2A, drop 1), whereas a droplet containing mutant NS 38-9 cells exhibited no wetting activity whatsoever (Fig. 2A, drop 2). Neither strain exhibited wetting activity after growth at 38°C (Fig. 2B) (7, 8).

To compare the hydrophobic surface properties of the strains, we used adhesion to hexadecane. The kinetics of adhesion (removal rate) of cells grown at 30°C to various hydrocarbon volumes are summarized in Fig. 3 to 5. Wild-type NS 38 cells (Fig. 3) were far less hydrophobic than cells of the corresponding serratamolide-deficient mutant were (Fig. 4). For example, whereas 84 ± 3% of the mutant cells were removed by 25 μl of hexadecane after 25 s of vortexing, only 34 ± 4% of the wild-type cells were removed under these conditions. Similarly, 5 μl of hexadecane removed 24 ± 2% of the mutant cells after 25 s of vortexing, as compared with 2 ± 1% of the wild-type cells.

When the adhesion (removal) rate is plotted as a function

![FIG. 1. Thin-layer chromatograms of lipids extracted from *S. marcescens* cells. The lipid possessing wetting activity (corresponding to serratamolide) is indicated by the arrow. Lanes: 1, strain NS 38 grown at 30°C; 2, strain NS 38 grown at 38°C; 3, mutant strain NS 38-9 grown at 30°C; 4, mutant strain NS 38-9 grown at 38°C.

20 ml of phosphate-buffered saline (8.0 g of NaCl, 0.2 g of KCl, 1.15 g of Na2HPO4, 0.2 g of KH2PO4, distilled water to 1,000 ml [pH 7.1]). Following a 15-min centrifugation (8,600 × g), the cell pellet was extracted with 10 volumes of ethanol. After removal of sediments by centrifugation as above and removal of the ethanol by evaporation, the dry material was further extracted with chloroform-methanol (2:1, vol/vol). The extracts were examined on a thin-layer chromatography plate of silica gel (DC-plastikfolien Kieselgel 60 F254; E. Merck AG, Darmstadt, Federal Republic of Germany) in a solvent system of chloroform-methanol-acetone-acetic acid (90:10:6:1 by volume). For detection of components the plate was sprayed with 50% H2SO4 in ethanol and heated briefly at ca. 200°C.

![FIG. 2. Wetting activity of bacterial suspension on polystyrene. The wetting activity of washed suspensions of *S. marcescens* grown at 30°C (panel A) or 38°C (panel B) was demonstrated by placing 10-μl droplets on untreated polystyrene disks. (A) Drop 1, strain NS 38; drop 2, mutant NS 38-9. (B) Drop 1, strain NS 38; drop 2, mutant NS 38-9.

![FIG. 3. Kinetics of adhesion of strain NS 38 to hexadecane. Following growth at 30°C, washed bacterial suspensions (1.6 ml) were mixed with hexadecane volumes (as indicated) for 5-s intervals as described in Materials and Methods. Results are presented as log A/\(A_0\) as a function of mixing time.](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5986608/figure3.png)
of the hexadecane-to-water volume ratio (Fig. 5), a slope is obtained which denotes the removal coefficient, a quantitative indication of the affinity of the cells for the hydrocarbon/water interface (13). The removal coefficients were 18 min⁻¹ for the wild-type cells, as opposed to 48 min⁻¹ for the wetting-agent-deficient mutant cells. Correlation coefficients for plots of removal rates and removal coefficients were significant in all cases ($P < 0.025$).

Following growth at 38°C, no adhesion was evident in either strain, as measured by the endpoint procedure. Previous investigations have shown that cell surface hydrophobicity is lost after growth at this temperature (11).

DISCUSSION

The data suggest that loss of serratamolide production in a mutant of S. marcescens (NS 38-9) is accompanied by a large increase in cell surface hydrophobicity. It may be inferred that when this amphipathic compound is present on the wild-type cell surface, it binds to hydrophobic sites, burying its nonpolar moieties toward the cell surface and exposing its polar sites into the bulk aqueous phase. Although we cannot completely rule out the possibility that the mutant strain lacks components in addition to serratamolide, it may be noted that outer surface protein profiles of mutant and wild-type cells were identical on sodium dodecyl sulfate-polyacrylamide gels (results not shown).

Recent studies of bacterial cell surface hydrophobicity suggest that hydrophobic properties are often determined by an interplay of "hydrophobins" (hydrophobicity-promoting components) and "hydrophilins" (hydrophobicity-reducing moieties), which coexist on the cell surface (12). Various investigators have suggested that the pigment prodigiosin acts as a hydrophobin in various Serratia cells (1, 3, 4, 11, 15). A 70-kilodalton outer surface protein may also act as a hydrophobin in S. marcescens (Bar-Ness and Rosenberg, submitted). The data presented here suggest that serratamolide may act as a hydrophilin, counteracting the hydrophobicity conferred by these components. In this context it is of interest that S. marcescens RZ exhibits high cell surface hydrophobicity, despite its production of a wetting agent, whereas its semihydrophobic nonpigmented mutant, strain 3162, is devoid of wetting activity (10; R. Bar-Ness and M. Rosenberg, unpublished data). It may be argued that the partial hydrophobicity exhibited by this mutant is the net result of loss of a hydrophobin (prodigiosin), counteracted to some extent by the absence of a hydrophilin (serratamolide). This hypothesis is currently under investigation.

Modulation of cell surface hydrophobicity has been suggested as a possible factor in host colonization by clinical S. marcescens isolates (11). The possibility that serratamolide plays a role in such a phenomenon warrants further study.

ACKNOWLEDGMENTS

We thank M. Harnick for valuable assistance and advice, Y. Mazor for excellent technical assistance, and L. Maman for photography.

This research was supported by grant 86-00263 from the United States-Israel Binational Science Foundation, Jerusalem, Israel.

LITERATURE CITED


