ISSN 0026-2617, Microbiology, 2011, Vol. 80, No. 4, pp. 573–575. © Pleiades Publishing, Ltd., 2011.
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SHORT COMMUNICATIONS

Hydrophobic and Donor–Acceptor Properties of the Surface of Warnerin-Sensitive or -Resistant Staphylococcus Cells

N. V. Pan'kova, T. V. Polyudova, L. M. Lemkina, and V. P. Korobov[1]

Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences, Perm, Russia

Received September 27, 2010

DOI: 10.1134/S0026261711040163

Hydrophobicity of the surface is a fundamental characteristic of bacterial cells that is responsible for the processes of bacterial adhesion at the interphase boundary, interaction with various substances, attachment to host tissues, biofilm formation, and virulence exertion [1, 2]. The hydrophobic properties of the cell surface are probably of particular importance for the contacts of bacteria with antibiotics, determining the levels of sensitivity or resistance to them [3]. However, data available on such relations are scarce. In particular, the development of resistance to oxacillin [4] and novobiocin [5] was shown to be accompanied by a decrease in hydrophobicity of the cell walls in the resistant strains. The role of the hydrophobic properties of the bacterial surface on the bacterial sensitivity towards peptide antibiotics has not been studied.

The goal of the present work was to study and compare how hydrophobicity and donor–acceptor properties of the cell surface of coagulase-negative staphylococci (CNS) affect their sensitivity to the low-molecular cationic peptide warnerin, showing a pronounced lytic effect on various gram-positive bacteria [7].

CNS strains from the collection of the Laboratory of Developmental Biochemistry of Microorganisms (Institute of Ecology and Genetics of Microorganisms, Ural Branch, Russian Academy of Sciences) were used in the work. The bacteria were grown in LB medium to the midexponential growth phase ($OD_{600} = 0.8$). Aliquots of the cultures were centrifuged (Sigma 3K30) at 10000 *g* for 10 min; the pellets were washed twice with 0.15 M NaCl and resuspended in the solution to OD_{600} of 0.18–0.22.

Studying the adhesion activity of bacterial cells was performed with a microbial adhesion to solvents (MATS) test [8] evaluating the cell surface hydrophobicity with nonpolar solvents (decane and hexadecane) and their electron donor–acceptor properties by comparison of the adhesive capacity of the microbial cells in the following pairs of polar and nonpolar solvents with equal surface tension: ethyl acetate (an electron donor)–decane and chloroform (an electron acceptor)–hexadecane. The differences in the results obtained in the experiments with different pairs of solvents indicate electron donor–acceptor interactions on the surfaces of bacterial cells and characterize the degree of hydrophobicity/hydrophilicity.

Cell suspensions (1.8 ml) were collected into calibrated glass tubes with the inner diameter of 10 mm, optical density was measured on a PD-303 digital spectrophotometer (Apel, Japan), and 0.3 ml of the solvent was added. The tubes were shaken for 90 s on a V-32 Multi-Vortex shaker (Biosan, Latvia) at the maximum amplitude. After phase separation (15–20 min), the optical density of the aqueous phases was measured again. The value of cell adhesion activity was calculated according to the formula: Ad, $\% = 100 \times [1 - (A/A_0)]$, where A_0 and A stand for the optical density of the aqueous phases of the bacterial suspensions before and after shaking, respectively.

The antibacterial peptide warnerin was isolated from the growth medium of the producer, *Staphylococcus warneri* IEGM KL-1, as was previously described [6]. Staphylococci sensitivity to warnerin was determined by the method of twofold serial dilutions of the peptide preparation (4 mg/ml) in polystyrene immunological plates using *S. epidermis* 33 suspension containing $1.2–1.5 \times 10^6$ CFU/ml as inoculum [6]. Statistical treatment of the data was carried out with the Excel 2003 software package (Microsoft Inc, 1999).

Analysis of the sensitivity of the CNS strains to the peptide revealed two groups differing in warnerin minimum inhibitory concentration (MIC), that is, sensitive strains with MIC < 64 μg/ml and nonsensitive strains with MIC > 2048 μg/ml.

As was demonstrated by the results of the study, hydrophobic properties of the bacterial surfaces of the two groups detected with nonpolar (Fig. 1) and polar solvents (Fig. 2) differed considerably. When suspended in hexadecane and decane, the cells of warnerin-sensitive strains (Fig. 1a) exhibited a medium or high level of hydrophobicity (26.0–65.3%), while the nonsensitive strains (Fig. 1b) were low- or medium-hydrophobic (4.0–54.3%). The highly warnerin-sensitive strain 2988 (MIC 32 μg/ml) turned out to be the

[1] Corresponding author; e-mail: korobov@iegm.ru.

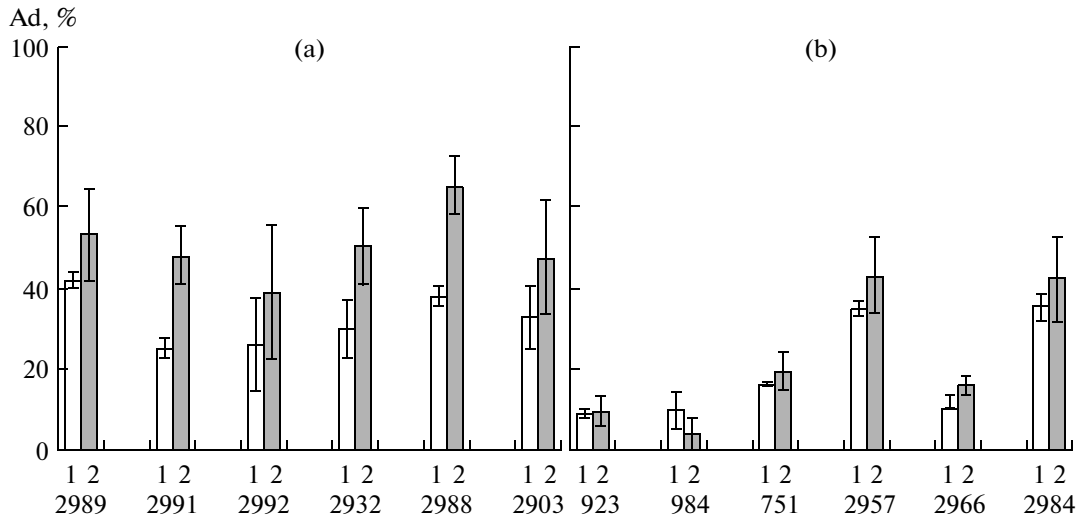

Fig. 1. Hydrophobic properties of warnerin-sensitive (a) and -nonsensitive (b) CNS strains. Ad, adhesion factor, %; decane (1) and hexadecane (2).

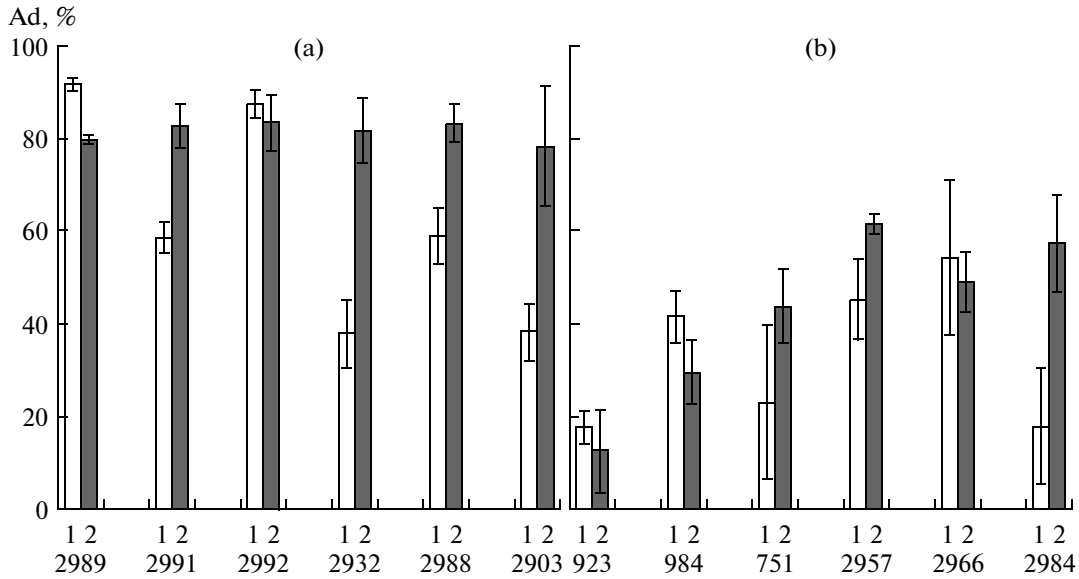

Fig. 2. Donor-acceptor properties of warnerin-sensitive (a) and -nonsensitive (b) CNS strains. Ad, adhesion factor, %; ethyl acetate (1) and chloroform (2).

most hydrophobic (65.3%), while the hydrophobicity of the less sensitive strain 2903 (MIC 64 μg/ml) was much lower, 47.5%. It is important to notice that the surface hydrophobicity of the test strain *S. epidermidis* 33 (MIC 0.25 μg/ml) was 77.9%. These experimental data make it possible to consider bacterial surface hydrophobicity to be an important factor determining sensitivity to warnerin and, probably, to other amphiphilic antibiotic agents.

Application of polar solvents revealed another characteristic feature of the bacteria sensitive to the cationic peptide. As follows from the data presented in Fig. 2a, bacteria of all strains of the group revealed high affinity to the protogenic solvent chloroform, apparently providing a source of electron-donor components. The level of bacterial adhesion was practically 80% in all strains. Importantly, two strains of the group (2989 and 2992) were also characterized by a high level of acception of ethyl acetate electrons

Table 1. Characteristics of the CNS strains in terms of their sensitivity to warnerin and hydrophobicity and donor–acceptor properties of the cells

Sensitivity of the bacteria to warnerin	Average hydrophobicity, Ad, %		Electron-donor properties, %	Electron-acceptor properties, %
	Decane	Hexadecane	$Ad_{chloroform}$–$Ad_{hexadecane}$	$Ad_{ethyl\ acetate}$–Ad_{decane}
Sensitive	32.1 ± 6.74	50.6 ± 8.65	31.0 ± 8.82	35.4 ± 19.22
Nonsensitive	19.7 ± 12.41	22.6 ± 16.69	20.1 ± 9.51	23.1 ± 17.52

revealing the presence of pronounced nucleophilic zones in the bacterial cell walls. Similar, although less pronounced, electron donor–acceptor features of the bacterial surface were detected in the bacteria of nonsensitive strains (Fig. 2b).

The donor–acceptor properties of the cell surface of the strains under study were also evaluated by comparing the results of the experiments with electron donor (ethyl acetate) and electron acceptor (chloroform) solvents with the bacterial behavior in the pairs of polar and nonpolar solvents of equal surface tension. This revealed involvement of the donor–acceptor mechanisms evaluated as a difference in the affinities of bacteria to polar and nonpolar solvents in the formation of the bacteria adhesive properties. As follows from Table 1, the surfaces of the bacteria of warnerin-sensitive and -insensitive strains are characterized by different levels of hydrophobicity and donor–acceptor properties, these features being more pronounced in the sensitive bacteria. However, the tendency requires further validation by comprehensive experimental data.

The results testify that the nature and specific features of the surface compounds and structures of bacteria under attack by a low-molecular cationic peptide warnerin largely determine its antibacterial activity. The warnerin-sensitive strains of staphylococci are characterized by pronounced hydrophobicity of the cell surface which, together with the amphiphilc nature of the peptide [6] promotes its adsorption and penetration through the cell wall to its target, the membrane. Under conditions of lowered hydrophobicity of the surface structures, the same role is played by the electron–acceptor properties of the bacterial cell walls.

ACKNOWLEDGMENTS

The work was supported by a grant of the Russian Foundation for Basic Research (project no. 10-04-96086-r_ural_a), the Molecular and Cell Biology program of the Presidium of the Russian Academy of Sciences (project no. 09-P-4-1019) and the Ural Branch, Russian Academy of Sciences (projects no. 09-M-14-2002 and 10-14-12-NAB).

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