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Note

Difference in cell surface hydrophobicity of *Halobacterium salinarium* strains

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The cell surface of halobacteria is remarkable for its ability to support the stressing conditions conferred by the high salt concentration of the surrounding medium. This is apparently due to an excess of surface charges that counteracts external ions[1–3] and to the internal accumulation of osmoregulators, which contributes to compensating for the high osmotic pressure from the exterior[4–7].

The balance between the charged and non-polar groups of the cell surface in halobacteria may be important in determining its degree of halo-tolerance and/or halophilism. This property necessarily varies from one species to another, and its evaluation may be an interesting approach for classification. As shown in this paper, the chromatographic behaviour on hydrophobic matrices of three well known extreme halophile strains, regarded as belonging to the same species[8], is distinct. It is concluded that hydrophobic interaction chromatography (HIC), a technique which takes advantage of the high salt concentration of the medium, is a useful alternative in the study of the cell surface hydrophobicity of halobacteria.

EXPERIMENTAL

The bacterial strains *Halobacterium halobium* (NRC 817), *H. cutirubrum* (CCM 2088) and *H. salinarium* (CCM 2148) were cultured in MH medium: 10 g/l $MgSO_4 \cdot 7H_2O$, 5 g/l KCl, 0.2 g/l $CaCl_2 \cdot 6H_2O$, 10 g/l yeast extract (DIFCO) and 2.5 g/l bacto tryptone (DIFCO), adjusted to 3.6 *M* NaCl at 37°C in a Lab-Line Orbit shaker at 120 rpm until the cell density reached a value of 0.45 optical units at 580 nm. Aliquots of 0.5 ml were reincubated in 50 ml as above and the bacteria from the mid-logarithmic phase were collected, washed twice with a saline solution (3.6 *M* sodium chloride) and kept at 4°C for chromatography. The adsorbent gels were obtained from Pharmacia (Uppsala, Sweden) and all other reagents from Sigma (St. Louis, MO, U.S.A.).

Hydrophobic adsorption

The hydrophobicity of the selected halobacteria was determined by adsorption on 1 ml of gel in test-tubes (0.9 × 11 cm) or on chromatographic columns (33.5 × 0.8 cm bed volume). A 1-ml volume of cell suspension (about 0.45 optical units at 580 or 2.25 optical units at 280 nm, equivalent to $6.4 \cdot 10^6$ cells when compared with a standard plot of *Halobacterium halobium*) was either mixed with the gel in the

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test-tube or applied to the column at a flow-rate of 35 ml/h, collecting samples of 2 ml per tube. The absorbance of the supernatant or column effluent was determined at 280 nm in a Spectronic 2000 spectrophotometer. All experiments were run in triplicate and the mean values determined.

RESULTS AND DISCUSSION

A number of techniques have been tried for estimating the cell surface hydrophobicity of bacteria. One example is based on its degree of aggregation at different concentrations of ammonium sulphate[9–11]; others utilize the distribution of microorganisms in a two-phase system of different polarity[12–15] or the measurement of the contact angle between the bacteria and a given solid surface[16]; the binding of hydrophobic probes to the cell surface has also been an approach towards this end[17].

Hydrophobic interaction chromatography (HIC)[18–20] has been applied successfully in the study of the hydrophobicity of many bacteria[9,21–23] and for the separation of eukaryotic cells from various sources[19]. HIC is based on the interaction between an immobilized hydrophobic ligand and a hydrophobic group of the sample. The strength of interaction increases with increasing salt concentration and temperature; the nature of the hydrophobic ligand, its density on the matrix and its accesibility also determine the extent of adsorption. Hence it is possible to establish the hydrophobic character of a substance by examining its chromatographic behaviour, varying the ligand and the experimental conditions.

In general, the cell surface possesses exposed hydrophobic sites that are responsible for bacterial adsorption on hydrophobic gels[24]. It has been shown, for instance, that enzymatic or chemical treatments modify the hydrophobicity of various kinds of microorganisms[25–27].

In this study we first chose the chromatographic approach for analysing the

TABLE I

EFFECT OF THE NATURE OF THE HYDROPHOBIC LIGAND ON THE ADSORPTION OF HALOBACTERIA ON HYDROPHOBIC GELS AT DIFFERENT SALT CONCENTRATIONS

Values correspond to mean percentages of three replicas.

Gel	*NaCl (M)*														
	H. cutirubrum					*H. halobium*					*H. salinarium*				
	3.6	*4.0*	*4.4*	*4.8*	*5.2*	*3.6*	*4.0*	*4.4*	*4.8*	*5.2*	*3.6*	*4.0*	*4.4*	*4.8*	*5.2*
Control															
Agarose (4B CL)	55	49	37	41	46	80	71	76	71	71	87	89	85	86	83
Aminoalkyl															
Agarose–ethane	69	62	44	41	46	78	78	69	68	71	89	86	84	84	86
Agarose–butane	68	61	41	41	44	72	71	69	68	66	88	83	80	83	83
Agarose–hexane	72	52	45	36	44	84	80	76	73	66	88	81	81	79	81
Alkyl															
Agarose–phenyl	31	52	52	56	56	86	89	89	83	86	99	98	99	99	99
Agarose–octane	76	60	53	46	56	79	82	83	80	80	92	89	88	88	92

behaviour of extreme halophiles, which gave very low elution yields, that is, only a fraction of the cell population (about 15%) eluted from the column. This problem was first attributed to the physcal entrapment of bacterial aggregates and therefore a batch-assay procedure was followed (Table I).

As shown in Table I, the percentage of adsorption of extreme halophiles (*H. halobium*, *H. salinarium* and *H. cutirubrum*) ranges from 30 to 99% according to the nature of the immobilized hydrophobic ligand and of the experimental conditions. *H. halobium*, *H. cutirubrum* and *H. salinarium* show important differences in their tendency to adsorb on the hydrophobic matrices employed in this study. This finding suggests that they posses different cell envelope properties and supports their designation as different strains of the *H. salinarium* species[8]. It is interesting to consider the cell surface hydrophobicity, which seems to be involved in the attachment of microorganisms to solid surfaces[28–33], for classification purposes; apparently, *H. cutirubrum* shows the weakest, *H. halobium* a moderate and *H. salinarium* the strongest hydrophobicity (Table I).

There is no clear pattern of the adsorption of *H. salinarium* strains on hydrophobic gels as a function of salt concentration (Table I). In this respect they do not follow the theoretical trend of most proteins and cell particles[18–20]. In other words, the adsorption tendency does not increase with increasing salt concentration. This does not necessarily mean that another mechanism of adsorption (*i.e.*, avidity for the carbohydrate matrix) is involved. In fact, the strains employed here do not utilize sugars as a carbon source[8] and their addition to the chromatographic buffer enhances, rather than diminishes, bacterial adsorption[35]. Bacterial hydrophobicity is the sum of a number of cell surface characteristics and its expression may be extremely complex. As reported[34], the tendency of 23 strains of *Staphylococci* to adhere to hydrophobic materials may vary within the same species; this behaviour appears to be related to the presence of capsules. Our observations, derived from the chromatographic patterns of halobacteria[35], show that differences in hydrophobic character also exist among extreme halophiles. Hence it may necessary to consider the biological significance of this property in order to interprete correctly these findings and establish their importance in bacterial classification.

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