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EXPERIMENTAL ARTICLES

Evaluation of the Hydrophobicity of Bacterial Cells by Measuring Their Adherence to Chloroform Drops

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Abstract—A simple method for measuring the hydrophobicity of bacterial cells is proposed, which is based on the spectrophotometric evaluation of cell adherence to chloroform drops in a biphasic water–chloroform mixture.

Key words: adhesive properties, hydrophobicity, hydrophilicity, hydrophobicity index, bacteria, biphasic system.

In recent years, there has been increasing interest in studying the adhesive properties of bacterial cells. The degree of the surface hydration of bacterial cells (or the degree of their hydrophobicity) is an important physicochemical parameter which largely determines the interaction of cells with solid surfaces and should be taken into account when investigating the cultivation, metabolic, and immobilization processes [1–4].

Data available in the literature indicate that the hydrophobicity of the cell surface depends on environmental conditions and is largely determined by the proportion between the hydrophobic and hydrophilic compounds constituting the outer membrane of bacteria, such as lipopolysaccharides, phospholipids, integral membrane proteins, metal ions, and so on. The hydrophobic–hydrophilic properties of vegetative bacterial cells and spores were described in a number of publications [1, 5–8].

The existing methods of evaluation of the adhesive properties of microbial cells depend greatly on the evaluation conditions [1, 9, 10] and, hence, fail to give adequate results. One of the most simple and rapid methods for measuring cell-surface hydrophobicity is based on the estimation of cell adherence to the hydrocarbon–water interphase in a biphasic system containing *n*-hexadecane, *n*-octane, or *p*-xylene [6]. The chief disadvantage of this method is that the hydrocarbons used by Rosenberg *et al.* are lighter than water. As a result, the aqueous phase used for colorimetric measurements cannot be collected without disturbing the hydrocarbon–water interphase, which reduces the accuracy of the method.

This work was undertaken to improve the Rosenberg method for estimating the surface hydrophobicity of microbial cells.

MATERIALS AND METHODS

The *Serratia marcescens* strain 184 used in this study was obtained from the collection at the Research Institute of Microbiology. The strain was grown aerobically at 30 ± 1°C for 48 h in a liquid synthetic medium or on a solid medium with fish-meal hydrolysate [11, 12].

Cells were suspended either in 0.85% NaCl (physiological saline solution) or in potassium phosphate buffer (pH 7.0) to an optical density (E_0) of 0.5–0.6. Optical density was measured at 540 nm in a KFK-2 photocolorimeter using a 10-mm-pathlength cuvette.

Experiments were carried out as follows. Cell suspensions (5 ml) contained in 10-mm-diameter test tubes were mixed with different volumes of chloroform. The mixtures were shaken for 1 min and allowed to separate into aqueous and chloroform phases for 4 min. The upper aqueous phase with cells was carefully transferred into the cuvette to determine a decrease in the optical density of the cell suspension due to a partition of cells between the aqueous (hydrophilic) and chloroform (hydrophobic) phases. The hydrophobicity index (HI) of microbial cells was calculated by the formula:

$$\mathrm{HI} = 100 - \frac{E \times 100}{E_0},$$

where E_0 is the initial optical density of the cell suspension, and E is the optical density of the aqueous phase after its separation from the chloroform phase.

The data presented in this paper are the means of 4–6 replicated measurements given for a confidence level of 0.95 [13].

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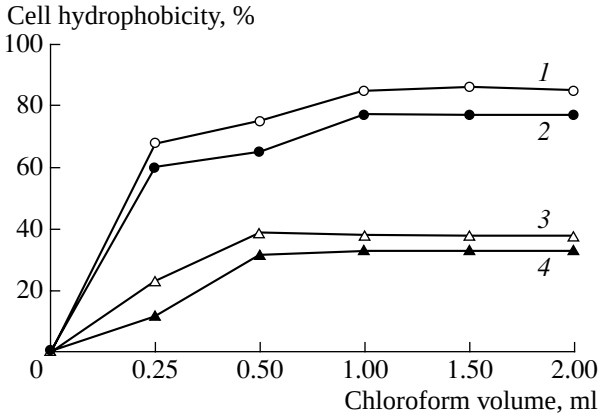

Fig. 1. The effect of the volume of the added chloroform on the evaluated hydrophobicity index of *S. marcescens* cells grown as (*1*, *2*) an agar culture or (*3*, *4*) a liquid culture. The cells were suspended either in (*1*, *3*) 0.85% NaCl or in (*2*, *4*) 1% potassium phosphate buffer.

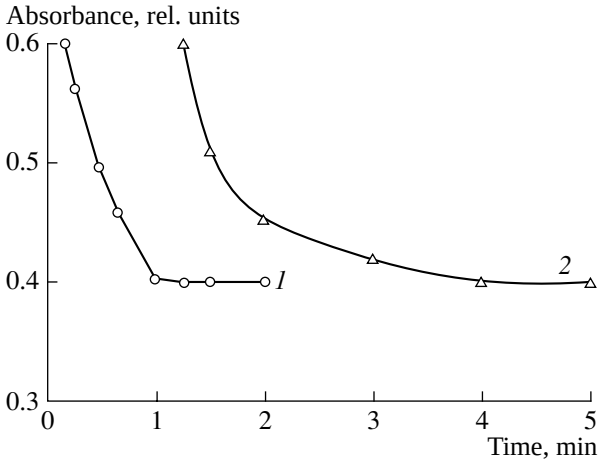

Fig. 2. Changes in the concentration of *S. marcescens* cells in the aqueous phase of the biphasic cell suspension–chloroform system: (*1*) at different shaking times and (*2*) at different separation times after the 1-min shaking of the system.

RESULTS AND DISCUSSION

Figure 1 shows the effect of the volume of the added chloroform on the evaluated hydrophobicity index of *S. marcescens* cells grown under different cultivation conditions (as a liquid or agar culture). It can be seen that, when the suspension of agar-grown cells (5 ml) was mixed with 0.5 ml chloroform, the chloroform phase after the mixture separation contained about 75% of the cells initially present in the cell suspension (Fig. 1, curve *1*). At the same time, this percentage was only 40% for cells grown in a liquid culture (Fig. 1, curve *3*). The increase in the volume of the added chloroform to 1 ml did not affect the partition of the liquid-culture cells between the aqueous and hydrophobic phases (curve *3*), but enhanced the partitioning of the agar-culture cells to the hydrophobic phase (curve *1*). Further increase in the volume of the added chloroform did not affect the results of determination of the hydrophobicity of either type of bacterial cells. The evaluation of cell hydrophobicity in potassium phosphate buffer (curves *2* and *4*) showed that K^+ and PO_4^{3-} ions make the cell surface less hydrophobic (or more hydrophilic), which is in agreement with the data available in the literature [4, 14].

The optimization of measurement conditions using agar-grown *S. marcescens* cells showed that the optimal time of shaking the cell suspension with chloroform is 1 min (Fig. 2, curve *1*), and that the time within which the biphasic aqueous cell suspension–chloroform system is completely separated into the particular phases is 4 min (Fig. 2, curve *2*).

Thus, to estimate cell hydrophobicity with chloroform, one has to prepare a cell suspension in 0.85% NaCl to an optical density of 0.5–0.6 (540 nm; 10-mm-pathlength cuvette). The volume ratio of the cell suspension to the added chloroform is 5 : 1. The optimal times of the biphasic cell suspension–chloroform system shaking and separation are 1 and 4 min, respectively. The described method for estimating cell hydrophobicity with the use of chloroform gives results which are comparable with those obtained using light hydrocarbons. The results of cell hydrophobicity analysis with chloroform and *n*-octane are especially close. At the same time, the accuracy of the determination of cell hydrophobicity with chloroform is about 5%, whereas it is no better than 20% with *n*-octane.

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