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SHORT COMMUNICATIONS

Cell Hydrophobicity as a Criterion of Selection of Bacterial Producers of Biosurfactants

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The main criteria of selection and assessment of the activity of microbial strains producing biosurfactants are the level of surface tension (ST) and emulsifying activity (EA) of liquid microbial cultures [1]. These parameters demonstrate the presence of surface-active substances (SAS) in liquid medium, and their values reflect SAS concentrations. In terms of their type of localization, biosurfactants of hydrocarbon-oxidizing bacteria may be extracellular or cell-bound [2]. In the case of extracellular bioSAS production, the ST and EA values may be to some extent considered as indices of surfactant concentrations in liquid media. On the other hand, direct chemical analogy cannot be applied in the case of synthesis of cell-bound bioSAS because the surface tension and emulsification index of such microbial cultures are mediated by the surfactant properties of cell biomass. The screening of bacterial producers of cell-bound bioSAS by chemical isolation of surfactants from cell biomass is labor-intensive and expensive. Therefore, it is important to determine a parameter that would reflect the surfactant properties of bacteria producing cell-bound bioSAS. For this purpose, we have chosen the cell hydrophobicity index (HI) demonstrating the degree of bacterial affinity to a hydrophilic or hydrophobic substrate.The surface tension of the samples was determined by plate separation, and the emulsifying activity was assayed as in [1]. The hydrophobicity index was determined by the modified method of Rosenberg [3]. The study was performed with ten strains of petroleum-oxidizing bacteria, including eight actinobacteria, from the collection of the Department of Genetics and Microbiology, Kuban State University. The microorganisms were grown in a liquid mineral medium [4] with 1% hexadecane. The hydrophobicity index of washed cells, the surface tension, and the emulsifying activity of liquid cultures and their supernatants were determined as in [1]. The initial surface tension of inoculated medium was 64 mN/m and the emulsifying activity was 0%. According to [5], a microorganism is considered a promising bioSAS producer if its liquid culture shows a decrease of ST < 40 mN/m.

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As is seen from Table 1, four of the ten strains were effective producers of biosurfactants. The comparison of the surfactant properties of liquid cultures and their supernatants demonstrated that the synthesized SAS were cell-bound. Two strains also produced extracellular surfactants. Characteristically, the cells of the strains exhibiting the maximal decrease of surface tension and high emulsifying activity in culture possessed the highest hydrophobicity, and vice versa. The Pearson's correlation coefficient was $r_{xy} = 0.74$ at $p = 0.000$ for the emulsifying activity of liquid cultures and cell hydrophobicity and –0.61 at $p = 0.000$ for the surface tension of liquid cultures and cell hydrophobicity. Thus, liquid cultures with more hydrophobic cells possess more expressed surfactant properties.

The three most active strains were selected for further studies: *Rhodococcus erythropolis* B2, *Rhodococcus* sp. F2, and *Rhodococcus* sp. J8. The comparison of the surfactant properties of their liquid cultures and supernatants showed that the bioSAS they synthesized were cell-bound. This was confirmed by the chemical isolation of total lipids from native cells by the method of Bligh and Dyer [6]. The obtained lipids were able to decrease the surface tension of water and to emulsify diesel fuel (Table 1), i.e., they were biosurfactants. The enhancement of surfactant properties of liquid cultures under increasing hydrophobicity of suspended cells was tested by cultivation of the bacteria on different media: liquid mineral medium with hexadecane (MM-C16), nutrient broth (NB), and nutrient agar (NA); in the latter case, the cells were washed off the solid medium with the buffer and the surfactant properties were measured in cell suspensions. All three strains exhibited increasing cell hydrophobicity and emulsifying activity in the following order of the media: NA—NB—MM-C16. The surface tension decreased in the opposite order (Table 2). The comparison of ST, EA, and HI dynamics of the cultures grown on MM-C16 demonstrated a similar tendency.

The possibility of using the bacterial hydrophobicity index for express selection of surface-active strains grown on solid media was assessed. The natural hydrophobicity of 38 out of 39 collection strains of petro-

Table 1. Surfactant properties of liquid cultures, their supernatants, and chemically isolated biosurfactants

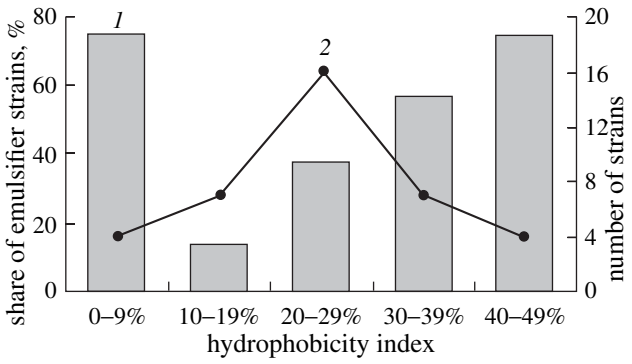
Strains	Properties of liquid cultures			Properties of chemically isolated bioSAS	
	HI, %	ST, mN/m	EA, %	ST, mN/m	EA, %
R. erythropolis F1	73	44.6	15	–	–
R. erythropolis B2	87	30.8	57	29.3	23
Rhodococcus sp. F2	90	34.5	45	27.8	29
Rhodococcus sp. J8	96	35.5	52	35.2	28
Gordonia amicalis K8	71	29.5	28	–	–
Gordonia sp. Z8	57	40.6	9	–	–
Nocardia sp. K5	42	42.0	26	–	–
Rhodococcus sp. Z5	13	53.0	0	–	–
Micrococcus sp. H1	70	57.8	23	–	–
Planococcus sp. S6	4	58.6	0	–	–

Table 2. Surfactant properties of producers of cell-bound biosurfactants at cultivation on different media

Strains that are producers of cell-bound bioSAS	Cell hydrophobicity index			Emulsifying activity of liquid cultures			Surface tension of liquid cultures, mN/m		
	NA	NB	MC-C16	NA	NB	MC-C16	NA	NB	MC-C16
R. erythropolis B2	22	29	87	3	39	57	56.8	46.9	30.8
Rhodococcus sp. F2	21	82	90	5	45	45	54.1	47.3	34.5
Rhodococcus sp. J8	88	90	96	14	40	52	54.1	52	35.5

leum-oxidizing bacteria grown on a solid nutrient agar in the absence of contact with hydrophobic hydrocarbon substrates was within 0–49%. There was a normal distribution of the strains by hydrophobicity index with the HI peak at 20–29%. The share of the strains which had emulsifying activity when grown in a liquid medium with a hydrocarbon was assessed in each interval of hydrophobicity (Figure). The share of bioemulsifiers increased from HI 10–19% to HI 40–49%; it was similarly high in the interval of HI 0–9%. The assessment of culture morphotypes showed that most of the strains with HI 0–9% were M forms, while those with HI 10–49% were R and S forms. The data obtained, in our opinion, provide evidence of different principles of bacteria–hydrocarbon interaction: direct contact, through hydrophobization of cell surface, for R and S forms, and emulsification of paraffins by extracellular biosurfactants for hydrophilic M forms.

The relation between the hydrophilic–hydrophobic properties of cells and their adhesive and foaming properties is well-known [7]. The presented results demonstrate the role of bacterial hydrophobicity in the formation of the surfactant properties of liquid cultures. Registration of the hydrophobicity index may be an additional criterion for selection of the bacteria which synthesize cell-bound bioSAS not excreted into aqueous medium. This group includes hydrocarbon-oxidizing rhodococci, nocardia, and other actinobacteria possessing a high biotechnological potential. The enhancement of the emulsifying properties of liquid cultures with increasing hydrophobicity of bacteria that produce cell-bound bioSAS should be taken into account in biotechnological production: in order to obtain biopreparations with surfactant properties, it is necessary to optimize the cultivation conditions towards the increase of cell hydrophobicity and biomass production.



Distribution of a sampling of 39 strains by natural hydrophobicity (*2*) and the share of emulsifiers for each interval of hydrophobicity (*1*).

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