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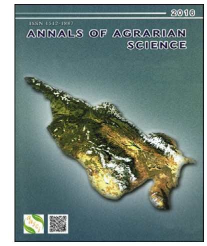

Selection of effective biosurfactant producers among *Bacillus* strains isolated from soils of Georgia

*Nino A. Gagelidze**, *Lia L. Amiranashvili*, *Khatuna I. Varsimashvili*, *Lela M. Tinikashvili*, *Lana L. Tolordava*, *Tinatin A. Sadunishvili*

Sergi Durmishidze Institute of Biochemistry and Biotechnology, Agricultural University of Georgia, 240, David Agmashenebeli Alley, Tbilisi, 0131, Georgia

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ABSTRACT

Application of biosurfactants of microbial origin and biosurfactant producing microorganisms in remediation technologies for cleaning sites polluted with organic and metal contaminants is environmentally friendly. Revealing the potential of microorganisms to synthesize surfactants and development of cost-effective bioprocesses for the biosurfactant production is an important direction of industrial biotechnology. *Bacillus* sp. BK34(7) and *Bacillus* sp. BK10(4) decreasing the indices of surface tension up to 29–30 mN/m were chosen as a result of multistep screening among 234 isolates from soils collected in Kakheti region of Georgia. The best result of biosurfactant synthesis was obtained on the 2nd day of cultivation. None of tested additional carbon sources (hexadecane, heptadecane, tetradecane, glycerol, molasses, sunflower, corn and olive oils) significantly enhances the ability of strains to reduce surface tension. Ability of these strains to decrease surface tension and emulsion capacity makes them as potential candidates for biosurfactant production.

Introduction

Increasing of human industrial activity has led to chemical contamination of the natural environment. Biosurfactants have been tested in environmental applications, such as bioremediation and dispersion of oil spills, enhanced oil recovery, and crude oil transfer, and are considered for surfactants of the future, especially in the food, cosmetics, and in agricultural chemicals [1,2].

Biosurfactant- or bioemulsifier-producers occurring in soils and sediments mainly belong to the genera *Pseudomonas*, *Bacillus*, *Sphingomonas* and *Actinobacteria* [3,1]. Application of biosurfactants produced by different strains of *Bacillus* for enhancement of the biodegradation of hydrocarbons and chlorinated pesticides, removal of heavy metals from a contaminated soil, sediment and water, increasing the effectiveness of phytoextraction has been intensively reported [3–6]. Revealing potential of microorganisms to synthesize surfactants as alternatives to synthetic surfactants and the

* Corresponding author. Tel.: +995 592 110 504.
E-mail address: n.gagelidze@agruni.edu.ge (N.A. Gagelidze).

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development of cost-effective bioprocesses for the biosurfactant production is an important task for industrial biotechnology.

The goal of the present work is isolation and selection of biosurfactant-producers among the strains belonging to the genus *Bacillus* and investigation of different substrates (sunflower (industrial, artisanal), corn, olive oils, glycerol, molasses (dilution with distilled water 1/3), C14, C15, C16) influence on the yields of biosurfactants for selected bacteria.

Objectives and methods

To isolate bacteria of the genus *Bacillus*, soil samples were collected in Kakheti region, Georgia, including sites polluted with crude oil in the vicinity of Sartichala borehole. Viable bacterial cultures were isolated by the method of serial 10-fold dilutions of soil suspension in phosphate-buffered saline (PBS) and plated on Petri dishes with Nutrient agar (Biolife, Italy). Incubation was conducted in thermostat at 30 °C for 3 days. Pure cultures of isolates were obtained by plating on Petri dishes containing the same medium in triplicate, using single colony isolation technique [7]. Separate pure colonies of isolates were transferred on slant agar for current use.

Deep cultivation of bacteria was conducted in 750-ml flasks containing 50 ml of Nutrient broth, on a rotary shaker (180 rpm) at 30 °C for 3 days. 10% of Bacterial suspension at the exponential phase of growth was introduced as inoculum into the liquid medium. Nutrient broth (Biolife, Italy) was used as a nutrient medium.

Blood hemolysis test, emulsification index (E 24) and surface tension of culture liquid were used for screening of biosurfactant producers.

For blood hemolysis test, fresh single colonies of the isolates were taken and streaked on Blood agar plates and incubated for 48 h at 37 °C. Bacterial colonies were observed for the presence of clear zone around the colonies.

The emulsifying capacity was estimated by emulsification index (E 24). The E 24 of culture samples was determined by adding 2 ml of any suitable hydrocarbons and 2 ml of the cell-free broth in test tube and vortexing at high speed for 2 min. The emulsion stability was determined after 24 h, and the emulsification index (E 24) was calculated by dividing the measured height of the emulsion layer by the mixture's total height and multiplying by 100 [8].

Surface tension of culture liquid was measured by tensiometric method. Culture liquid was centrifuged at 8000 rev/min for 10 min and surface tension was measured at 20 °C by tensiometer (Tensiometer K6, KRUSS, GmbH, Germany).

In order to increase the yield of biosurfactants, hexadecane, pentadecane, tetradecane, glycerol, molasses, sunflower (artisanal and industrial), corn and olive oils in the amount of 1% were added to the Nutrient broth.

Experiments were conducted in duplicate and the results reported are averages of three independent experiments.

Results and analysis

Biosurfactant-producing microorganisms are ubiquitous, inhabiting water (sea, fresh water, ground water) and land

Table 1 – Selected *Bacillus* strains increasing surface tension.

Conditional#	Intensity of clear zones	Surface tension, mN/m	Conditional#	Intensity of clear zones	Surface tension, mN/m	Conditional#	Intensity of clear zones	Surface tension, mN/m
BK1(4)	4+	34	BK40(3)	4+	37	BK20(3)	3+	35
BK2(4)	4+	55	BK40(5)	4+	63	BK22(3)	3+	36
BK3(4)	4+	54	BK40(7)	4+	34	BK22(10)	3+	34.5
BK4(2)	4+	54	BK41(1)	4+	36	BK25(7)	3+	37
BK8(2)	4+	49	BK1(2)	3+	60	BK28(7)	3+	38.5
BK9(1)	4+	55	BK3(1)	3+	42	BK29(1)	3+	38.5
BK12(2)	4+	55	BK3(3)	3+	37	BK29(2)	3+	39.5
BK12(4)	4+	51	**BK4(3)**	**3+**	**32.5**	BK30(1)	3+	53
BK13(5)	4+	60	BK4(5)	3+	41	BK30(2)	3+	50
BK15(2)	4+	60	BK6(1)	3+	35.5	BK31(1)	3+	48
BK15(4)	4+	50	BK6(3)	3+	41.5	BK32(6)	3+	35
BK16(2)	4+	47	**BK6(4)**	**3+**	**33**	BK34(2)	3+	52
BK17(3)	4+	47	BK7(1)	3+	38.5	BK34(3)	3+	61
BK18(4)	4+	45	BK7(4)	3+	33.75	**BK34(7)**	**3+**	**29**
BK21(5)	4+	52	BK7(5)	3+	34	BK35(2)	3+	34
BK25(1)	4+	51.5	BK9(2)	3+	44	BK35(5)	3+	50
BK27(3)	4+	51	**BK10(4)**	**3+**	**33.5**	BK35(6)	3+	51
BK27(4)	4+	48	**BK13(1)**	**3+**	**33.5**	BK36(2)	3+	54
BK27(5)	4+	52	BK14(1)	3+	38.5	BK37(2)	3+	34
BK29(3)	4+	53	BK15(1)	3+	35.5	BK37(7)	3+	47
BK32(1)	4+	56	**BK15(5)**	**3+**	**33.5**	BK39(1)	3+	50
BK32(2)	4+	47	BK16(1)	3+	38	BK42(1)	3+	34
BK33(1)	4+	56	BK19(3)	3+	51	BK40(8)	3+	50
BK34(5)	**4+**	**33**	BK19(4)	3+	34.5	BK41(4)	3+	36