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ORIGINAL ARTICLE

Characteristics of *Bacillus Licheniformis 9/2* Biosurfactant Producer

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ABSTRACT
Biosurfactants, being biodegradable, environmentally friendly and multifunctional biomolecules, are widely used in various sectors of human activity. Biosurfactants synthesized by bacteria of the genus Bacillus are considered one of the most promising due to their physicochemical and biological properties. Therefore, the purpose of this study was to study a new bacterial strain of Bacillus sp. 9/2 biosurfactant producer isolated from an oil and gas well in the Kashkadarya region (Uzbekistan). The strain was identified as Bacillus licheniformis 9/2. After optimization of the nutrient medium and strain cultivation conditions, the biosurfactant yield was 3.4 g/LL.
Keywords *Bacillus licheniformis, biosurfactant, screening, lipopeptide, optimization*

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INTRODUCTION

Surfactants (surfactants) are surface-active amphiphilic molecules that, when accumulated at the interface of immiscible liquids, reduce the surface or interfacial tension between two phases, increasing the solubility or mobility of one phase in the other. At the oil/water interface, surfactants attach to the hydrocarbon (HC) through their hydrophobic tail and form a monolayer around the HC particle with a hydrophilic head facing the aqueous phase. This increases the surface area of the hydrocarbons and leads to emulsification, increasing the bioavailability of the hydrocarbons to microbes and, therefore, biodegradation [6]. Most surfactants currently used in various industries are chemically synthesized, are fast-acting but usually toxic, and remain in the environment for a long time. Biosurfactants (BS), synthesized by microorganisms, are promising biomolecules that can replace chemical surfactants due to their physicochemical and biological properties (bioavailability, structural diversity, low toxicity, biodegradability, resistance to elevated temperatures, NaCl concentrations, and ability to function in a wide pH range). They are used in practical applications as foaming agents, moisturizers, emulsifiers, and additives in food, detergents, and cosmetics [12]. Most of the biosurfactant glycolipids studied to date are rhamnolipids, produced by the bacteria *Pseudomonas aeruginosa*, and trehalolipids synthesized by bacteria of the genus *Rhodococcus*, *Arthrobacter* sp., and *Mycobacterium* sp., respectively [13], as well as various lipopeptides and lipoproteins (for example, sophorolipids, viscose, and surfactin), the producers of which are the bacteria *Torulopsis bombicola*, *Pseudomonas fluorescens*, and *Bacillus subtillus* [3]. Bacteria such as *Acinetobacter radioresistens, Acinetobacter calcoaceticus,* and *Candida lipolytica* are producers of polymeric surfactants such as alasan, emulsan, liposan, and biodispersan, respectively [2]. Currently, the potential commercial applications of BS are becoming increasingly in demand because they are environmentally friendly and non-toxic biomolecules. Lipopeptide BS, the main producers of which are bacteria of the genus *Bacillus*, is one of the promising groups of BS [6-11]. Previously, a bacterial strain belonging to the genus *Bacillus* sp. *9/2,* a BS producer, was isolated from a contaminated sample of an oil and gas well in the Kashkadarya region [14].

Considering the promising use of biosurfactants and their biotechnological potential, the purpose of the research is to study and characterize the biosurfactant producer strain *Bacillus* sp. 9/2.

MATERIAL AND METHODS

Microorganism

A biosurfactant producer, *Bacillus* sp. 9/2 was previously isolated from an oil and gas well in the Kashkadarya region by the method of enrichment culture [14].

Morphology, physiology, and chemotaxonomy

The selected strain was identified based on morphological, biochemical, and molecular genetic methods. The strain's morphological-cultural and physiological-biochemical properties were studied per Bergey's "Manual of Systematic Bacteriology" [4]. Thus, the morphology and tinctorial properties of the strain were studied by Gramme staining of vegetative cells under a microscope (Model N300M (NDCE-X5) with magnification (10 x 100); bacterial motility was determined by the crushed drop method using a 16–18-hour broth culture. The growth pattern of the strain in liquid and solid nutrient media was studied for its cultural properties. Biochemical properties of an 18–20 hours culture on the Hiss nutrient medium using differential diagnostic carbohydrate discs (Himedia), the crops were kept for two days at 35–37°C and the presence or absence of carbohydrate fermentation was determined by changes in the color of the medium. The following biochemical tests were used: catalase test, methyl red test, Voges-Proskauer test, strain resistance to various NaCl concentrations, starch hydrolysis, citrate utilization, and nitrate reduction test. To identify the strain, a proteomic analysis of bacterial cells was carried out using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

DNA extraction and PCR amplification of the 16S rRNA gene

The genomic DNA of *Bacillus* sp. 9/2 was extracted using a modified Marble technique F. [15]. PCR amplification was performed using universal oligonucleotide primers for the 16S rRNA gene: 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) (Lane, D.J. 1991). Amplification via PCR of DNA samples collected from bacterial strains has been carried out using the GenPak® PCR core. The reaction dose was 20 μL, and it contained 10 μl of dilution, 8.2 μl of water that was double-distilled, 0.4 μL of each primer (27F and 1492R), and one μl of DNA sample. PCR amplification consisted of some steps: initial denaturation at 94°C for 3 minutes, denaturation at 94°C for 40 seconds, primer annealing at 55°C for 40 seconds, elongation at 70°C for 90 seconds for 35 cycles, final elongation at 70°C for 7 minutes, and kept at 4°C. The amplicons were separated using electrophoresis on a 2% agarose gel stained with ethidium bromide. The PCR product was purified from the gel, quantified using Nanodrop, and adjusted to 20 ng. The samples were sequenced by the SINTHOL company in Russia.

16S rRNA phylogeny

Phylogenetic analysis was performed using MEGA X. The 16S rRNA gene sequences obtained in the present study were submitted to NCBI GenBank to obtain an accession number. In addition, the identified bacterial isolate was routinely maintained on nutrient agar (Himedia) before each screening experiment. The original pure culture was maintained on nutrient agar (Himedia) agar slants and stored at 4°C for use. For long-term storage, pure bacterial cultures were maintained in glycerol broth (≈18% v/v) and stored at −20°C.

Selection of optimal nutrient medium and cultivation conditions for BS biosynthesis

To prepare the inoculum, the bacterial strain was grown in 5 mLmL of TSB (tryptone soy broth, Himedia) for 14–18 hours at a temperature of 30°C and a 150-rpm rotation of the incubator shaker. Then, in an amount of 2% (containing $0.8x10^7$ CFU/mL), the initial OD_{600} from 0.1 to 0.8 was added to 100 mLmL of liquid nutrient medium (LMM), with the following composition (g/LL): Na_2HPO_4 - 2,2; KH_2PO_4 - 1.4; $MgSO_4 \cdot 7H_2O$ - 0.6; $FeSO_4 \cdot 7H_2O$-0.01; NaCl - 0.3; $CaCl_2$ - 0.02 and 0.1% solution of trace elements containing (g/LL); $ZnSO_4 \cdot 7H_2O$ - 2.32; $MnSO_4 \cdot 4H_2O$ - 1.78; H_3BO_3 - 0.56; $CuSO_4 \cdot 5H_2O$ - 1.0; $NH_4MoO_4 \cdot 2H_2O$ - 0.39 and KI - 0.66. The pH of the medium is 7.02 ± 0.02.

To study the effect of various sources of carbon and nitrogen and their optimal concentration on the biosynthesis of BS, the strain was cultivated with various sources of carbon (sucrose, glucose, starch, glycerol, and n-hexadecane) at a concentration of 20 g/LL and nitrogen at a concentration of 1.0 g/LL (urea, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2HPO_4$, $(NH_4)\ HCO_3$, $(NH_4)_2SO_4$) at 30°C and 150 rpm on the incubator shaker for 72 hours. After 72 hours of cultivation, the cultural suspension (CS) was centrifuged at 10,000 rpm for 10 minutes at 4°C. The resulting biomass was washed with 10 mM phosphate buffer pH 7.0, centrifuged, and the resulting supernatant was combined with the supernatant obtained after the first centrifugation of the CS. The amount of biomass was then determined by drying it in an oven at a temperature of 100°C to a constant dry weight. The amount of BS in the cell-free supernatant was

investigated, and measures of the emulsification index (E24), pH change, and supernatant surface tension (ST) were carried out.

Bacillus sp. 9/2 strain was cultivated in MSM medium with glycerol concentrations ranging from 20 to 60 g/L to select the optimal nutrient medium. The selection of the optimal urea concentration was studied in the range of 1.0–4.0 g/L. To select optimal cultivation conditions, the strain was grown on the optimal nutrient medium MSM in 1000-mL flasks with a nutrient medium volume of 500 mL and a starting pH of 6.0 to 8.0, at 150 rpm, 25–40°C temperature range for 72 hours.

Determination of the optical density (OD)

The OD value of the cell suspension was determined using a UV-5100 spectrophotometer (Metash, China) using a 1.0 cm cuvette. Bacterial growth was assessed by the optical density of the cell suspension at λ=600 nm, taking into account dilution, as well as by measuring the absolutely dry biomass obtained by centrifuging the CS for 10 min at 10,000 rpm.

Measurement of surface tension

The surface tension of the CL supernatant was measured using an automatic tensiometer (Model GD8541A, China). Measurements were carried out at 20°C. Surface tension was calculated using the formula: σ = mg/2l, where m is the plate separation mass, g (average of three measurements); g = 9.8 m/s2 – free fall acceleration; l = 15.7 mm – length of the tear line (width of the plate). The decrease in surface tension (Δσ) was calculated as the difference between the surface tension values of the sterile medium (control) and the test culture sample.

Analysis of extracellular BS production

The oil displacement analysis test was conducted in a Petri dish containing 20 milliliters of distilled water, 5 milliliters of oil, and 30 microliters of supernatant on top. The diameter of the clear zone on the oil surface was measured and compared with the negative control; distilled water served as the negative control. The CTAB agar test and hemolytic activity were performed according to the method of Goswani and Dec [1, 8]. The emulsification index (IE), which shows the ability of strains to emulsify hydrocarbons in an aqueous medium, was determined by the Cooper and Goldenberg method [5].

The isolation of BS was carried out using acid precipitation and organic solvent extraction, according to Vater J. *et al.* [16]. To do this, the supernatant was acidified with 6N HCl to pH 2–4, after which it was kept for 14–18 hours at a temperature of 4°C to enhance the precipitation of BS. The precipitate was separated by centrifugation at 12,000 rpm, then dissolved in 10 mM phosphate buffer pH = 7.0 and extracted several times with an equal volume of a chloroform-methanol mixture in a ratio of 2:1. The aqueous fraction was separated from the chloroform-methanol fraction, lyophilized, and weighed to quantify the polar part of the BS.

In the chloroform-methanol fraction (the nonpolar part of the BS), the solvent was removed on a rotary evaporator at a temperature of 45°C, and the yield of the BS was determined by weighing.

RESULTS AND DISCUSSION

The strain decreased surface tension when growing on MSM medium from 81.7 to 49.4 mN/m. When determining the emulsification index (E_{24}) of the CS supernatant, E_{24} was more than 60% and was stable for 5 days (Figure 1A). Analysis of oil displacement (with a density of 0.9 g/cm^2): when adding 10 µl of a sample, the oil displacement zone varied in the range of 20–25 mm (Fig. 1B). In the blood hemolysis test of the *Bacillus* sp. 9/2 strain on blood agar medium, a hemolysis zone was observed around the colonies (Figure 1C). According to the literature, almost all BS producers have positive hemolytic activity, while not all hemolytic species are BS producers. Thus, studies conducted by Noha H. Youssef *et al.* showed that out of 205 strains belonging to various genera, forty-nine percent of the strains did not lyse blood agar; however, when using other screening methods, they showed the ability to biosynthesize BS; lysis of blood agar also did not correlate with surface tension [19]. Therefore, hemolytic activity should be considered as an unreliable criterion for the detection of BS in microbial culture.

A methylene blue agar plate can be used to confirm the presence of anionic BS; it is a semi-quantitative method for detecting extracellular BS. In the study, a pure culture of the *Bacillus* sp. *9/2* strain was streaked onto a medium containing CTAB. On the plate, a dark blue halo appeared around the colony (Fig.1D), indicating the anionic form of BS in the form of an insoluble complex with the cationic bromide salt, and the complex is detected using methylene blue present in the agar.

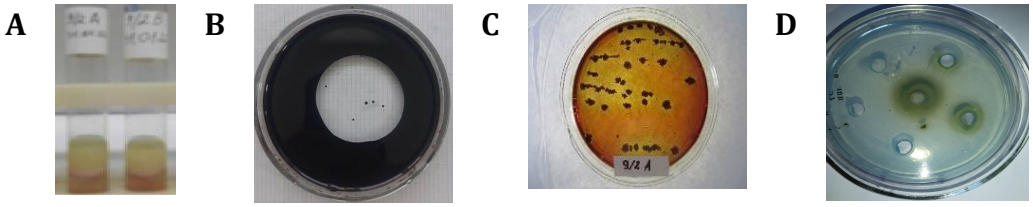

Figure 1. Screening by (A) emulsification index E24; (B) oil displacement analysis test; (C) test for hemolytic activity; (D) test on CTAB agar of the BS producer strain *Bacillus* sp. 9/2.

In this study, the species identification of the *Bacillus* sp. 9/2 strain was studied, the influence of various carbon and nitrogen sources was investigated, and the optimal nutrient medium and conditions for cultivating the *Bacillus* sp. 9/2 strain on the biosynthesis of BS was selected, and the resulting BS was characterized.

Morphological, physiological, and biochemical characteristics of the *Bacillus* sp. 9/2

The study of morphological-cultural and physiological-biochemical properties showed that the strain, when growing on TSB scum, causes turbidity in the medium; when shaken, flocculent sediment is formed; when a film is formed, the medium becomes clear and releases a pink pigment. When the strain grows on nutrient agar medium, the colonies are round, greyish-white in color, matte, with an uneven wavy edge, and have small grooves in the center of the colony with a maximum diameter of 0.6–1.4 cm. All colonies are dry, do not grow on agar, are easily separated, are gram-positive, and s spore-forming (Fig. 2).

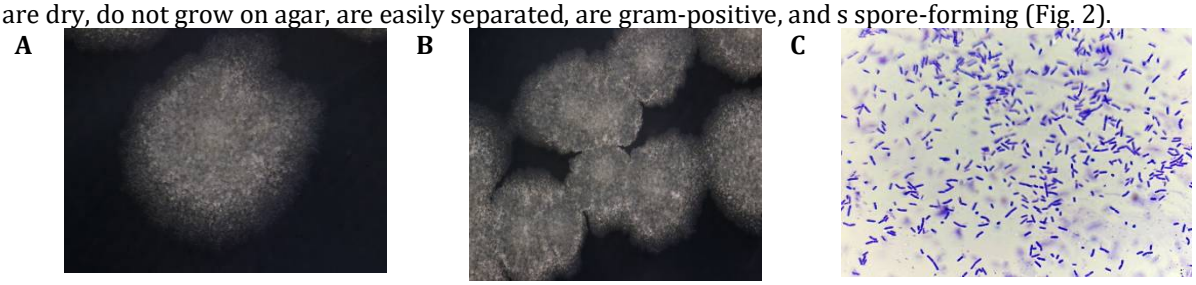

Figure 2. (A) Morphology of *Bacillus* sp. 9/2 colonies on nutrient agar; Microscopic preparation of *Bacillus* sp. 9/2 (B) - strain spores (magnitude 10×100)) – (C) Gram stain (magnification 10×100);

Based on physiological and biochemical characteristics, it was established that the strain grows under aerobic conditions and hydrolyzes urea. It gives a positive reaction with the Voges-Proskauer methyl red test and grows at a NaCl concentration of 8–10%. Hydrolyzes starch and casein, liquefies gelatin, and does not break down tyrosine. Produces catalase, protease, and amylase. Does not form lecithinase or lipase. Reduces nitrates to nitrites. Does not form an indole. Fermenting glucose, arabinose, xylose, maltose, fructose, cellobiose, and sorbitol does not ferment raffinose (Table 1, Fig. 3).

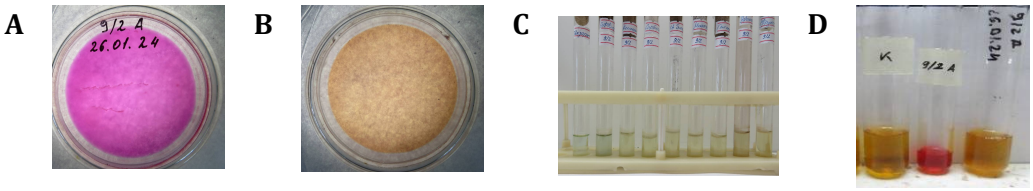

Fig. (3) Physiological and biochemical characteristics of the strain *Bacillus sp 9/2*, BS producer. Hydrolysis of urea (A) – experience (B) – control; Carbohydrate series; Methyl red and (FP) test.

Table (1) Morphological, physiological, and biochemical characteristics of the *Bacillus* sp. 9/2 strain, a BS producer

Sign	*Bacillus* sp. 9/2
Gram stain	Gram positive
Morphology	Colonies are round in shape, grayish-white in color, matte, with an uneven wavy edge, with small grooves in the center of the colony
Mobility	Mobile
Spore	The spores are oval in shape and located centrally in the cell
Size	0.6–1.0 μm in length
Pigment	The culture produces a dark pink pigment in the medium
Acid formation:	
Glucose	Forms acid
Arabinose	Forms acid
Xylose	Forms acid
Maltose	Forms acid
Fructose	Forms acid
Cellobiose	Forms acid
Sorbitol	Forms acid
Raffinose	Does not form acid
Biochemical reaction	
Indole	Negative
Voges Proskauer	Positive
Nitrates to nitrites	Positive

Strain identification by molecular genetic methods

As can be seen in Figure 4A, high-quality and pure genomic DNA of *Bacillus* sp. 9/2 strain was extracted. Figure 5B shows the formation of a PCR product of the 16S rRNA gene with a size of 1500 bp; as a result of sequencing, a nucleotide sequence of 1213 base pairs were obtained. The nucleotide sequence of the 16S rRNA gene of the strain was checked in the National Center for Biotechnology Information (NCBI) database, and as a result, 100% homology with the species *Bacillus licheniformis* was found. In addition, 99.91% similarity with the species *Bacillus subtilis, Bacillus velezensis*, and *Bacillus amyloliquefaciens* was found. The phylogenetic tree was constructed using the maximum likelihood statistical method using Mega4 bioinformatics software (Fig. 5).

A B

Fig (4). Genomic DNA (A) and PCR product (B) of the 16S rRNA gene of the *Bacillus* sp. 9/2 strain.

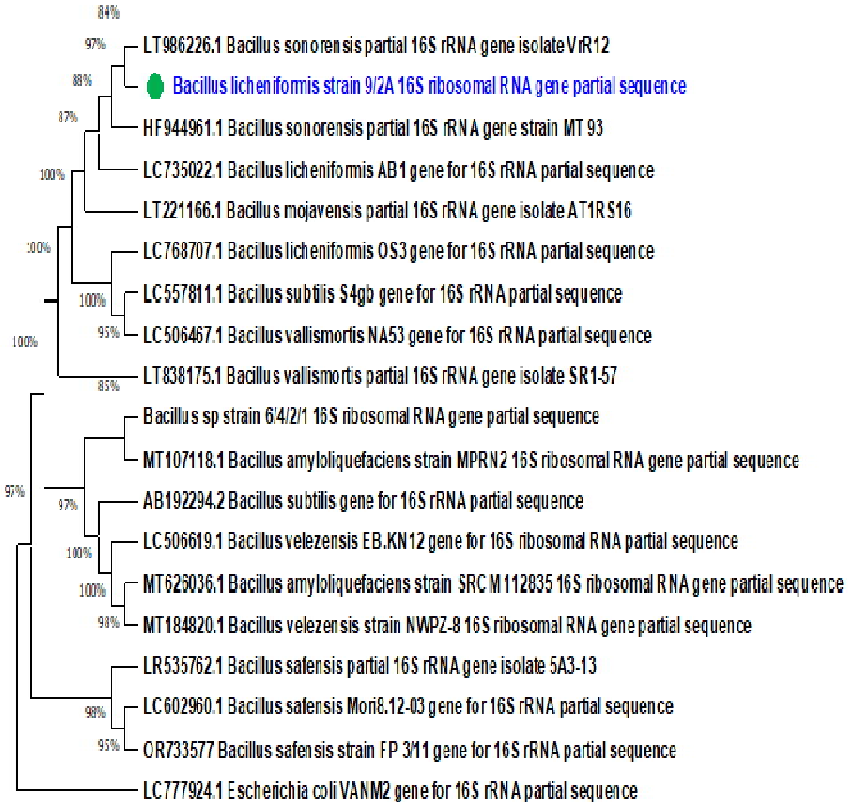

Fig. (5) Phylogenetic tree of a *Bacillus* strains using the maximum likelihood statistical method in Mega4 bioinformatics software.

The nucleotide sequence of the 16S rRNA gene of this strain was placed in the GenBank and NCBI databases, and the identification number PP335299 was obtained. Based on morphological-cultural, physiological-biochemical, and molecular-genetic properties, the bacterial strain *Bacillus* sp. 9/2 was identified as a *B. licheniformis* 9/2A.

Effect of carbon and nitrogen sources on the biosynthesis of BS by *B. licheniformis* 9/2A

The selection of optimal nutrient medium and cultivation conditions is important for the biosynthesis of BS, since the maximum biosynthesis of BS depends on the composition of the nutrient medium and optimal cultivation conditions, which in turn will lead to a reduction in the cost of the final BS product. To achieve maximum biosynthesis of BS, it is necessary to monitor the main cultivation parameters (substrate, C/N ratio, aeration, pH, and temperature).

To select the optimal source of carbon and nitrogen, we used glucose, sucrose, starch, or glycerol at a concentration of 20 g/L as a carbon source and urea, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2HPO_4$, (NH_4) HCO_3 as a nitrogen source, and $(NH_4)_2SO_4$ at a concentration of 1.0 g/L.

When studying the influence of various carbon sources on the biosynthesis of BS, it was found that the strain can use all substrates except n-hexadecane (HD).

Bacteria of the genus *Bacillus* differ in their ability to use hydrocarbons (n-HD or paraffin) as a carbon source; that is, for some strains, these substrates are the only carbon sources for growth and biosynthesis of BS, and for others, they limit or completely inhibit growth (Wang, D. *et al.* 2019).

In our studies, limited growth of the strain was observed when n-HD was used in the medium as a carbon source. Biosynthesis of BS by strain *B. licheniformis 9/2A* was observed on a medium with sucrose, glucose, and glycerol, while the surface tension of the supernatant of BS in all three media decreased and was 47.3 mN/m when cultivated with sucrose, 46.3 mN/m with glucose, and on glycerol, 33.8 mN/m. It should be noted that when the strain is cultivated on a medium with sucrose and glucose, the strain synthesizes cell-associated polysaccharides to a greater extent; therefore, the cell biomass was 11.4 g/LL and 4.68 g/L, respectively, whereas, on a medium with glycerol, biosynthesis of biosurfactant is observed (Fig. 6).

The ability of bacteria of the genus *Bacillus* to use glycerol as a carbon source and biosynthesis of crude BS is described in a study conducted by Walaa A. Eraqi *et al.* [18].

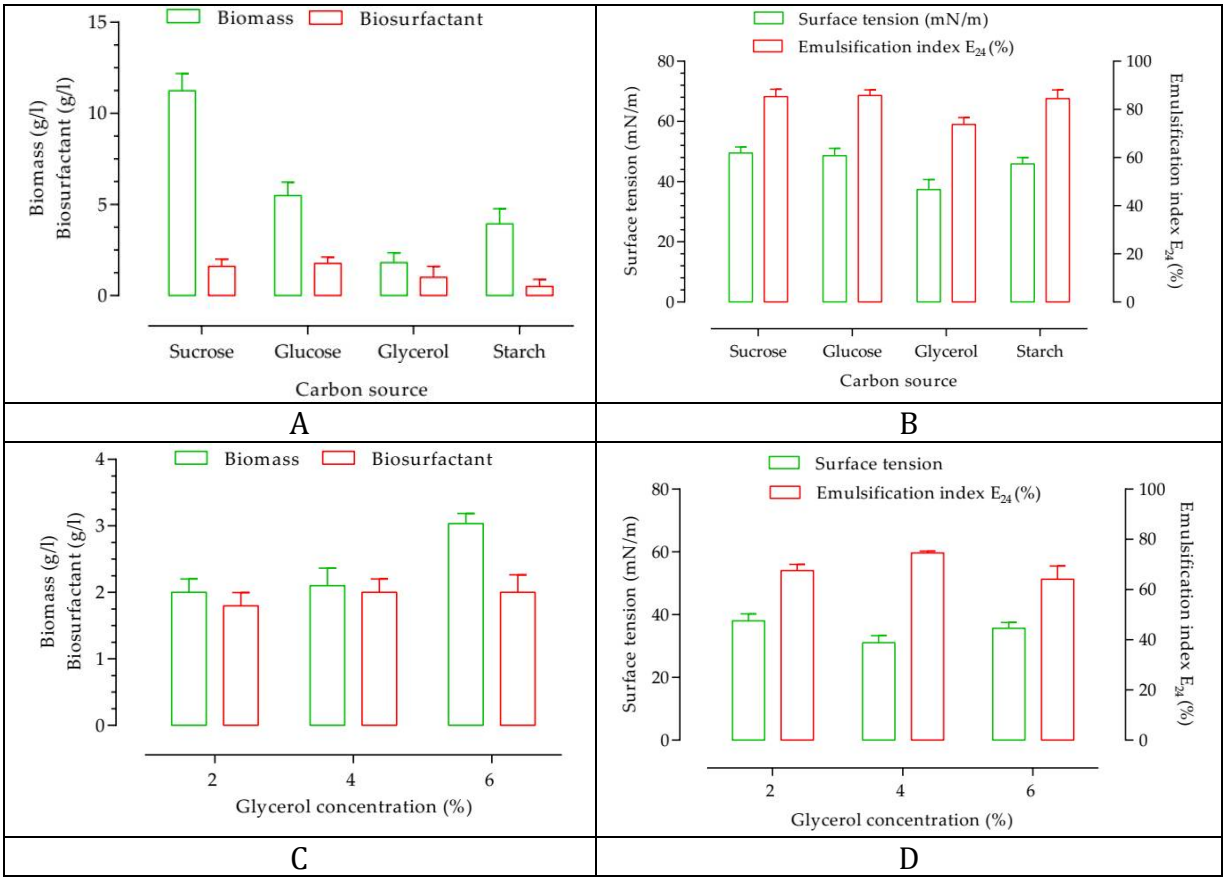

Fig. (6) The influence of various carbon sources on the biosynthesis of BS by *B. licheniformis* 9/2 (A, B) and influence of different glycerol concentration on the biosynthesis of BS by *B. licheniformis* 9/2 A after 48 hours of cultivation at a temperature of 30°C. (C, D).

When selecting the optimal concentration of glycerol, it was found that with an increase in the concentration of glycerol in the medium to 6%, a linear increase of biomass is observed in the medium. Maximum BS biosynthesis and a decrease in surface tension are observed at a glycerol concentration of 4%. At higher concentrations of glycerol, the biomass increases, and the biosynthesis of BS increases, but not significantly, which indicates the biosynthesis of physiologically inactive cells for the biosynthesis of crude BS On the other hand, when synthesizing BS using the produced cellular biomass, it is necessary to take into account the balance between carbon and nitrogen sources.

Because nitrogen is necessary for microbial growth and the production of some primary and secondary metabolites [7]. The type of nitrogen source found in the production environment affects the production of BS by microbes after selecting the optimal carbon source, glycerol, at a concentration of 4%. To select the optimal nitrogen source, the strain was cultivated in a medium containing urea, $NaNO_3$, NH_4Cl, NH_4NO_3, $(NH_4)_2HPO_4$, (NH_4) HCO_3, and $(NH_4)_2SO_4$ at a concentration of 2.0 g/L. It was experimentally established that the smallest decrease in the surface tension of the CL supernatant of 27.2 mN/m, with an increased EI of 68%, was observed when the strain was cultivated on a medium with urea (Fig. 7). When using other nitrogen sources in combination with glycerol, the smallest decrease in surface tension was observed $(NH_4)_2HPO_4$ and (NH_4) HCO_3 and amounted to 66, with an EI of 5–18%. Zhang *et al.*, in their study [17, 20], obtained the highest BS production at 3.0 g/L urea for the related species *Bacillus atropaeus*.

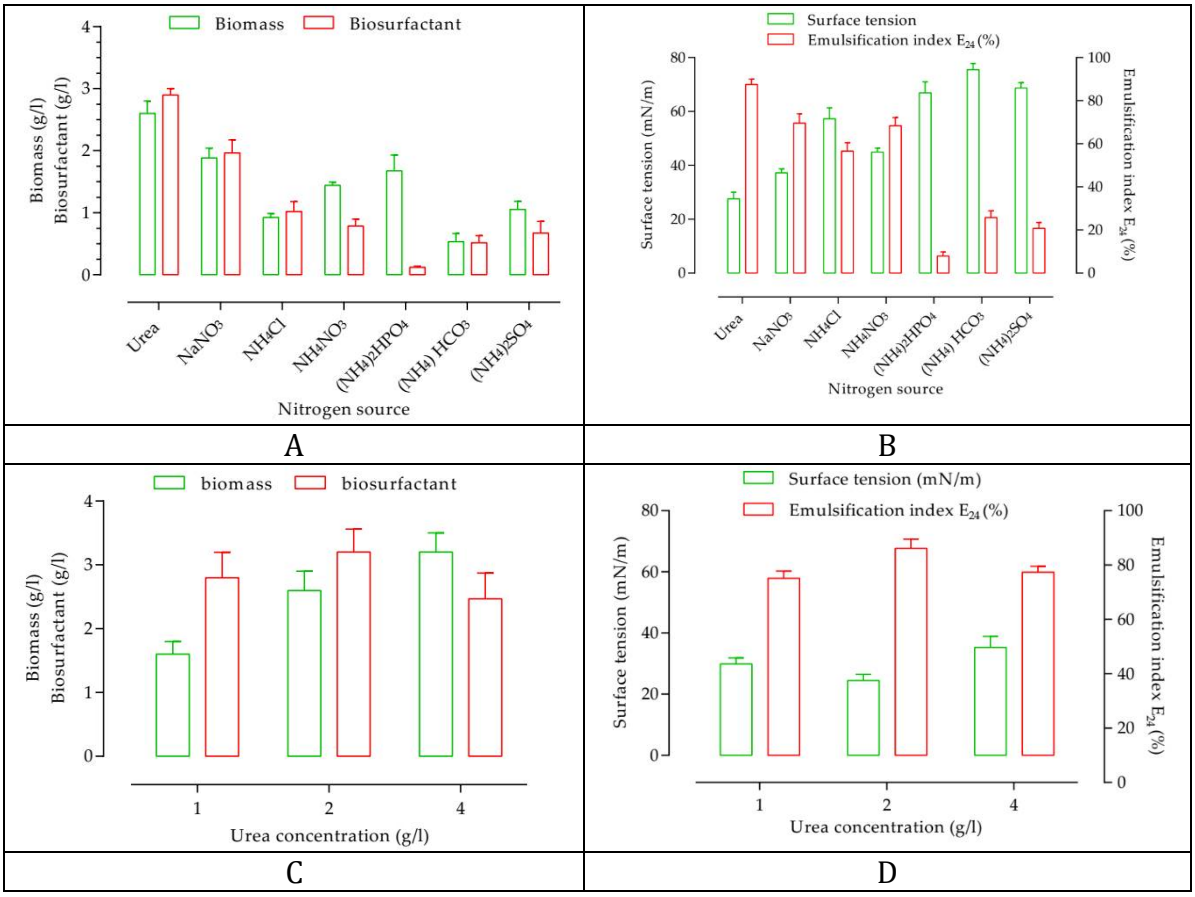

Fig. (7) The influence of different concentrations nitrogen sources (A, B,) and different concentration urea (C, D) on the biosynthesis of crude BS by *B. licheniformis* 9/2 after 48 hours of cultivation at a temperature of 30°C.

When studying the effect of various urea concentrations on the biosynthesis of BS by *B. licheniformis 9/2* it was found that the maximum biosynthesis of crude BS was observed in a medium with a urea concentration of 2 g/L. It was shown that the glycerol/urea (C/N) ratio of 20 times contributed to obtaining the maximum yield of crude BS while achieving the lowest surface tension (34.15 ± 0.6 mN/m) and maximum EI $_{24}$ (55 ± 0.3%) (Fig. 7). Consequently, the use of optimal concentrations of urea as a source of organic nitrogen and the use of the optimal C/N ratio effectively influenced cellular metabolism and BS biosynthesis. Overall, in this study, urea in glycerol played a significant role in improving the biosynthesis of BS. It has also been shown that optimal seeding density is important for the high biosynthesis of BS. According to the research results, it was established that with an initial OD_{600} of 0.2, the yield of BS was quite low and amounted to no more than 0.53 g/L, while with an OD_{600} in the range of 0.6–0.8, the strain on the MSM nutrient medium with a glycerol concentration of 40 g/L and a urea concentration of 2 g/L had a yield of 3.1 g/L. A study of the influence of the initial pH value of the CL on the growth and biosynthesis of BS showed that the maximum biosynthesis of BS was observed at pH = 7.0–7.5; as the pH of the medium increased, a decrease in BS biosynthesis was observed. When researching the temperature effect on BS biosynthesis, the maximum EI_{24} with minimum surface tension was observed at a temperature of 30°C, and the yield of BS was 2.92 g/L. At low (25°C) and increased (35–40°C) cultivation temperatures, the biosynthesis of BS decreases (Fig.8).

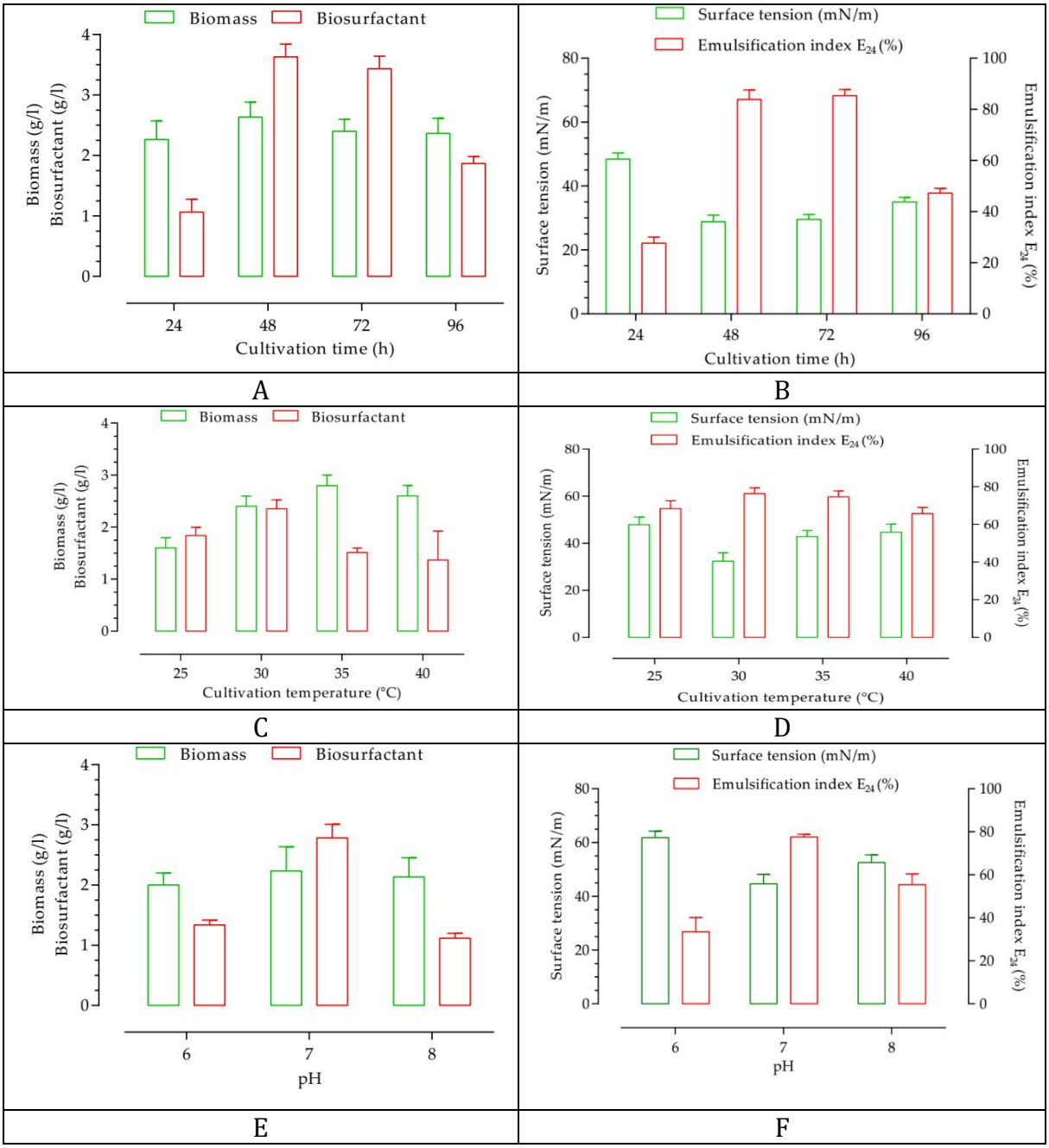

Fig. (8) The optimal cultivation time (A, B), temperature (C, D) and pH (E, F) strain B. licheniformis 9/2 for biosynthesis of BS.

The influence of an optimal nutrient medium on the growth and biosynthesis of BS by *B. licheniformis* 9/2

The growth kinetics and biosynthesis of BS by *B. licheniformis* 9/2 were studied on the optimal nutrient medium MSM with an inoculum OD_{600} = 0.6. Glycerol at a concentration of 40 g/L was used as a carbon source, and urea at a concentration of 2 g/L was used as a nitrogen source at a temperature of 30°C. with an initial pH value of 7.5 at 150 rpm.

The strain was cultivated in 1000-mL flasks in a nutrient medium volume of 500 mL with strain growth dynamics for 96 hours. As the research results showed, the maximum decrease in surface tension was observed at 48–72 hours of incubation, with a biomass of 2.4 g/L.

Analysis of literature data shows that for the biosynthesis of secondary metabolites; the process of cultivating microorganisms must be completed when 1 mL of CS contains at least 70% of cells in the vegetative form and 30% in the spore form [9]. As the research results have shown, when the strain is cultivated on an optimal nutrient medium, sporulation is observed after 48–60 hours of cultivation, which

corresponds to the maximum decrease in the surface tension of the CL, the maximum EI_{24}, and the biosynthesis of BS. Thus, as a result of the research, the species identity of the strain was established, and the optimal nutrient medium and strain cultivation conditions were selected for maximum biosynthesis of BS. Statistical analysis of the results based on Student's and Duncan's tests, performed after ANOVA analysis, showed that the highest BS production was obtained when using glycerol at a concentration of 4%. Under such conditions, the yield of BS was 3.4 g/L.

CONCLUSION

This study characterizes the strain *Bacillus licheniformis* 9/2*,* as a BS producer. The strain was isolated from an oil and gas well in the Kashkadarya region (Uzbekistan). Based on the study of morphological-cultural, physiological-biochemical, molecular genetic methods of the strain *Bacillus* sp. 9/2 was identified as *Bacillus licheniformis* 9/2A. The optimal nutrient medium (source of carbon and nitrogen) and cultivation conditions for the biosynthesis of BS by the strain *Bacillus licheniformis* 9/2 were selected; it was shown that the optimal source of carbon is glycerol at a concentration of 4%, and urea at a concentration of 2 g/L as a source of nitrogen. When optimizing strain cultivation conditions, temperature and pH, the yield of BS increased and amounted to 3.4 g/L.

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REFERENCES

1. Adetunji, A. I., & Olaniran, A. O. (2021). Production and potential biotechnological applications of microbial surfactants: An overview. Saudi journal of biological sciences, 28(1), 669–679.
2. Adejumo, S. A., Oli, A. N., Okoye, E. I., Nwakile, C. D., Ojiako, C. M., Okezie, U. M., Okeke, I. J., Ofomata, C. M., Attama, A. A., Okoyeh, J. N., & Esimone, C. O. (2021). Biosurfactant Production Using Mutant Strains of *Pseudomonas aeruginosa* and Bacillus subtilis from Agro-industrial Wastes. Advanced Pharmaceutical Bulletin, 11(3), 543–556.
3. Ahmadi-Ashtiani, Hamid-Reza, Anna Baldisserotto, Elena Cesa, Stefano Manfredini, Hossein Sedghi Zadeh, Mostafa Ghafori Gorab, Maryam Khanahmadi, Samin Zakizadeh, Piergiacomo Buso, and Silvia Vertuani. (2020). "Microbial Biosurfactants as Key Multifunctional Ingredients for Sustainable Cosmetics" Cosmetics 7, No. 2: 46.
4. WB Whitman. (1984). Bergey's manual of systematic bacteriology Publisher: Williams & Wilkins, Baltimore, MD, 1984-989.
5. Cooper, D. G., & Goldenberg, B. G. (1987). Surface-active agents from two bacillus species. Applied and Environmental microbiology, 53(2), 224–229.
6. Fenibo, E. O., Ijoma, G. N., Selvarajan, R., & Chikere, C. B. (2019). Microbial Surfactants: The Next Generation Multifunctional Biomolecules for Applications in the Petroleum Industry and Its Associated Environmental Remediation. Microorganisms, 7(11), 581.
7. Ghribi, D., & Ellouze-Chaabouni, S. (2011). Enhancement of Bacillus subtilis Lipopeptide Biosurfactants Production through Optimization of Medium Composition and Adequate Control of Aeration. Biotechnology research international, 2011, 653654.
8. Goswami, M., & Deka, S. (2019). Biosurfactant production by a rhizosphere bacteria *Bacillus altitudinis* MS16 and its promising emulsification and antifungal activity. Colloids and surfaces. B, Biointerfaces, 178, 285–296.
9. Khilko T.V. (2004). Optimization of nutrient media for growth and sporulation of bacteria *Bacillus subtilis* and *Bacillus licheniformis* // Microbiol. T. 66, No. 1. – P. 36–41.
10. Lane, D.J. (1991) 16S/23S rRNA Sequencing. In: Stackebrandt, E. and Goodfellow, M., Eds., Nucleic Acid Techniques in Bacterial Systematic, John Wiley and Sons, New York, 115-175.
11. Meena, K. R., & Kanwar, S. S. (2015). Lipopeptides as the antifungal and antibacterial agents: applications in food safety and therapeutics. BioMed research international. 473050.
12. Santos, D. K., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: Multifunctional Biomolecules of the 21st Century. International journal of molecular sciences, 17(3), 401.
13. Shu, Q., Lou, H., Wei, T., Liu, X., & Chen, Q. (2021). Contributions of Glycolipid Biosurfactants and Glycolipid-Modified Materials to Antimicrobial Strategy: A Review. Pharmaceutics, 13(2), 227.
14. Sayliev M.U., Alimova B.Kh., Pulatova O.M., Makhsumkhanov A.A., Davranov K.D. (2022). Isolation, characterization and selection of the optimal nutrient medium for the biosynthesis of biosuractants by bacteria of the genus Bacillus. Scientists. Uz International Scientific Journal Science and Innovation Issue Dedicated to the 80th Anniversary of The Academy of Sciences of The Republic of Uzbekistan. DOI:10.5281/zenodo.8370396

15. Francisco S-Serra, Margarita G, Liselott Svensson-S, Antonio B, Daniel Jaén-L, Roger K, Edward R. B. Moore. (2018). A protocol for extraction and purification of high-quality and quantity bacterial DNA applicable for genome sequencing: a modified version of the Marmur procedure. Biology. DOI:. Corpus ID: 92382694. https://dx.doi.org/10.1038/protex.2018.084
16. Vater, J., Kablitz, B., Wilde, C., Franke, P., Mehta, N., & Cameotra, S. S. (2002). Matrix-assisted laser desorption ionization-time of flight mass spectrometry of lipopeptide biosurfactants in whole cells and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum sludge. Applied and environmental microbiology, 68(12), 6210–6219.
17. Wang, D., Lin, J., Lin, J., Wang, W., & Li, S. (2019). Biodegradation of Petroleum Hydrocarbons by *Bacillus subtilis* BL-27, a Strain with Weak Hydrophobicity. Molecules (Basel, Switzerland), 24(17), 3021.
18. Walaa A. Eraqi, Aymen S. Yassin, Amal E. Ali, and Magdy A. Amin. (2016). Utilization of Crude Glycerol as a Substrate for the Production of Rhamnolipid by *Pseudomonas aeruginosa* Hindawi Publishing Corporation Biotechnology Research International . Article ID 3464509, 9 pages.
19. Youssef, N. H., Duncan, K. E., Nagle, D. P., Savage, K. N., Knapp, R. M., & McInerney, M. J. (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. Journal of microbiological methods, 56(3), 339–347.
20. Zhang, J., Xue, Q., Gao, H., Lai, H., & Wang, P. (2016). Production of lipopeptide biosurfactants by *Bacillus atrophaeus* 5-2a and their potential use in microbial enhanced oil recovery. Microbial cell factories, 15(1), 168. .