ISSN 0003-6838, Applied Biochemistry and Microbiology, 2023, Vol. 59, No. 1, pp. 1–13. © Pleiades Publishing, Inc., 2023.
Russian Text © The Author(s), 2023, published in Prikladnaya Biokhimiya i Mikrobiologiya, 2023, Vol. 59, No. 1, pp. 00000–00000.

Surfactin: Its Biological Activity and Possibility of Application in Agriculture

O. V. Kisil[a], *, V. S. Trefilov[b], V. S. Sadykova[a], M. E. Zvereva[b], and E. A. Kubareva[c]

[a] *Gause Institute of New Antibiotics, Moscow, 119021 Russia*

[b] *Department of Chemistry, Moscow State University, Moscow, 119991 Russia*

[c] *Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, 119991 Russia*

**e-mail: olvv@mail.ru*

Received August 12, 2022; revised August 30, 2022; accepted September 2, 2022

Abstract—In this review, the current information about surfactin (a cyclic lipopeptide), which is one of the most studied microbial biosurfactants, is summarized and analyzed. The mechanism of surfactin biosynthesis, the spectrum of its natural and synthetic isoforms, the biological activity of surfactin, as well as its role in the regulation of the life processes of producers, have been presented. The potential of using surfactin and biopreparations based on surfactin-producing bacteria of the genus *Bacillus* to protect and stimulate plant immunity has been shown.

Keywords: surfactin, *Bacillus* spp., plant protection

DOI: 10.1134/S0003683823010027

Microbial control strategies using antibiotics are constantly faced with the problem of the emergence of resistant bacterial strains, including those embedded in biofilm formations. In recent years, the attention of researchers has been riveted on bacterial surfactants (biosurfactants). They represent a heterogeneous group of amphiphilic compounds: with hydrophilic (amino acid or peptide, di- or polysaccharide, and anionic or cationic) fragments and hydrophobic (residues of saturated or unsaturated aliphatic acids) fragments. Directly interacting with the components of the matrix of pathogenic bacteria and fungi, they change the physical properties of the biofilm surface and initiate its degradation. The dispersing properties of biosurfactants were not inferior to those of modern pharmaceuticals against bacterial and yeast biofilms, as well as the viral membrane structures. This makes them potential candidates for new-generation antimicrobial agents and/or adjuvants for other antibiotics. Microbial biosurfactants have several advantages over synthetic surfactants (**SS**): biodegradability, low toxicity, and physicochemical stability at high temperatures or extreme pH values [1]. Some biosurfactants are currently used in the clinical, food, pharmaceutical, and environmental sectors, while others remain under study and development.

Surfactin is the best-studied biosurfactant produced by *Bacillus* spp. [2]. In 1968, while studying the effect of microbial metabolites on the fibrinolytic system and the blood coagulation system, Arima et al. [3] found a powerful clotting inhibitor secreted into the culture fluid by several *Bacillus subtilis* strains. The inhibitor was isolated as white needles. Due to its high surface activity exceeding that of sodium lauryl sulfate, it was named "Surfactin" (this derives from the English word "surface-active"). In Japan, it is patented as a powerful biosurfactant that inhibits the formation of blood clots [4]. To date, surfactin has been found to be produced by several *Bacillus* species, including *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. mojavensi*, *B. licheniformis*, *B. circulan*, *B. natto*, *B. tequilensis*, *B. inaquosorum*, *B. spizizenii*, *B. vallismortis*, *B. subtilis* subsp. *subtilis*, and *B. velezensis* (The National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov) [5–7]. Studies of 35 wild-type and commercial strains carried out by Hse et al. [8] indicated that the wild-type strains of *B. amyloliqufaciens* and *B. subtilis* show the highest level of surfactin production among all cultures: 452.5 and 125.6 mg/L, respectively.

Surfactin is an amphiphilic molecule, which determines its unique physicochemical properties: the ability to foam, emulsify, modify hydrophobic surfaces, and chelate [9, 10]. It can disperse oil spills on the water surface, increasing the efficiency of their utilization by native marine microorganisms, as well as improve the adhesion of bacteria to the oil slick to increase the productivity of bioremediation [11]. The emulsifying properties of surfactin suggest the possibility of using it in the cosmetic industry and biopharmaceuticals [12]. Surfactin induces cytotoxicity against cell lines of many types of malignancies, such as breast

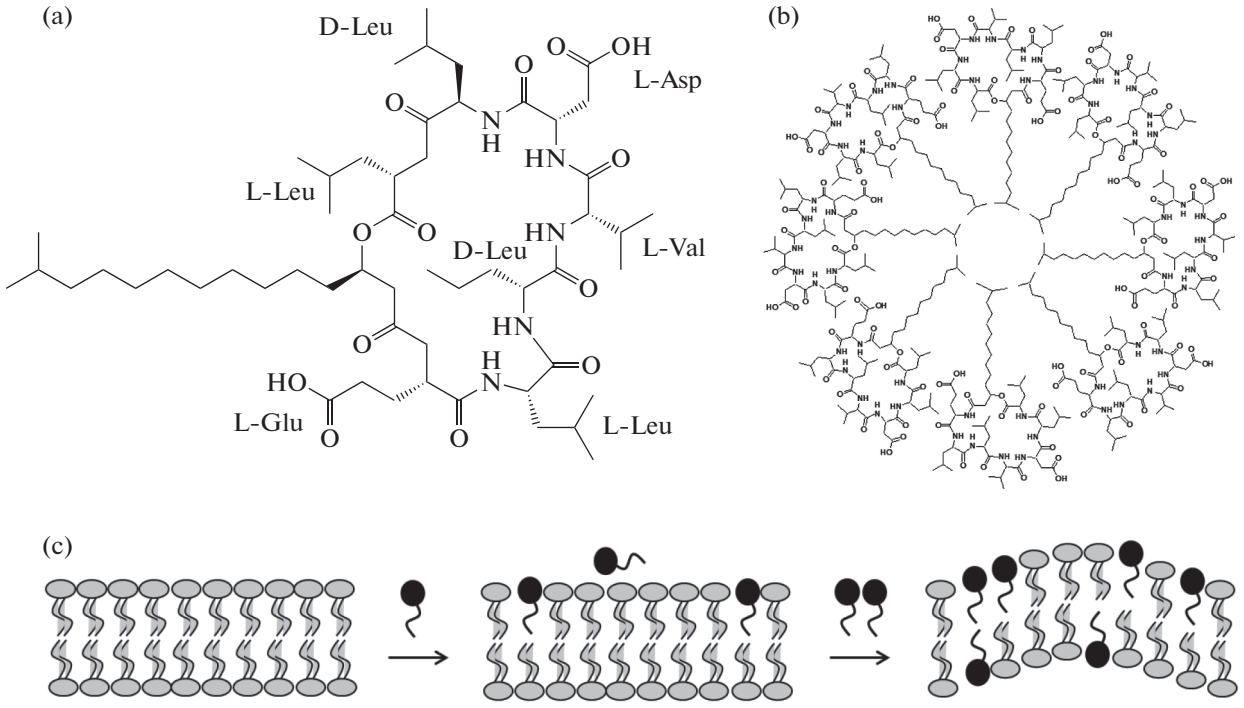

Fig. 1. Surfactin: its structure and interaction with the cell membrane: (a) structural formula of surfactin; (b) proposed structure of a micelle formed by surfactin molecules (adapted from [2]); (c) schematic model of phospholipid membrane destabilization (lipids are shown in gray) by surfactin molecules (black); adapted from [16].

and colon cancer, leukemia, and hepatoma [2]. The amphiphilic nature of surfactin makes it easy to introduce surfactin into nanopreparations (polymer nanoparticles, micelles, microemulsions, and liposomes), which allows optimization of the delivery of surfactin to malignant tumors and increases the effectiveness of antitumor therapy. Unfortunately, the commercial potential of surfactin as a therapeutic agent cannot be fully realized due to its hematotoxicity (hemolysis of erythrocytes).

The structural diversity of surfactin. Initially, the structure of surfactin was established by hydrolysis of the molecule into fragments, followed by their identification and determination of the sequence order, first, along the amino-acid sequence and then along the chain of aliphatic acids [13, 14]. Surfactin consists of a closed peptide chain formed by seven α-amino-acid residues connected to a β-hydroxy aliphatic acid residue. A typical sequence of amino-acid residues in a cyclopeptide fragment (Fig. 1a) is as follows: L-Glu1–L-Leu2–D-Leu3–L-Val4–L-Asp5–D-Leu6–L-Leu7 [15]. It is noteworthy that positions 3 and 6 contain D-form amino-acid residues. The polar part of surfactin consists of two negatively charged amino-acid residues Glu and Asp (in natural surfactin).

The study of the three-dimensional structure of surfactin by ^{1}H NMR showed the presence of a minor polar and a major hydrophobic domain. The minor domain is formed by the main chain of the cyclic peptide and its two negatively charged amino-acid residues: Glu1 and Asp5. The hydrophobic domain is formed by the hydrocarbon chain of the fatty acid residue and the amino acids Leu2, Leu3, Val4, Leu6, and Leu7. Such a two-domain structure determines the amphiphilic nature of surfactin and, as a result, its high surface activity [15]. Based on data from circular dichroism spectroscopy and Fourier transform infrared spectroscopy, Wass et al. [17] showed that surfactin in a solution has a pronounced ability to self-assemble with the formation of micelles and larger aggregates, while the morphology of the formed micelles is significantly affected by environmental conditions, such as pH, metal ions, and temperature (Fig. 1b).

The relative complexity and variability of the structure determine the coexistence of a large number of surfactin isomers in the samples isolated from the cultural liquid of bacteria [18]. Due to the wide range of isomers, the molecular weight of surfactins varies in the range of 993–1049 Da [5]. The main structural analogs of surfactin can be divided into two groups: (1) isomers that differ in the amino-acid sequence of the hydrophilic "head" and (2) isomers that differ in the number of carbon atoms in the aliphatic part of the fatty acid, i.e., the length of the "tail." Computer analysis of gene clusters for secondary metabolites showed that most surfactin variants encoded in the genomes of members of the genus *Bacillus* differed in positions 1 and 7 of the peptide ring. The surfactin biosynthesis

genes of *B. subtilis* and *B. amyloliquefaciens* encode Leu at position 7, while *B. atrophaeus* genes encode Ile; *B. licheniformis* genes encode Gln and Ile at positions 1 and 7, respectively [19]. Natural isoforms of surfactin were identified with variations in hydrophobic amino-acid residues at positions 2 (Leu is substituted with Val or Ile) and 4 (Val changes to Leu, Ala, or Ile) [5]. The hydrophilic residue of aspartic acid (position 5) can be modified: it is methylated and converted into a relatively hydrophobic aspartic acid 4-methyl ester [20]. A putative reason for such a wide range of variations may be amino-acid substitutions in the adenylation domains of surfactin synthetase subunits (see the section on "Surfactin Biosynthesis") [21].

The length of the hydrophobic "tail" of surfactin is also subject to changes: it can vary from 13 to 18 carbon atoms. The main isoforms have 14 and 15 carbon atoms [5]. Not only the length of the carbon chain but also its branching can vary. The existence of the *iso*-C12, *iso*-C13, *anteiso*-C13, *iso*-C14, *n*-C14, *iso*-C15, *n*-C15, *anteiso*-C15, *anteiso*-C16, and *anteiso*-C17 isomers has been shown [22].

The ratio of various isomers in surfactin synthesized by bacteria can be influenced by changing the cultivation conditions, namely, adding various low-molecular-weight compounds to the nutrient medium. Bartal et al. [5] showed that the isoform composition of surfactin depended on the carbon source in the nutrient medium, with the greatest effect observed when using fructose and xylose. The introduction of metal ions (Mn^{2+}, Cu^{2+}, and Ni^{2+}) into the culture liquid led to the formation of both new aspartate-methylated forms of surfactin and isomers with longer fatty acid chains: two-thirds of the molecules were C16, C17, or C18 homologs. Related studies in this area have shown that the presence of amino acids in the nutrient medium also affects the isomeric composition of the synthesized surfactin [22]. As an example, the addition of the Arg, Gln, or Val amino acids to the nutrient medium of *B. subtilis* TD7 increases the proportion of surfactin isomers with an even length of the hydrophobic "tail," while the addition of Cys, His, Ile, Leu, Met, Ser, or Thr increases the proportion of isomers with odd length.

The structure–activity relationship of surfactin. Differences in the structure (the length and structure of the hydrocarbon tail, as well as the amino-acid composition) affect the biological activity of surfactins. With increasing chain length, the surface and interfacial activity of surfactins also increases. Therefore, a lipopeptide with a short hydrocarbon chain tends to form small micelles, while an increase in chain length results in a tendency for micelle enlargement and aggregation (Fig. 1b) [15]. The level of penetration of surfactin into the phospholipid cell membrane is directly proportional to the length of the hydrocarbon chain. Surfactin with a C15 "tail" was shown to possess a greater antitumor activity than that with a C13 or C14 hydrocarbon chain [23]. Similar data were obtained upon the inactivation of enveloped viruses with surfactin. Surfactin C13 showed very low antiviral activity compared to C14 and C15 isoforms [24]. Unfortunately, with an increase in the hydrophobicity of fatty acids (length of the "tail"), not only the antiviral activity but also the hemolytic effect of surfactin increased.

The cyclicity of the peptide fragment is also critical for the adsorption of surfactin on the lipid surface and its penetration into the lipid film. It has been shown that linear surfactin obtained by chemical cleavage of the ring has a lower surface activity than natural surfactin [25]. As for the role of the nature of amino-acid residues in the biological activity of surfactin, the experimental results have shown that the surface activity increases, while the critical micelle concentration decreases when amino acids are replaced by more hydrophobic ones [15]. A crucial role in the amphiphilic properties of surfactin belongs to two negatively charged residues (Glu and Asp). The natural Glu-methylated C15 surfactin (C15-surfactin-O-methyl ester) has a higher surface activity and lower antitumor activity than C15-surfactin [26]. If the Glu or Asp residues are methylated or amidated, the surface tension lowering activity of surfactin is increased by 20%, although the lipopeptide solubility in water decreases. Upon modification of Glu1 and Asp5 residues with aminomethanesulfonic acid, the surface activity of surfactin decreases abruptly due to electrostatic and steric factors [26].

Studies have shown that the structure of surfactin could be modified to enhance its desirable effects and reduce its undesirable effects. One of the main disadvantages of using surfactin as an antitumor and antibacterial drug is its hemolytic activity. To overcome this, linear forms of surfactin were developed, for which, unlike cyclic forms, no significant hemolysis was observed [28]. Surfactin variants lacking Leu3 or Leu6 residues in the ring also showed reduced hemolytic activity. At the same time, Asp5-free surfactin retained hemolytic properties but demonstrated better antibacterial properties compared to natural surfactin against *Staphylococcus aureus* and *Bacillus cereus*. The minimum inhibitory concentration (MIC) that suppresses the development of these pathogens was 25 μg/mL for surfactin lacking Asp5 and 50 μg/mL for native surfactin [29].

Biosynthesis of surfactin. The secondary metabolic apparatus of bacteria is known to be organized into gene clusters, within which the genes responsible for the biosynthesis of compounds produced by bacteria are located close to each other [30]. A similar cluster for surfactin was described by different groups of scientists at the same time [31]. The database (Minimum Information about a Biosynthetic Gene cluster, http://mibig.secondarymetabolites.org) contains a

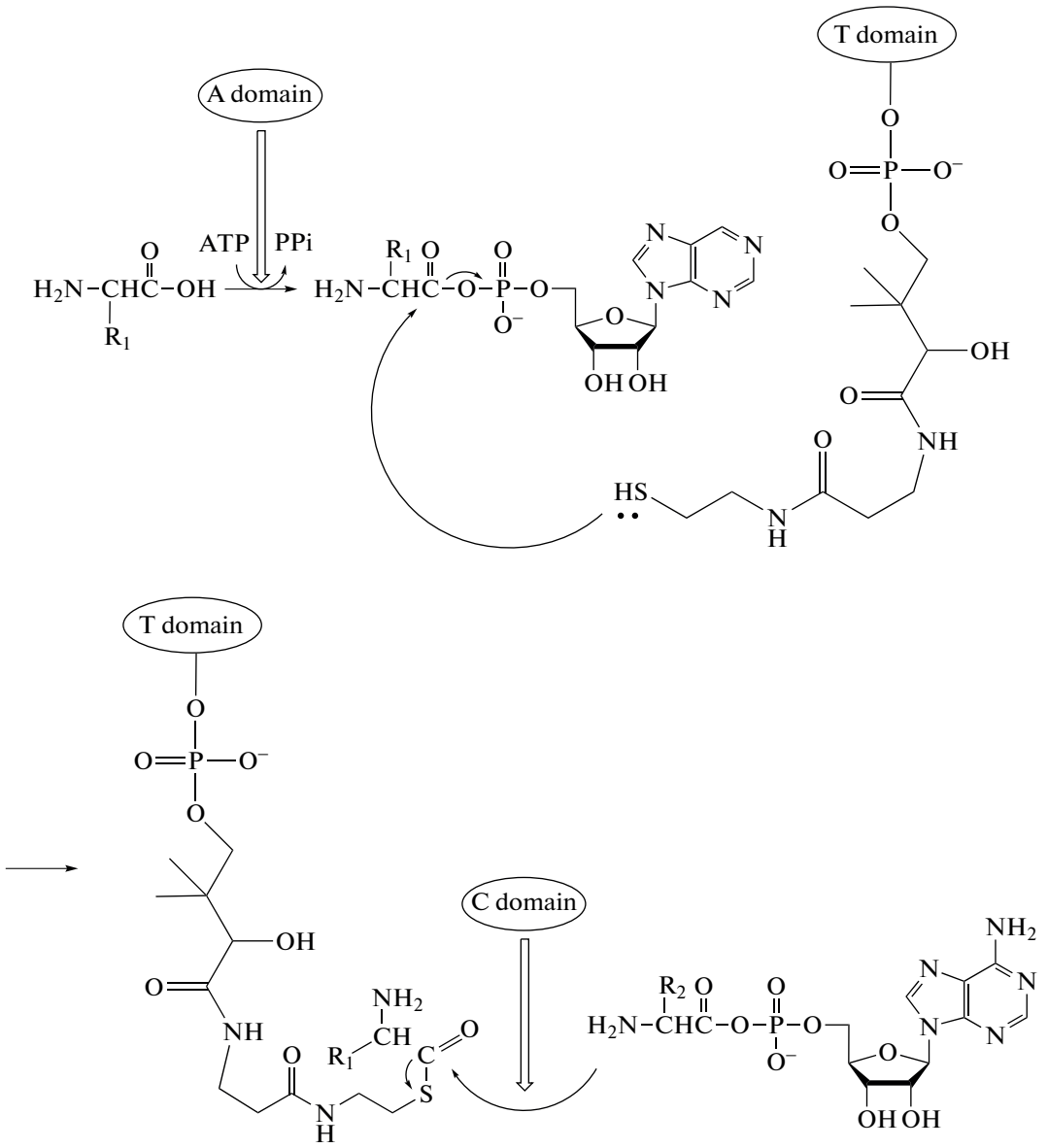

Fig. 2. The scheme of the reaction of surfactin biosynthesis by one of the modules of nonribosomal peptide synthetases. R_1 and R_2 are side chains of amino-acid residues.

cluster of surfactin biosynthesis genes for *Bacillus velezensis* FZB42 (MiBIG identifier: BG0000433) [32].

The biosynthesis of surfactin, like most other cyclic lipopeptides, is carried out by a nonribosomal mechanism with special systems, that is, nonribosomal peptide synthetases (NRPSs). NRPS are multi-enzyme complexes, which can be conditionally divided into modules, i.e., sections of NRPSs responsible for the incorporation of a certain amino acid into the peptide chain [2, 6]. In turn, each module can be divided into several domains: an adenylation domain (A), a thiolation domain (T), and a condensation domain (C). The A domain is responsible for the recognition and activation of a specific amino acid through the formation of aminoacyl adenylate (the process is similar to the activation of the carboxyl group of an amino acid by tRNA synthetase during ribosomal peptide synthesis). Further, the mobile and flexible 4'-phosphopantetheinyl part of the T-domain forms a thioether bond with the carboxyl group of the amino acid and, due to its relatively large length (20 Å), transfers the amino-acid residue from the active center of the T domain to the C domain. The C domain, in turn, catalyzes the formation of a new peptide bond and the movement of the extended peptide to the next module (Fig. 2) [31]. The linear arrangement of several modules in the form of an assembly line provides a coordinated elongation of the peptide chain. After modules 3 and 6, there are additional epimerization domains (E domains) respon-

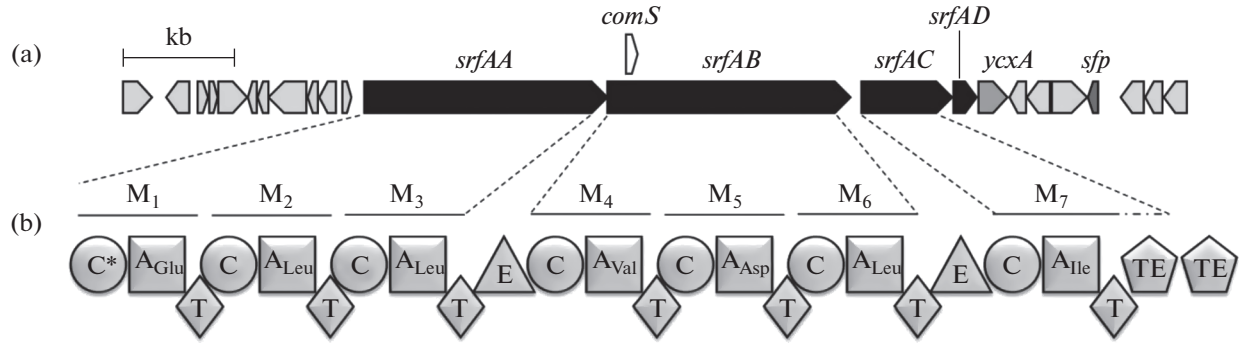

Fig. 3. A gene cluster for the proteins involved in surfactin biosynthesis (a) and a classical scheme of the surfactin assembly line (b). NRPS subunit genes are highlighted in black; M, assembly line module; the rest of the cluster genes are shown in gray. The cluster gene size scale is given in thousand bp (kb). The regulatory gene *comS* is co-transcribed with *srfAB*; (adapted from [31]).

sible for the isomerization of the natural L-Leu residue associated with the T domain in modules 3 and 6 into D-isomers, since the surfactin molecule contains D-Leu at these positions. In most cases, nonribosomal peptide synthesis is completed by macrocyclization, with the parts of the molecule removed in the constructed linear peptide chain covalently bonding to each other. The cyclization process is most often catalyzed by thioesterase domains at the C-terminus of the NRPS assembly line. The β-hydroxy aliphatic acid residue contained in surfactins and other microbial lipopeptides is incorporated into the molecule at the beginning of biosynthesis. The N-terminus of the NRPS starter module contains an additional domain (C*) responsible for the formation of a bond between the CoA-activated β-hydroxy aliphatic acid residue and the first amino acid.

The genes encoding surfactin NRPS subunits (*srfAA*, *srfAB*, *srfAC*, and *srfAD*) are combined into a single *srfA* operon (Fig. 3). The SrfAA and SrfAB proteins consist of three modules, while SrfAC consists of one module and a thioesterase (**TE**) domain responsible (as mentioned above) for the release of the heptapeptide chain and its macrocyclization. Moreover, the SrfAA subunit also contains the abovementioned additional domain at the N-terminus, which catalyzes the attachment of the residue of β-hydroxy aliphatic acid to the first amino acid (most often, it is Glu in the case of surfactin) of the lipophilic "tail." SrfAD is a TE domain that is responsible for the regeneration of mismatched T domains in the modules of the first three subunits [31].

All four genes encoding surfactin NRPS are included in one *srfA* operon, whose transcription is controlled by the P_{srfA} promoter. P_{srfA} activity, in turn, is regulated by the ComA transcription factor, which is part of the ComP/ComA two-component system. When the concentration of *B. subtilis* bacterial cells reaches a certain value, the membrane ComP histidine kinase phosphorylates the ComA protein; as a result, it is activated and induces the transcription of the *srfA* operon, initiating surfactin biosynthesis. In [33], the P_{srfA} promoter, whose activity is not constant over time due to the dependence on the ambient cell concentration, was replaced by the native constitutive P_{veg} promoter from the *B. subtilis* genome to increase the amount of synthesized surfactin. Such a substitution turned out to increase the synthesis of surfactin only in inefficient producer strains, while in the strains with increased natural production of surfactin, its yield decreased. In another study, a group of scientists replaced the P_{srfA} promoter in the *B. subtilis* THY-7 producer strain with P_{groE} (another "strong" natural promoter for the *B. subtilis* THY-7 strain) but obtained the same result: the production of surfactin in the modified strain decreased markedly. At the same time, when P_{srfA} was replaced by an artificial Pg3 promoter induced exogenously by isopropyl-β-D-1-thiogalactopyranoside (**IPTG**), the production of surfactin increased by 18 times compared to the native strain [34].

In addition to the structural genes for surfactin synthetase, the cluster of biosynthetic surfactin genes includes one built-in and several adjacent additional genes encoding transporters and regulatory proteins (Fig. 3, upper panel, gray arrows) [32]. Among them, the *sfp*, *ycxA*, *krsE*, *yerP*, and *comS* genes, whose transcription is directly related to the efficiency of surfactin production, should be noted separately. The *sfp* gene, which codes for phosphopantetheinyl transferase (an enzyme that catalyzes the transfer of the phosphopantetheinyl residue to the inactive T domain of NRPS subunits and thereby activates them), deserves special attention [35]. The presence of an intact *sfp* gene in the bacterial genome was shown to be strictly necessary for the production of surfactin. As an example, the *Bacillus subtilis* 168 strain systematically used as a model system for gram-positive organisms does not produce surfactin; at the same time, the *srfA* operon genes are identical to the genes in surfactin-producing strains. It turned out that due to a mutation in the *sfp* gene, one extra nucleotide appeared (A at position 634) in *B. subtilis* 168, which caused the inac-

tivation of the Sfp protein and the blocking of the surfactin synthesis. At the same time, its production is restored when a vector containing an intact *sfp* gene isolated from the producer strain is introduced into the *B. subtilis* 168 strain [36]. An important role both in the process of surfactin biosynthesis and in the mechanism of resistance of producer cells to it belongs to the system of surfactin export through the membrane, which makes it possible to avoid the intracellular accumulation of surfactin. Three genes that are involved in surfactin efflux have been identified: *ycxA*, *krsE*, and *yerP* [37]. It has been shown that the main exporter is the YerP protein, an increase in the expression of which increases the amount of surfactin in the culture liquid by 145% [37].

The *comS* gene located within the *srfA* operon in the open reading frame of the *srfAB* gene has several functions. On the one hand, ComS is involved in the positive regulation of the cell genetic competence (the ability to capture exogenous genetic material and assimilate it); on the other hand, it is part of the *comQXPA* system responsible for recognizing related bacteria surrounding the cell ("quorum sensing") and regulating surfactin biosynthesis [33, 38].

The biological activity of surfactin. *Surfactin as a surfactant.* Biosurfactants, which possess the properties of surfactants, can change the conditions at the interfaces between two phases that differ in polarity and the number of hydrogen bonds (for example, water/oil or water/air) [2]. There are two mechanisms proposed for the effect of biosurfactants on the viability of producer cells: (1) increasing the ability to emulsify, which, in turn, increases the availability of hydrophobic compounds as a source of nutrients and (2) helping microorganisms to attach and detach from surfaces [6]. Surfactin is known as one of the most powerful surfactants. It reduces the surface tension of water from 72 mN/m to 27 mN/m at a concentration of only 10 μM, which is well below its critical micelle concentration in water (23 mg/L) and about two orders of magnitude less than that for most detergents [39]. The mosaic polarity distribution and branched ring structure allow surfactin to adopt a spherical micellar structure to facilitate close packing at interfaces (Fig. 1b). On average, the mass of a micelle is 179000 and the aggregation number is $n = 173$ [40]. The lipid "tail," which moves freely in the solution, is actively involved in hydrophobic interactions in supramolecular structures at the water/air interface. At the phase boundary, surfactin molecules are very closely aligned and the adsorption properties of surfactin depend not only on the concentration but also on the length of the hydrocarbon chain [15].

Surfactin in "quorum sensing." Bacterial communities can "sense" environmental changes and adjust their behavior accordingly using a quorum recognition system. This process depends on cell density and is regulated by signaling molecules, that is autoinducers. The concentration of the latter increases in the extracellular environment as the density of bacterial cells increases, and when it reaches a certain threshold level, a group of cells begins to act synchronously. Signaling molecules cause cell differentiation into different types to adapt to environmental changes. As a jungle, under adverse conditions, surfactin initiates the development of a subpopulation of *B. subtilis* known as cannibals. They secrete special toxins that destroy neighboring cells, while the cannibal cells themselves are immune to toxins. It is proposed that surfactin is a signaling molecule in microbial communication and is involved in the activation of the membrane-associated sensory receptor histidine kinase (KinC). In turn, KinC activates the expression of the early sporulation protein Spo0A, which ultimately activates sporulation, biofilm formation, and colonization of plant roots [41].

Surfactin and destabilization of biological membranes. The biological activity of surfactin is associated with its interaction with the lipid part of the biological membranes of all bacterial species. Due to their amphiphilic structure, surfactin molecules can easily be incorporated into lipid layers and firmly adhered to them: the hydrophobic part of surfactin interacts with the hydrocarbon chains of membrane phospholipids, while the peptide part interacts with the polar groups of lipids (Fig. 1c). Penetration of surfactin into the phospholipid bilayer results in the impairment and/or creation of channels in the cytoplasmic membrane, which, in turn, can activate a cascade of molecular events leading to defense reactions [42, 23]. Surfactin destabilizes the membrane through several mechanisms: (1) incorporation into lipid bilayers, (2) formation of channels/pores or diffusion of singly and doubly charged ions across the membrane barrier, and (3) solubilization of the membrane, like the action of a detergent. Which mechanism will be involved depends on the concentration of surfactin [43]. At low concentrations, antimicrobial peptides tend to penetrate the membrane and integrate into the lipid bilayer [44]. This causes unilamellar vesicle formation on the outer membrane, which leads to cell deformation and, ultimately, cell death (Fig. 1c) [45].

With increasing concentration, surfactin begins to form aggregates in the lipid bilayer, which ultimately leads to the formation of pores in the cell membrane. These pores create a path for the release of nucleic acids, essential ions, and ATP from the cell, which leads to cell death. Ca^{2+} ions were shown to promote deeper penetration of surfactin into the membrane by neutralizing the charges of both surfactin (shielding the negative charges of Glu1 and Asp5 residues of the peptide cycle) and charged lipid parts [46, 47]. At high concentrations, the detergent mechanism of membrane destruction, which is based on the solubilization of phospholipids by surfactin molecules, predominates, while the level of penetration of surfactin is directly proportional to the length of the "tail." Liu et al. [48] have shown that surfactin inhibits *Staphylo-*

coccus aureus biofilm formation by reducing the percentage of alkali-soluble polysaccharides and suppressing the expression of the *icaA* and *icaD* genes involved in biofilm formation.

Surfactin, biofilm formation, and plant root colonization. *B. subtilis* is a motile, Gram-positive, spore-forming, facultative aerobic soil bacterium. For the genus *Bacillus*, cell migration over the surface of media, swarming, and the ability to colonize plant roots through the formation of biofilms on their surface are directly related to the production of surfactin [6, 49]. In the immobile subpopulation of *B. subtilis*, surfactin activates the transcription of the Spo0A protein gene by interacting with KinC, which leads to the impairment of the motor-rotary flagellar mechanisms [38, 50]. In plants, surfactin can promote the wettability of its hydrophobic cuticle, which contributes not only to the increased motility of bacterial cells but also to the solubility and diffusion of growth substrates. It has been shown that surfactin can change the viscosity of surfaces, thereby affecting cell motility [51, 52]. Analysis of *B. amyloliquefaciens* subsp. *plantarum* FZB42, which is commercially used as a biofertilizer in agriculture and as a model bacterium for studying interactions with plants, showed that almost 10% of the genome of this producer is associated with the synthesis of antimicrobial metabolites [53]. However, studies of plants indicate that, except for surfactin, the amount of antimicrobial metabolites found near plant roots is relatively small. Thus, surfactins allow bacterial colonies of the genus *Bacillus* to form biofilms [54].

Bacteria initiate biofilm formation in response to certain environmental factors such as nutrient and oxygen availability. When moving from free-living organisms to attached colonies in a biofilm, they undergo dynamic changes, including the specific production of secondary metabolites and a significant increase in resistance to biological, chemical, and physical effects. The experience of the successful use of surfactin to increase plant resistance to pathogens in the laboratory is summarized in Table 1. Surfactin is known to affect the ability of *B. subtilis* to stimulate plant development through biofilm formation [55]. Therien et al. [56] showed that surfactin production by *B. subtilis* was not essential for biofilm formation; however, the absence of surfactin reduced colony growth.

Bais et al. [59] demonstrated the ability of surfactin from *B. subtilis* to colonize *Arabidopsis* roots, with the formation of a stable biofilm and, thus, to protect them from *Pseudomonas syringae* both in vitro and in soil. The MIC of surfactin against *P. syringae* was 25 μg/mL, which was relatively high for an antimicrobial agent but acceptable for a commercial biopesticide. In experiments with *Arabidopsis* roots that were pre-inoculated with a suspension of *B. subtilis*, the levels of surfactin in the washed roots were significant: 151.6 μg/mL per 50 mg wet weight of roots. The concentration of dissolved surfactin on the root surface may be significantly higher than the in vitro MIC against *P. syringae*. Interestingly, after inoculation with the pathogenic strain of *P. syringae*, the production of surfactin doubled. The authors tested a mutant strain with a deletion in the *sfp* gene, which was deficient in surfactin production. It was ineffective as a biological control agent for *P. syringae* and did not form strong biofilms either on *Arabibopsis* roots or inert surfaces. Similar results were reported by Luo et al. [61] who obtained mutant *B. subtilis* 916 strains with deletions in the *srfAA* gene encoding the first subunit of the surfactin NRPS.

B. subtilis 916 is a key ingredient of the commercially available "Wenquning" biofungicide, which is popular in China. Mutant strains that are deficient in surfactin production demonstrated a change in swarm mobility, as well as a decrease in antagonistic activity and the efficiency of biofilm formation [61]. At the same time, swarm mobility was restored after the addition of surfactin (10 μg/mL), while the biofilm formation could not be restored even when a 50 μg/mL dose was added. In the study carried out by Fan et al. [62], a mutant strain of *B. subtilis* characterized by reduced production of surfactin showed a decrease in the biofilm formation and swarm mobility and did not inhibit the growth of *Acidovorax citrulli* [62].

Surfactin isoforms are species-specific and mediate species-specific signaling, resulting in different ecological behaviors. A mutant strain of *B. atrophaeus* characterized by reduced production of surfactin responded to the related exogenous surfactin C obtained from the native strain by the formation of strong biofilms, while in the presence of surfactin A produced by *B. subtilis*, only a loose biofilm was observed [21]. Likewise, the related surfactin A promoted the formation of persistent biofilms in a surfactin-deficient *B. subtilis* strain, while surfactin C derived from *B. atrophaeus* induced loose biofilms in *B. subtilis*.

The research by Debois et al. [73] showed that surfactin was the main bacterial metabolite accumulated in plants during the first hours of interaction between bacteria and plant roots. The synthesis of surfactin is specifically stimulated by the interaction of bacteria with plant cell wall polymers: xylan or arabinogalactan, leading to a rapid accumulation of micromolar amounts of lipopeptide in the root system. At such concentrations, surfactin not only enhances the ability of the producer strain to colonize roots but also activates the induced systemic resistance of the host plant.

Surfactin and induced systemic resistance in plants. In addition to direct antagonism, some bacteria can defend plants indirectly by stimulating inducible defense mechanisms that make the host plant more resistant to pathogen entry. This inducible defense is observed throughout ontogeny and is close to natural immune responses. The described phenomenon is called induced systemic resistance (ISR). It is believed

Table 1. The use of surfactin to increase plant resistance to pathogens

Strain	Active lipopeptide	Object of protection	Treatment method	Pathogen	Reference
Plant protection against pathogens via direct antimicrobial action					
B. subtilis RB14	A mixture of lipopeptides: iturin and surfactin	Tomato (leaves)	Soil inoculation before planting germinated seeds, plant treatment	*Rhizoctonia solani*	[57]
B. subtilis GA1	A mixture of lipopeptides: iturin, fengycin, and surfactin	Apples (fruits)	Fruit treatment	*Botrytis cinerea*	[58]
B. subtilis 6051	Surfactin	*Arabidopsis* (roots)	Inoculation of roots and soil	*Pseudomonas syringae pv tomato*	[59]
B. subtilis UMAF6614	A mixture of lipopeptides: bacillomycin, fengycin, and surfactin (surfactin is a major determinant of antipathogenic response)	Melon (leaves)	Spraying leaves	*Podosphaera fusca, Pectobacterium carotovorum* subsp. *carotovorum, Xanthomonas campestris* pv. cucurbitae	[60]
B. subtilis 916	Mixture of surfactin isoforms (from C13 to C15)	Rice (stem and leaves)	Spraying the stem and leaves	*Rhizoctonia solani*	[61]
B. atrophaeus 176s, *B. atrophaeus* 1942, *B. subtilis* OKB105, *B. amyloliquefaciens* FZB42	Surfactins A, B, and C. A mixture of isoforms, from C13 to C16	Tomatoes (roots), lettuce (roots), and sugar beet (roots)	Root system treatment	*Rhizoctonia solani*	[21]
B. subtilis 9407	Surfactin A. A mixture of isoforms, from C13 to C16	Melon (leaves)	Soaking germinated seeds	*Acidovorax citrulli*	[62]
B. velezensis Bvel1	A mixture of lipopeptides: iturin A2, surfactin isoforms C13 and C15, oxydifficidin, bacillibactin, and L-dihydro-anticapsin	Grapes (clusters)	Berry treatment	*Botrytis cinerea*	[63]
Stimulation of induced systemic resistance of plants to diseases					
B. subtilis 2500, *B. subtilis* 2508	Mixture of isoforms, from C13 to C16	Beans (leaves), tomato (leaves)	Seed treatment. Addition of the bacterial suspension to the soil, and root watering after a week.	*Botrytis cinerea*	[42]

Table 1. (Contd.)

Strain	Active lipopeptide	Object of protection	Treatment method	Pathogen	Reference
B. subtilis UMAF6639	Mixture of lipopeptides, including surfactin. Surfactin is the main determinant of the stimulation of the immune response.	Pumpkin (leaves)	Root inoculation and root watering after a week	*Podosphaera fusca*	[64]
B. amylolequifaciens S499	A mixture of lipopeptides: iturin, fengycin, and surfactin	Sugar beet (roots)	Watering of plants	*Polymyxa betae*	[65]
B. amyloliquefaciens S499, *B. amyloliquefaciens* FZB42, *B. amyloliquefaciens* QST713, *B. subtilis* 98S	Surfactin	Tomato (leaves), tobacco (leaves)	Adding surfactin to hydroponic solution.	*Botrytis cinerea*	[66]
B. subtilis ABS-S14	Surfactin. The synergism of a mixture of lipopeptides (iturin, fengycin, and surfactin) is shown	Orange (fruit)	Fruit treatment	*Penicillium digitatum*	[67]
B. amyloliquefaciens FZB42-AK3	A mixture of surfactin isoforms from C12 to C15	Perennial ryegrass (leaves)	Seed soaking in bacterial cell suspension/ surfactin solution. Addition of bacterial inoculum/ surfactin solution to soil, and root watering after three weeks	*Magnaporthe oryzae*	[68]
B. amyloliquefaciens S13-3	Mixture of lipopeptides, including surfactin Surfactin commercial drug	Strawberries (leaves)	Spraying leaves	*Colletotrichum gloeosporioides*	[69]
B. subtilis	Surfactin commercial drug	Peanut (stalk)	Immersion of seedling roots in surfactin solution	*Sclerotium rolfsii*	[70]
B. subtilis 26D	Surfactin	Wheat (leaves)	Seed soaking	*Septoria nodorum*	[71]
B. subtilis GLB19.1	Blend of lipopeptides: surfactin and fengycin	Grapes (leaves)	Spraying the leaves	*Plasmopara viticola*	[72]
B. velezensis BBC023, *B. velezensis* BBC047	Mixture of surfactin isoforms, from C12 to C17	Tomato (roots and leaves)	Root or leaf surface inoculation	*Botrytis cinerea*	[49]

that surfactin does not induce a plant defense response associated with significant genetic reprogramming and adaptation but rather activates host defense mechanisms to initiate systemic resistance [73]. The potential of surfactin to induce plant systemic resistance was first shown in bean and tomato samples [42]. The use of the pure form of lipopeptide in micromolar concentrations led to a significant reduction in the incidence of plants infected with *Botrytis cinerea*. Cawoy et al. [66] conducted a study with a large number of natural isolates of the genus *Bacillus*, differing in their ability to produce lipopeptides under specified conditions in vitro. Some isolates did not produce any lipopeptides, others produced two or all three families of lipopeptides, including iturins, fengycins, and surfactins, in varying proportions. A strong correlation between the protective activity of bacterial extracts against *Botrytis cinerea* and the amount of surfactin produced by them was observed.

The treatment of plants in the first 15–20 days of ontogeny or seeds (before sowing them) with certain biologically active substances can induce metabolic changes that are unfavorable for pathogens [74]. Soaking perennial ryegrass seeds in a suspension of surfactin-producing *B. amyloliquefaciens* bacterial cells resulted in the activation of induced systemic plant resistance against *Magnaporthe oryzae* [68].

The basis of the Fitosporin-M commercial drug, which is widely used in Russia and contains *B. subtilis* bacteria, is a surfactin-producing strain of *B. subtilis* 26D. In the genome of the strain *B. subtilis* 26D, the genes encoding subunits of surfactin synthetase were identified, while the genes responsible for the synthesis of iturine and fengycin synthetases were not found [71]. The authors have shown that surfactin from *B. subtilis* 26D protects the plant from grass septoria not only via a direct fungicidal action but also indirectly through increased expression of plant defense protein genes and activation of the immune potential in host plants. The treatment of perennial ryegrass roots with surfactin and a suspension of *B. amyloliquefaciens* FZB42-AK3 cells (the strain produces only surfactin but not the bacillomycin D or fengycin antifungal compounds) significantly reduced the disease incidence with *M. oryzae* [68]. Surfactin causes multilevel activation of ISR in ryegrass due to enhanced accumulation of hydrogen peroxide in plant roots and subsequent H_2O_2-mediated defense reactions. A rapid increase in peroxidase activity was observed in the extracellular fluid of the treated plants. Deposition of callose and phenolic compounds on ryegrass leaf blades at *M. oryzae* infection sites was shown. The H_2O_2-dependent, peroxidase-mediated interrelationship of phenolic compounds localized in the cell wall plays a significant role in strengthening plant cell walls and limiting the penetration of pathogens. In the study by Ongena et al. [42], the initiation of ISR by surfactin led to an increase in the activity of all enzymes that decompose hydroperoxides. In the work by Rodriguez et al. [70], peroxidase activity and deposition of phenolic compounds under the zone of fungal infection were significantly higher in infected peanut samples pre-treated with surfactin than in untreated samples.

The exact mechanism for the direct action of surfactin against bacterial pathogens is unknown. The published data indicate that the inhibitory effect of surfactin is not associated with its direct impact on the viability of target cells but results from the interference with key pathogen developmental processes, such as *Pseudomonas syringae* biofilm formation [59] or aerial hyphae development, as shown for free-living soil bacteria *Streptomyces coelicolor* [75]. At the same time, surfactin does not inhibit the growth of substrate hyphae of *S. coelicolor*, which would be expected if it acted as an antibiotic.

Almost all antibiotics that increase plant resistance to pathogens penetrate deep into plant tissues, increasing the risk of harmful effects of the fruits of this plant on humans. However, surfactin is not distributed in plant tissues. In a study conducted by Ongena et al. [42], no living cells of the isolate belonging to the genus *Bacillus* were found in bean and tomato leaf samples after the treatment of the root system with a bacterial suspension; this indicates that bacteria do not migrate into the intercellular space of plants. Thus, for surfactin, inhibition of the development of phytopathogens occurs due to the induction of resistance in the host plant, since bacilli and the pathogen remain localized on different parts of the plant. In this case, the bacterial population on the roots is within the concentrations required to initiate induced systemic resistance.

In recent years, research on the possibility of using aerobic endospore-forming bacteria in agriculture has led to the development of various products based on strains of the genus *Bacillus* for commercial use as microbial pesticides, fungicides, or fertilizers [72]. Bacilli are able to form spores that allow them to withstand adverse environmental conditions; they can be safely transported, stored, and suspended in liquid for ease of use [76]. Several commercial products based on *B. amyloliquefaciens, B. licheniformis, B. pumilus*, and *B. subtilis* are sold as biofungicides.

According to literature sources, different strains of the genus *Bacillus* produce different types of lipopeptides and, as a result, have different activity even against the same pathogen. The molecular and physiological mechanisms by which bacilli exhibit biofungicidal activity are in many cases not fully understood. The bioregulatory activity of bacilli is probably the result of the concurrent effects of their antibacterial activity and plant colonization. Recent studies summarized in this review prove the key role of surfactin in root colonization among plant-associated *Bacillus* spp. species and biofilm formation, as well as during extracellular matrix formation (as a signaling mole-

cule). For a wide range of host plants, low concentrations of surfactin were shown to be the starting signal for the activation of a complex cascade of defense mechanisms of the induced systemic resistance to phytopathogens. Considering that this defense mechanism in plants is more environmentally friendly than pesticide application, it is worth considering the use of surfactin in agriculture as a natural biopesticide and plant growth promoter. All experiments on the use of surfactin presented in this review were performed in the laboratory. Currently, the widespread use of surfactin in agriculture is not discussed. However, the fact that surfactin is the "leading" lipopeptide among lipopeptides of the genus *Bacillus* should encourage researchers to screen for effective surfactin-producing strains among bacilli to select candidates for the production of biopesticides.

FUNDING

This study was supported by the Ministry of Science and Higher Education of the Russian Federation, project on "Analysis of Microbiomes in Plants and Invertebrates of Extreme Habitats to Develop Strains Producing New Metabolites and Enzymes" no. 075-15-2021-1396 dated October 26, 2021.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflict of interest. This article does not contain any studies involving animals or human participants performed by the authors.

REFERENCES

1. Fracchia, L., Banat, J.J., Cavallo, M., Ceres, C., and Banat, I.V., *AIMS Bioeng.*, 2015, vol. 2, no. 3, pp. 144–162. https://doi.org/10.3934/bioeng.2015.3.144
2. Wu, Y.S., Ngai, S.C., Goh, B.H., Chan, K.G., Lee, L.H., and Chuah, L.H., *Front. Pharmacol.*, 2017, vol. 8, p. 76. https://doi.org/10.3389/fphar.2017.00761
3. Arima, K., Kakinuma, A., and Tamura, G., *Biochem. Biophys. Res. Commun.*, 1968, vol. 31, pp. 488–494. https://doi.org/10.1016/0006-291X(68)90503-2
4. Lilge, L., Ersig, N., Hubel, P., Aschern, M., Pillai, E., Klausmann, P., Pfannstiel, J., Henkel, M., Heravi, K.M., and Hausmann, R., *Microorganisms*, 2022, vol. 10, no. 4, p. 779. https://doi.org/10.3390/microorganisms10040779
5. Bartal, A., Vigneshwari, A., Kredics, L., Manczinger, L., Varga, M., Vágvolgyi, C., and Szekeres, A., *Molecules*, 2018, vol. 23, no. 10, p. 2675. https://doi.org/10.3390/molecules23102675
6. Stein, T., *Mol. Microbiol.*, 2005, vol. 56, no. 4, pp. 845–857. https://doi.org/10.1111/j.1365-2958.2005.04587.x
7. Caulier, S., Nannan, C., Gillis, A., Licciardi, F., Bragard, C., and Mahillon, J., *Front. Microbiol.*, 2019, vol. 10, p. 302. https://doi.org/10.3389/fmicb.2019.00302
8. Hsieh, F.C., Li, M.C., Lin, T.C., and Kao, S.S., *Curr. Microbiol.*, 2004, vol. 49, pp. 186–191. https://doi.org/10.1007/s00284-004-4314-7
9. Long, X., He, N., He, Y., Jiang, J., and Wu, T., *Bioresour. Technol.*, 2017, vol. 241, pp. 200–206. https://doi.org/10.1016/j.biortech.2017.05.120
10. Marcelino, L., Puppin-Rontani, J., Coutte, F., Machini, M.T., Etchegaray, A., and Puppin-Rontani, R.M., *Amino Acids*, 2019, vol. 51, pp. 1233–1240. https://doi.org/10.1007/s00726-019-02750-1
11. Banat, I.M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M.G., Fracchia, L., Smyth, T.J., and Marchant, R., *Appl. Microbiol. Biotechnol.*, 2010, vol. 87, no. 2, pp. 427–444. https://doi.org/10.1007/s00253-010-2589-0
12. Varvaresou, A. and Iakovou, K., *Lett. Appl. Microbiol.*, 2015, vol. 61, no. 3, pp. 214–223. https://doi.org/10.1111/lam.12440
13. Kakinuma, A., Hori, M., Isono, M., Tamura, G., and Arima, K., *Agric. Biol. Chem.*, 1969, vol. 33, pp. 971–972. https://doi.org/10.1080/00021369.1969.10859408
14. Kakinuma, A., Sugino, H., Isono, M., Tamura, G., and Arima, K., *Biol. Chem.*, 1969, vol. 33, pp. 973–976. https://doi.org/10.1080/00021369.1969.10859409
15. Liu, J.F., Mbadinga, S.M., Yang, S.Z., Gu, J.D., and Mu, B.Z., *Int. J. Mol. Sci.*, 2015, vol. 16, no. 3, pp. 4814–4837. https://doi.org/10.3390/ijms16034814
16. Liu, J., Zou, A., and Mu, B., *Colloids Surf., A*, 2010, vol. 361, pp. 90–95. https://doi.org/10.1016/j.colsurfa.2010.03.021
17. Vass, E., Besson, F., Majer, Z., Volpon, L., and Hollosi, M., *Biochem. Biophys. Res. Commun.*, 2001, vol. 282, no. 1, pp. 361–367. https://doi.org/10.1006/bbrc.2001.4469
18. Bonmatin, J.-M., Laprevote, O., and Peypoux, F., *Comb. Chem. High Throughput Screen.*, 2003, vol. 6, no. 6, pp. 541–556. https://doi.org/10.2174/138620703106298716
19. Aleti, G., Sessitsch, A., and Brader, G., *Comput. Struct. Biotechnol. J.*, 2015, vol. 13, pp. 192–203. https://doi.org/10.1016/j.csbj.2015.03.003
20. Kecskemeti, A., Bartal, A., Boka, B., Kredics, L., Manczinger, L., Shine, K., Alharby, N.S., Khaled, J.M., Varga, M., Vagvolgyi, C., and Szekeres, A., *Molecules*, 2018, vol. 23, p. 2224. https://doi.org/10.3390/molecules23092224
21. Aleti, G., Lehner, S., Bacher, M., Compant, S., Nikolic, B., Plesko, M., Schuhmacher, R., Sessitsch, A., and Brader, G., *Environ. Microbiol.*, 2016, vol. 18, no. 8, pp. 2634–2645. https://doi.org/10.1111/1462-2920.13405
22. Liu, J.F., Yang, J., Yang, S.Z., Ye, R.Q., and Mu, B.Z., *Appl. Biochem. Biotechnol.*, 2012, vol. 166, no. 8, pp. 2091–2100. https://doi.org/10.1007/s12010-012-9636-5
23. Liu, X., Tao, X., Zou, A., Yang, S., Zhang, L., and Mu, B., *Protein Cell*, 2010, vol. 1, no. 6, pp. 584–594. https://doi.org/10.1007/s13238-010-0072-4

24. Kracht, M., Rokos, H., Ozel, M., Kowall, M., Pauli, G., and Vater, J., *J. Antibiot. (Tokyo)*, 1999, vol. 52, no. 7, pp. 613–619. https://doi.org/10.7164/antibiotics.52.613
25. Eeman, M., Berquand, A., Dufrene, Y.F., Paquot, M., Dufour, S., and Deleu, M., *Langmuir*, 2006, vol. 22, no. 26, pp. 11337–11345.
26. Liu, X., Yang, S., and Mu, B., *Proc. Biochem. Soc.*, 2009, vol. 44, no. 1, pp. 1144–1151. https://doi.org/10.1016/j.procbio.2009.06.014
27. Morikawa, M., Hirata, Y., and Imanaka, T., *Biochim. Biophys. Acta*, 2000, vol. 1488, no. 3, pp. 211–218. https://doi.org/10.1016/s1388-1981(00)00124-4
28. Dufour, S., Deleu, M., Nott, K., Wathelet, B., Thonart, P., and Paquot, M., *Biochim. Biophys. Acta*, 2005, vol. 1726, no. 1, pp. 87–95. https://doi.org/10.1016/j.bbagen.2005.06.015
29. Jiang, J., Gao, L., Bie, X., Lu, Z., Liu, H., Zhang, C., Lu, F., and Zhao, H., *BMC Microbiol.*, 2016, vol. 16, p. 31. https://doi.org/10.1186/s12866-016-0645-3
30. Medema, M.H., Kottmann, R., Yilmaz, P., Cummings, M., Biggins, J.B., Blin, K., de Bruijn, I., Chooi, Y.H., Claesen, J., and Coates, R.C., *Nat. Chem. Biol.*, 2015, vol. 11, pp. 625–631. https://doi.org/10.1038/nchembio.1890
31. Theatre, A., Cano-Prieto, C., Bartolini, M., Laurin, Y., Deleu, M., Niehren, J., Fida, T., Gerbinet, S., Alanjary, M., Medema, M.H., Leonard, A., Lins, L., Araboloza, A., Gramajo, H., Gross, H., and Jacques, P., *Front. Bioeng. Biotechnol.*, 2021, vol. 9, p. 623701. https://doi.org/10.3389/fbioe.2021.623701
32. Koumoutsi, A., Chen, X.H., Henne, A., Liesegang, H., Hitzeroth, G., Franke, P., Vater, J., and Borriss, R., *J. Bacteriol.*, 2004, vol. 186, no. 4, pp. 1084–1096. https://doi.org/10.1128/JB.186.4.1084-1096.2004
33. Willenbacher, J., Mohr, T., Henkel, M., Gebhard, S., Mascher, T., Syldatk, C., and Hausmann, R., *J. Biotechnol.*, 2016, vol. 224, pp. 14–17. https://doi.org/10.1016/j.jbiotec.2016.03.002
34. Jiao, S., Li, X., Yu, H., Yang, H., Li, X., and Shen, Z., *Biotechnol. Bioeng.*, 2017, vol. 114, pp. 832–842. https://doi.org/10.1002/bit.26197
35. Quadri, L.E., Weinreb, P.H., Lei, M., Nakano, M.M., Zuber, P., and Walsh, C.T., *Biochemistry*, 1998, vol. 37, no. 6, pp. 1585–1595. https://doi.org/10.1021/bi9719861
36. Nakano, M.M., Corbell, N., Besson, J., and Zuber, P., *MGG Mol. Gen. Genet.*, 1992, vol. 232, pp. 313–321. https://doi.org/10.1007/BF0028001
37. Li, X., Yang, H., Zhang, D., Li, X., Yu, H., and Shen, Z., *J. Ind. Microbiol. Biotechnol.*, 2015, vol. 42, pp. 93–103. https://doi.org/10.1007/s10295-014-1527-z
38. Rahman, F.B., Sarkar, B., Moni, R., and Rahman, M. S., *Biotechnol. Rep.*, 2021, vol. 32, p. e00686. https://doi.org/10.1016/j.btre.2021.e00686
39. Seydlova, G. and Svobodova, J., *Cent. Eur. J. Med.*, 2008, vol. 3, pp. 123–133. https://doi.org/10.2478/s11536-008-0002-5
40. Ishigami, Y., Osman, M., Nakahara, H., Sano, Y., Ishiguro, R., and Matsumoto, M., *Colloids Surf.*, vol. 4, pp. 341–348.
41. Chen, B., Wen, J., Zhao, X., Ding, J., and Qi, G., *Front. Microbiol.*, 2020, vol. 11, p. 631. https://doi.org/10.3389/fmicb.2020.00631
42. Ongena, M., Jourdan, E., Adam, A., Paquot, M., Brans, A., Joris, B., Arpigny, J.L., and Thonart, P., *Environ. Microbiol.*, 2007, vol. 9, no. 4, pp. 1084–1090. https://doi.org/10.1111/j.1462-2920.2006.01202.x
43. Deleu, M., Lorent, J., Lins, L., Brasseur, R., Braun, N., El Kirat, K., Nylander, T., Dufrene, Y.F., and Mingeot-Leclercq, M.P., *Biochim. Biophys. Acta*, 2013, vol. 1828, no. 2, pp. 801–815. https://doi.org/10.1016/j.bbamem.2012.11.007
44. Li, T., Li, L., Du, F., Sun, L., Shi, J., Long, M., and Chen, Z., *Molecules*, 2021, vol. 26, no. 11, p. 3438. https://doi.org/10.3390/molecules26113438
45. Tran, C., Cock, I.E., Chen, X., and Feng, Y., *Antibiotics* (Basel), 2022, vol. 11, no. 1, p. 88. https://doi.org/10.3390/antibiotics11010088
46. Maget-Dana, R. and Ptak, M., *Biophys. J.*, 1995, vol. 68, pp. 1937–1943. https://doi.org/10.1016/S0006-3495(95)80370-X
47. Maget-Dana, R. and Ptak, M., *J. Colloid Interface Sci.*, 1992, vol. 153, pp. 285–291. https://doi.org/10.1016/0021-9797(92)90319-H
48. Liu, J., Li, W., Zhu, X., Zhao, H., Lu, Y., Zhang, C., and Lu, Z., *Appl. Microbiol. Biotechnol.*, 2019, vol. 103, no. 11, pp. 4565–4574. https://doi.org/10.1007/s00253-019-09808-w
49. Stoll, A., Salvatierra-Martinez, R., Gonzalez, M., and Araya, M., *Microorganisms*, 2021, vol. 9, no. 11, p. 2251. https://doi.org/10.3390/microorganisms9112251
50. Marahiel, M.A., Nakano, M.M., and Zuber, P., *Mol. Microbiol.*, 1993, vol. 7, no. 5, pp. 631–636. https://doi.org/10.1111/j.1365-2958.1993.tb01154.x
51. Raaijmakers, J.M., De Bruijn, I., Nybroe, O., and Ongena, M., *FEMS Microbiol. Rev.*, 2010, vol. 34, no. 6, pp. 1037–1062. https://doi.org/10.1111/j.1574-6976.2010.00221.x
52. Sachdev, D.P. and Cameotra, S.S., *Appl. Microbiol. Biotechnol.*, 2013, vol. 97, pp. 1005–1016. https://doi.org/10.1007/s00253-012-4641-8
53. Chowdhury, S.P., Hartmann, A., Gao, X., and Borriss, R., *Front. Microbiol.*, 2015, vol. 6, p. 780. https://doi.org/10.3389/fmicb.2015.00780
54. Hofemeister, J., Conrad, B., Adler, B., Hofemeister, B., Feesche, J., Kucheryava, N., Steinborn, G., Franke, P., Grammel, N., Zwintscher, A., Leenders, F., Hitzeroth, G., and Vater, J., *Mol. Genet. Genomics*, 2004, vol. 272, no. 4, pp. 363–378. https://doi.org/10.1007/s00438-004-1056-y
55. Morikawa, M., *J. Biosci. Bioeng.*, 2006, vol. 101, no. 1, pp. 1–8. https://doi.org/10.1263/jbb.101
56. Therien, M., Kiesewalter, H.T., Auria, E., Charron-Lamoureux, V., Wibowo, M., Maroti, G., Kovacs, A.T., and Beauregard, P.B., *Biofilm*, 2020, vol. 2, p. 100021. https://doi.org/10.1016/j.bioflm.2020.100021
57. Asaka, O. and Shoda, M., *Appl. Environ. Microbiol.*, 1996, vol. 62, no. 11, pp. 4081–4085. https://doi.org/10.1128/aem.62.11.4081-4085.1996

58. Toure, Y., Ongena, M., Jacques, P., Guiro, A., and Thonart, P., *J. Appl. Microbiol.*, 2004, vol. 96, no. 5, pp. 1151–1160. https://doi.org/10.1111/j.1365-2672.2004.02252.x

59. Bais, H.P., Fall, R., and Vivanco, J.M., *Plant Physiol.*, 2004, vol. 134, no. 1, pp. 307–319. https://doi.org/10.1104/pp.103.028712

60. Zeriouh, H., de Vicente, A., Perez-Garcia, A., and Romero, D., *Environ. Microbiol.*, 2014, vol. 16, no. 7, pp. 2196–2211. https://doi.org/10.1111/1462-2920.12271

61. Luo, C., Zhou, H., Zou, J., Wang, X., Zhang, R., Xiang, Y., and Chen, Z., *Appl. Microbiol. Biotechnol.*, 2015, vol. 99, no. 4, pp. 1897–1910. https://doi.org/10.1007/s00253-014-6195-4

62. Fan, H., Zhang, Z., Li, Y., Zhang, X., Duan, Y., and Wang, Q., *Front. Microbiol.*, 2017, vol. 8, p. 1973. https://doi.org/10.3389/fmicb.2017.01973

63. Nifakos, K., Tsalgatidou, P.C., Thomloudi, E.E., Skagia, A., Kotopoulis, D., Baira, E., Delis, C., Papadimitriou, K., Markellou, E., Venieraki, A., and Katinakis, P., *Plants* (Basel), 2021, vol. 10, no. 8, p. 1716. https://doi.org/10.3390/plants10081716

64. Garcia-Gutierrez, L., Zeriouh, H., Romero, D., Cubero, J., de Vicente, A., and Perez-Garcia, A., *Microbiol. Biotechnol.*, 2013, vol. 6, no. 3, pp. 264–274. https://doi.org/10.1111/1751-7915.12028

65. Desoignies, N., Schramme, F., Ongena, M., and Legreve, A., *Mol. Plant Pathol.*, 2013, vol. 14, no. 4, pp. 416–421. https://doi.org/10.1111/mpp.12008

66. Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., Dommes, J., and Ongena, M., *Mol. Plant Microbe Interact.*, 2014, vol. 27, no. 2, pp. 87–100. https://doi.org/10.1094/MPMI-09-13-0262-R

67. Waewthongrak, W., Leelasuphakul, W., and McCollum, G., *PLoS One*, 2014, vol. 9, no. 10, p. e109386. https://doi.org/10.1371/journal.pone.0109386

68. Rahman, A., Uddin, W., and Wenner, N.G., *Mol. Plant Pathol.*, 2015, vol. 16, no. 6, pp. 546–558. https://doi.org/10.1111/mpp.12209

69. Yamamoto, S., Shiraishi, S., and Suzuki, S., *Lett. Appl. Microbiol.*, 2015, vol. 60, no. 4, pp. 379–386. https://doi.org/10.1111/lam.12382

70. Rodriguez, J., Tonelli, M.L., Figueredo, M.S., Ibanez, F., and Far, A., *Eur. J. Plant Pathol.*, 2018, vol. 152, pp. 845–851. https://doi.org/10.1007/s10658-018-1524-6

71. Cherepanova, E.A., Blagova, D.K., Burkhanova, G.F., Sarvarova, E.S., and Maksimov, I.V., *Ekobiotekh*, 2019, vol. 2, no. 3, pp. 339–346. https://doi.org/10.31163/2618-964X-2019-2-3-339-346

72. Li, Y., Heloir, M.C., Zhang, X., Geissler, M., Trouvelot, S., Jacquens, L., Henkel, M., Su, X., Fang, X., Wang, Q., and Adrian, M., *Mol. Plant Pathol.*, 2019, vol. 20, no. 8, pp. 1037–1050. https://doi.org/10.1111/mpp.12809

73. Debois, D., Fernandez, O., Franzil, L., Jourdan, E., de Brogniez, A., Willems, L., Clément, C., Dorey, S., De Pauw, E., and Ongena, M., *Environ. Microbiol. Rep.*, 2015, vol. 7, no. 3, pp. 570–582. https://doi.org/10.1111/1758-2229.12286

74. Poliksenova, V.D., *Vestn. BGU, Ser. 2*, 2009, no. 1, pp. 48–60.

75. Straight, P.D., Willey, J.M., and Kolter, R., *J. Bacteriol.*, 2006, vol. 188, no. 13, pp. 4918–4925. https://doi.org/10.1128/JB.00162-06

76. Pérez-Garcia, A., Romero, D., and de Vicente, A., *Curr. Opin. Biotechnol.*, 2011, vol. 22, no. 2, pp. 187–193. https://doi.org/10.1016/j.copbio.2010.12.003

Translated by A. Panyushkina

SPELL: OK