EXPERIMENTAL ARTICLES

Cytophysiological Characteristics of the Vegetative and Dormant Cells of *Stenotrophomonas* sp. Strain FM3, a Bacterium Isolated from the Skin of a *Xenopus laevis* Frog

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Abstract—A bacterial strain (FM3) that is closely related to *Stenotrophomonas acidaminiphila* and *S. maltophilia*, was isolated from the skin surface of the frog *Xenopus laevis*. Cytophysiological studies on vegetative cells and cyst-like cells (CLCs) that were obtained in model experiments addressed the dynamics of transition of vegetative cells to the dormant state and their reversion to vegetative growth. The ultrastructural organization of the vegetative and dormant cells of strain FM3 possesses unique properties. Cultures that developed after inoculating vegetative cells were characterized by: (1) resistance to physical factors and sterilization procedures; (2) high antimicrobial activity with respect to some gram-positive and gram-negative bacteria; (3) resistance to polypeptide antibiotics; (4) the presence of an easily detaching S-layer on the cell surface; (5) the ability to secrete outer membrane vesicles into the intercellular space; and (6) formation of S-layer-derived tubular structures associated with outer membrane vesicles that are regularly arranged within the tubes. Dormant cells were characterized by: (1) resistance to dehydration; (2) resistance to high temperatures; and (3) the preservation of the S-layer on the surface of cystlike cells (CLCs). Depending on experimental conditions, strain FM3 formed three CLC morphotypes, which differed in their abundance and ultrastructural organization. The experimental conditions used for CLC formation approximated those under which bacteria survive in hospitals. A model of intermicrobial parasitism is suggested that applies to motile FM3 cells during the development of their populations (cultures).

Keywords: ultrastructural organization, transmission electron microscopy, S-layers of bacterial cells, cyst-like dormant cells, cell surface, sterilizing factors, bacterial resistance to antibiotics, intermicrobial parasitism, antibacterial activity

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The skin of amphibians is a unique ecological niche for the development of microorganisms. The composition of the mucus that covers amphibian bodies is relatively complex. It includes a large number of polysaccharides, various antibodies, lysozyme, alkaloids, and a number of other biologically active substances such as short peptides. These substances that are frequently present in high concentrations on the frog skin may exhibit antifungal, antiviral, and antibacterial activity (Zasloff, 1987; Bevins and Zasloff, 1990; Conlon, 2011; King et al., 2012). It was established that the amphibian skin surface is inhabited by representatives of over 30 bacterial genera such as *Pseudomonas*, *Brevundimonas*, *Pseudoclavibacter*, *Rhodococcus*, and others (Bevins and Zasloff, 1990; Belden and Harris, 2007; Brucker et al., 2008; Becker and Harris, 2010; Bell et al., 2013; Antwis et al., 2014; Becker et al., 2015;

De Assis et al., 2017). Many culturable symbionts produce a number of biotechnologically important biologically active substances. The isolation and characterization of these microorganisms currently receives much attention in view of pressing medical and environmental issues, especially the task of synthesizing peptides with high antimicrobial activity.

We isolated strain FM3, together with a number of small gram-negative bacteria, from the skin of the common clawed frog *Xenopus laevis*. Based on the results of analysis of its 16S rRNA gene sequence, strain FM3 was placed in the genus *Stenotrophomonas*. According to the data on the similarity degree of the nucleotide sequence of the 16S rRNA gene (over 99%), it was very closely related to the species *S. maltophilia* and *S. acidaminiphila*. Phylogenetically, representatives of the *Stenotrophomonas* genus belong

to the class *Gammaproteobacteria* (LPSN). Species of this genus are widespread in natural habitats. They inhabit various water bodies, soils, and plant rhizospheres.

The bacterium *S. maltophilia* is the most extensively investigated member of the genus *Stenotrophomonas*. This species is of special interest, chiefly because of its physiological and biochemical properties, including its ability to cause infectious diseases in humans under certain conditions.

A distinctive feature of *S. maltophilia* strains is their high resistance to diverse antibiotics (Sánchez, 2015; Çıkman et al., 2016), which causes additional problems for the patients that suffer from nosocomial infections in hospitals.

Of special note is also the biotechnological potential of *S. maltophilia*. It was revealed that the organism can produce a variety of biotechnologically important enzymes including those degrading keratin, atrazine, trichloroethylene, and dichlorodiphenyltrichloroethane (DDT). It was suggested that strain *S. maltophilia* should be used for bioremediation of heavy metals-polluted soil, plant roots colonization, and for biological control of development of fungi, bacteria, and insects (Mukherjee and Roy, 2016).

Very limited data are available concerning the ultrastructural organization of the bacteria *S. maltophilia* and *S. acidaminiphila*, the closest relatives of strain FM3. It is only known that the cells of *S. maltophilia* C17 display the cell wall structure that is typical of gram-negative bacteria; they are characterized by the presence of the surface S-layer (Debettei and Prensier, 1989).

The significant biotechnological potential and the medical and biotechnological importance of *S. maltophilia*, one of the most widespread opportunistic agents causing nosocomial (intrahospital) infections (Denton and Kerr, 1998; Shaginyan and Chernukha, 2005; Ryan et al., 2009; De Mauri et al., 2014; Trivedi et al., 2015; Brooke et al., 2017) and the interesting features of its ultrastructural organization and physiology that gave grounds for conducting research with a new isolate, strain FM3, which is closely related to *S. maltophilia*.

The goal of the present work was to conduct comparative studies on the structural and physiological adaptive alterations in bacterial cells during their transition to the dormant state and reactivation and to elucidate the possible structural mechanisms that provide for their resistance to sterilizing factors.

MATERIALS AND METHODS

Isolation of strain FM3. The studied bacterium was isolated from the mucosa of the skin cover of the common clawed frog *Xenopus laevis*. Mucous skin layer samples were collected by scraping the skin after washing the frog three times in sterile tap water to remove

contaminating bacterial cells. The samples were placed in 5 mL of phosphate buffer (pH 6.9) that contained (g/L) NaH_2PO_4 , 6.0 and Na_2HPO_4 , 22.0, homogenized, and plated on the IBFM RAN (5/5) trypton–soybean medium containing the following (g/L): soybean extract, 30; casein hydrolysate, 5.0; yeast extract, 1.0; aminopeptide, 60.0 mL/L; and agar, 20.0 (pH 8.0).

Pure cultures were isolated using the following media: (a) the IBFM RAN (5/5) trypton–soybean medium; (b) Difco (BBL) nutrient agar; (c) the Luria–Bertani medium (LB); (d) a mineral medium containing the following (g/L): NaH_2PO_4 , 6.0; KH_2PO_4 , 3.0; NaCl , 0.5; NH_4Cl , 1; MgSO_4 , 0.25; CaCl_2 , 0.01; and agar, 20.0; (e) the soil agar medium containing the following (g/L): soil, 300.0; yeast extract, 1.0; and agar, 20.0.

Molecular genetic techniques. Genomic DNA was isolated from the cells using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, United States) in compliance with the manufacturer's instruction.

The 16S rRNA gene was amplified in a PCR system using the universal prokaryotic 16S rRNA primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525r (5'-AAGGAGGTGATCCAGCC-3') (Weisburg et al., 1991).

The amplified DNA was purified using the QIAEX II Gel Extraction Kit (QIAGEN, United States). The PCR fragments of the DNA were sequenced with an automatic sequencer, the Applied Biosystems 3130 Genetic Analyzer.

Phylogenetic analysis. Primary phylogenetic analysis of the sequences obtained was carried out with the BLAST software [http://www.ncbi.nlm.nih.gov/blast] using the EzBioCloud database (www.ezbiocloud.net). The 16S rRNA gene sequences from the GenBank database (www.ncbi.nlm.nih.gov) were used for phylogenetic analysis. The nucleotide sequences of the 16S rRNA gene obtained for strain FM3 were manually aligned with those of the reference strains of the taxonomically closest microorganisms using the CLUSTAL W software package (Thompson et al., 1994).

Phase contrast microscopy. Microscopic studies were conducted using a Nikon Eclipse Ci microscope (Nikon, Japan) with a ProgRes SpeedXT camera (Jenoptic, Germany).

Transmission electron microscopy. To prepare ultrathin sections, the cells of the tested variants including CLCs were concentrated by centrifugation (10000 g, 15 min) and fixed in 2% glutaraldehyde solution in 0.05 M cacodylate buffer (pH 7.2) for 1 h at 4°C. Thereupon, the samples were washed three times with 0.05 M cacodylate buffer (pH 7.2) and additionally fixed with a 2% OsO_4 solution in the same buffer for 4 h at 18–20°C. Glutaraldehyde–osmium fixation

in the presence of ruthenium red was used for contrasting cell wall polysaccharides and the S- layer (Luft, 1964). Dehydrated samples in agar blocks were subsequently encapsulated in Epon 812 epoxy resin.

The sections were mounted on support grids, contrasted for 30 min in 3% uranyl acetate solution in 70% alcohol, and additionally contrasted with lead citrate according to Reynolds (1963).

Ultrathin sections were examined under a JEM-1400 transmission electron microscope (JEOL, Japan) at an accelerating voltage of 80 kV.

Cell volume calculation was based on the data of morphometric analysis of the electron microscopic images of negatively stained or total bacterial preparations. While calculating cell volume (V), it was assumed that they represented spheres or ellipsoids of revolution. The following formula was used:

$$V = \frac{1}{6}\pi l d^2,$$

where l is cell length and d is cell diameter.

Characterization of the physiological properties of the tested strain. The liquid complete 5/5 medium was used for determining optimum growth conditions for the tested strain. The temperature optimum was established by varying the temperature within the 4–42°C range, and the effect of pH on growth was assessed within the 4.0–11.0 range. The medium pH was changed by adding 3 M NaOH or 1 M HCl. Halotolerance was determined by cultivation in the media containing 0–15% NaCl. Growth intensity was assessed as optical density determined at 590 nm in a 5-mm cuvette with an UV Specord spectrophotometer (Carl Zeiss, Germany).

To determine the spectrum of utilized substrates and enzyme activities of the isolate, we used the API ID32 E and CH 50 tests (bioMérieux, France) in compliance with the manufacturer's instructions, as well as colored Hiss medium with carbohydrates that contained the following (g/L): peptone, 10.0; NaCl, 5.0; carbohydrate, 7.0; and 1.6% bromothymol blue solution, 1.0 mL.

The presence of catalase was qualitatively determined by monitoring bubble formation upon adding a drop of 3% (vol/vol) H₂O₂. Oxidase activity was determined by the technique based on the color reaction that is associated with the oxidation of tetramethyl-*p*-phenylene diamine (Kovacs, 1956).

Hydrolysis of cellulose, casein, starch, and Twin 20, 40, 60, and 80 was performed as described by Gonzalez et al. (1978).

Resistance to antibiotics. The DI-PLS-50-01 indicator disks were used for determining bacterial resistance to antibiotics (Bauer et al., 1966).

Antagonistic activity of the strain was assessed by the well diffusion method (Egorov, 1965). For this purpose, strain FM3 culture was dispensed (15 µL)

into wells; extracellular metabolites were allowed to diffuse into the agar; and semiliquid nutrient agar (0.7%) containing 5–7 × 10⁷ cells/mL of the test culture was layered over them. The plates were incubated at 24°C for 72 h. Antagonistic activity was visually estimated at 24-h intervals by documenting the presence and measuring the size of test culture growth inhibition areas around the wells.

Obtaining the dormant forms. Dormant forms capable of long-term survival have not yet been described for strain FM3 tested in this work. Therefore, several different approaches to obtaining them were developed. The following techniques were used: (i) long-term (up to 4 months long) incubation of the culture grown in a rich medium (LB broth); (ii) long-term (1 to 4 months long) incubation of a culture grown in a synthetic medium for *Arthrobacter* that contained the following: (g/L tap water): glucose, 2; KH₂PO₄, 0.01; (NH₄)₂SO₄, 0.2; MgSO₄ · 7H₂O, 0.15; K₂HPO₄, 1.0; CaCl₂ · 6H₂O, 0.2; pH 7.2; the N source concentration was fivefold decreased to 0.04 g/L; and (iii) slow dehydration on fivefold-diluted agar media.

RESULTS AND DISCUSSION

Description of the colonies and cell morphology of *Stenotrophomonas* sp. FM3. Generally, there is much similarity between the colonies of vegetative cells and cystlike dormant cells (CLCs) that have been planted on an agar medium after returning to active growth (the first transfer) (Table 1). However, the variant spectrum became wider in the first transfer; colonies with a wrinkled, crater-shaped center were detected, apart from the typical colonies of vegetative cells.

A peculiar feature of cell morphology of strain *Stenotrophomonas* sp. FM3 was that growth on agar and in a liquid medium resulted in the cells that significantly differ in size. The size of the vegetative cells from agar medium-grown cultures (1.5–2.0 × 0.35–0.6 µm) (Fig. 1a) was approximately 1.5 times smaller than that of the cells from liquid medium-grown cultures (2.2–2.8 × 0.6–0.68 µm) (Fig. 1b). The average vegetative cell volume in a liquid medium was 0.45 µm³, whereas the cell volume on agar varied from 0.16 to 0.35 µm³ (not exceeding this value).

In *Stenotrophomonas* sp. FM3 cultures with limitation by essential nutrients, transition to the dormant state during a long-term (1.5–7 month) storage period resulted in a considerable (over threefold in some CLCs) decrease in the linear size (length/width ratio) of rod-shaped cells that dropped to 0.6–1.0 × 0.33–0.6 µm. Phase contrast microscopy revealed a considerable change in their shape: short rods and spherical forms were observed (Fig. 1c). The volume of dormant cells varied from 0.03 to 0.25 µm³. The volumes of the majority (over 80%) of the dormant cells were less than 0.1 µm³. This implies that surviving unfavorable

Table 1. Colony characteristics of strain FM3

Colony features	Inoculum	
	vegetative cells*	CLCs, the first transfer**
Diameter, mm	1.5–3	1.5–3
Shape	Round	Round
Transparency	Semitransparent	Semitransparent
Relief	Convex	Convex with a wrinkled crater-shaped center
Surface pattern	Smooth, glossy	Smooth, glossy
Color	Yellow, more intense in the center	Yellow, more intense in the center
Edge	Even	Even
Structure	Homogeneous	Homogeneous

* Hereinafter, cells from a regularly reinoculated culture.

** Hereinafter, cells from the CLCs of the first subculture.

conditions in a dormant state in the form of ultrasmall cells is characteristic of *Stenotrophomonas* sp. FM3.

Upon reactivation, the suspension of young vegetative cells in the first transfer in the liquid medium contained rod-shaped cells that were homogeneous in size ($1.7\text{--}2.5 \times 0.33\text{--}0.37 \mu\text{m}$) and in volume (0.1 to $0.18 \mu\text{m}^3$) (Fig. 1d).

The images of the above cell variants that were obtained using the same magnification are combined in one picture, facilitating the comparison of their size, shape, and ultrastructural organization (Fig. 2).

Ultrastructural organization of the vegetative cells of strain FM3. The structure of the cells of the tested bacterium *Stenotrophomonas* sp. FM3 was typi-

cal of gram-negative bacteria. The cell wall was characterized by the presence of an outer membrane lined by a thin underlying murein layer. Additional distinctive features of strain FM3 cells were the presence of an S-layer on the cell surface and the ability to form outer membrane vesicles (OMVs) (Fig. 3). When released into the environment, outer membrane vesicles were attached to the S-layer fragments. The fragments detached from the cell surface and rolled up, forming tubular structures (T). Their wall was formed by the S-layer and the internal space was filled by OMVs (Figs. 3, 4).

The S-layer of strain FM3 cells is characterized by a tetragonal symmetry of the subunits it is comprised

Fig. 1. Cell morphology of *Stenotrophomonas* sp. FM3: vegetative cells grown on agar-containing 5/5 medium for 15 h (a); vegetative cells grown in liquid 5/5 medium for 15 h (b); dormant cell forms (CLCs) obtained by long-term (~7 months-long) starvation in a key growth factors-limited medium (c); and cells grown in a liquid medium after inoculating CLCs (d). Scale bar, 10 μm .

of (Fig. 4a'). Outer membrane vesicles that are in close contact with the S-layer are evenly distributed over its surface; they are regularly arranged without contacting one another, and the distance between them is 8–10 nm (Fig. 4b). OMVs contain granules consisting of an electron-transparent substance that has not been identified yet. However, the fact that the strain possesses antagonistic properties against a number of bacteria (see below), gives grounds for suggesting that the biological function of the granule-forming substance is related to antimicrobial activity and it may form a part of the mechanism of suppressing or lysing extraneous bacteria.

It is also possible that the S-layer in complex with OMVs enhances the advantages of strain FM3 in terms of intermicrobial interaction with bacterial species that compete for the substrate. Bacterial S-layers are known to have a high absorption capacity. Presumably, the S-layers shed by a cell form “rafts”/“trawls” whose entire surface attaches to the surface of an extraneous bacterium upon contacting it. Hence, strain FM3 is likely to implement the following antimicrobial mechanism: S-layer fragments form a complex with antimicrobial agents-containing OMVs, adhere to the surface of extraneous cells, and efficiently damage target bacteria in several cell envelope parts at the same time. Strain FM3 is motile due to a polar flagella. While actively moving in the intercellular space in an aqueous environment, *Stenotrophomonas* sp. FM3 distributes these “rafts”/“trawls” that attach to target cells and inactivate or destroy them.

Strain FM3 was isolated from the skin surface of the common clawed frog *Xenopus laevis*. Representatives of this frog species are known to synthesize low-molecular weight peptides with high antimicrobial activity (Zasloff, 1987; Bevins and Zasloff, 1990; Conlon, 2011; King et al., 2012). It was established that bacterial S-layers prevent the entry of antimicrobial low-molecular weight peptides into the cells, providing for the resistance of bacteria to antibiotics (de la Fuente-Núñez et al., 2012).

Ultrastructural organization of the dormant cells of strain FM3. Three morphotypes of cystlike dormant cells of the tested FM3 strain were obtained under different experimental conditions. They differed in their ultrastructural organization and viability. Special attention was given to supramolecular alterations in the surface ultrastructures, due to the presence of an S-layer in strain FM3 cells. It is assumed that one of the S-layer's functions is protecting the cells from environmental factors and low-molecular weight antibiotics of natural origin that may also be synthesized by the host organism (the common clawed frog *Xenopus laevis* in the system tested). It was established that spherical or ovoid cystoids with a significantly expanded, electron-transparent periplasmic space (CLCs of morphotype I) were formed in the liquid incubation medium under nitrogen limitation (Figs.

Fig. 2. Ultrastructure of strain FM3 vegetative cells grown in a liquid medium (a); CLCs formed by cultivation under nitrogen limitation for 7 months (b); and vegetative cells of the first transfer after germinating CLCs (c). Transmission electron microscopy. Ultrathin sections. Cell images obtained at the same magnitude are shown for comparison. Scale bar, 1 μ m.

5a, 5b). The cytoplasm of morphotype I CLCs was distinguished by a high degree of heterogeneity and an unique structural reorganization of the DNA filaments in the nucleoid. It differed from the DNA reorganization that was earlier described in dormant bacterial cells and involved DNA compactization (Suzina et al., 2004; Mulyukin et al., 2008, 2009). The nucleoid of morphotype I CLCs lost its characteristic fibrillar and granular structure and represented complexes

Fig. 3. Ultrastructural organization of *Stenotrophomonas* sp. FM3 cells. Transmission electron microscopy. Ultrathin sections. Designations: OM, outer membrane; N, nucleoid; V, outer membrane vesicles; S, S-layer. Scale bar, 0.3 μ m.

Fig. 4. The S-layer and outer membrane vesicles that are characteristic of *Stenotrophomonas* sp. FM3 cells. Transmission electron microscopy. Negative contrast. Designations: V, outer membrane vesicles; S, S-layer; T, tubular structure at an early formation stage. Scale bar, 0.3 μ m.

that were composed of a homogeneous, low electron density substance located both in the central part of the cell and on its periphery.

CLCs of two other morphotypes were formed while the culture was grown on diluted agar media with slow dehydration (for 6–8 months) at room temperature (20–22°C). The ultrastructure of morphotype II CLCs was typical of a large number of gram-negative CLCs that were described earlier. They were characterized by nucleoid compactization, decreased ribosome content, and an increase in cell envelope thickness, which mainly was due to murein layer thickening (Figs. 5c, 5d). Morphotype II CLCs were also formed during long-term (up to 8 months long) incubation at room temperature (20–22°C) in the rich LB medium.

Morphotype III CLCs were a principally new cystoid morphotype. They were represented by thick-

walled spaghetti-shaped forms with a homogeneous cytoplasmic content that were not described earlier (Fig. 5e). Morphotype III CLCs were detected after 7–12 months of growth on diluted agar media with slow dehydration. Morphotype III CLCs completely lost the rod-like shape that is characteristic of the vegetative cells. No symptoms of cell lysis were revealed, and all envelope components including the S-layer remained intact.

It was established that the S-layers that are characteristic of the tested strain were retained in all the three morphotypes of cystoids formed. However, it should be noted that the most intense formation and detachment of the S-layers (associated with outer membrane vesicles) by strain FM3 vegetative cells proceeded during the first 2–3 months of cultivation after isolation of the strain from the frog skin surface. This phe-

Fig. 5. Ultrastructural organization of the cyst-like dormant cells (CLCs) of *Stenotrophomonas* sp. FM3. Transmission electron microscopy. Ultrathin sections: (a, b), morphotype I CLCs; (c, d), morphotype II CLCs; (e), morphotype III CLCs. Designations: OM, outer membrane; CM, cytoplasmic membrane; PS, periplasmic space; M, murein; N, nucleoid; S, S-layer. Scale bar, 0.5 μ m.

nomenon became subsequently much less often, even though all vegetative and dormant cells retained the S-layer. A high S-layer fragment concentration was detected in the incubation medium of nitrogen-limited strain FM3 cells after 7 months of starvation. The forming CLCs of strain FM3 actually floated in the S-layer fragment “broth.” The growth dynamics during the mid-stationary phase (over 70 h of cultivation) was characterized by a high content of a fibrillar material associated with large conglomerations of S-layer fragments.

The numbers of the colonies that were generated under various growth conditions in the three CLC morphotypes were variable. The highest CFU values were obtained with morphotype I CLCs formed during long-term incubation of strain FM3 in the nitrogen-limited liquid medium and with morphotype II CLCs that was generated during long-term (up to 8 months long) incubation at room temperature (20–22°C) in the rich LB medium. It was impossible to

separately monitor morphotype II and III CLC numbers (CFUs) because these dormant cells form a mixed population and may be in close contact. Importantly, the experimental conditions under which the CLC morphotypes were obtained maximally approximated those under which bacteria survive in hospitals.

Physiological and biochemical properties of *Stenotrophomonas* sp. FM3 bacterial cells. The isolate is an aerobic, chemoorganotrophic, and catalase- and oxidase-positive bacterium. *S. maltophilia* and *S. acidaminiphila* that are the most closely related species are oxidase-negative. However, there is evidence that some *S. maltophilia* strains belong to oxidase-positive bacteria (Carmody et al., 2011). FM3 utilizes a wide spectrum of amino acids and other organic acids and carbohydrates as carbon and energy sources; it does not degrade organic polymers such as cellulose, agar, pectin, and Tween-20, -40, -60, or -80. However, FM3 breaks down starch and casein. It was noted that the cells lose the capacity to degrade starch after about

Table 2. Resistance to physical factors

Influencing factors	Strain FM3	
	vegetative cells	CLCs
UV light within the sterilizing irradiation range, 1 h	+	—
Heating, 80°C, 20 min	—	—
Heating, 70°C, 20 min	Sporadic colonies	—
Dehydration, 10 days	+	++
Alcohol (50%, 10 min)	—	—
Surfactant (concentrated solution)	+ (lawn)	+ (lawn)

one year of frequent transfers under laboratory conditions. However, upon reactivation from the dormant state, the cells that germinate from morphotype I CLCs reacquired the ability to degrade starch. Strain FM3 grows within the 15–38°C temperature range (with an optimum at 37°C); at pH values of 6.0–8.0 (with an optimum at 7.0); and at NaCl concentrations of 0–1.0%.

It was revealed that strain FM3 vegetative cells were resistant to severe conditions such as long-term UV-irradiation within the sterilizing range (applied for more than one hour) (Table 2), which is of considerable importance in clinical terms. This accounts for the development of *S. maltophilia*-caused nosocomial infections. Surprisingly, the morphotype I and II cyst-like dormant cells of strain FM3 that were obtained under experimental conditions lacked the aforementioned feature. Moreover, both the vegetative cells and the morphotype II CLCs of strain FM3 were not resistant to alcohol solutions, but they were characterized by a high resistance to dehydration for 10 days (Table 2). According to the CFU data, the viability of

the CLCs was significantly higher than that of the vegetative cells during long-term dehydration.

It was revealed that the high antimicrobial activity against some gram-positive and gram-negative bacteria, which was highly pronounced during the first 2–3 month of transfers of strain FM3 after its isolation (Table 3), subsequently decreased considerably and was completely lost after 3 years. However, after reactivating CLCs (stored for 7–8 months), the germinating vegetative cells of the first transfer reacquired antimicrobial activity against some gram-positive bacteria (Table 4). A positive correlation was revealed between the intensity of formation of OMVs-associated S-layer fragments, their release into the intercellular space, and the antimicrobial activity of strain FM3. A decrease in antimicrobial activity after 3 years of cultivation of strain FM3 correlated with a marked reduction in unattached S-layers, so that they were difficult to detect on the cell surface. This positive relationship indirectly testifies to the involvement of S-layers in the mechanism of antagonistic intermicrobial interaction. It is bacterial motility that presumably plays an important part in this mechanism because it provides for the spreading of S-layer fragments associated with OMVs that contain biologically active substances producing an antimicrobial effect. This enables the strain to deal with a large number of target bacteria within a short time.

It was established that strain FM3 was resistant to a number of antibiotics (Table 5). Resistance to polypeptide antibiotics such as bacitracin and a lack of resistance to other kinds of antibiotics apparently represents a strain-specific feature and is related to the source used for isolating strain FM3. Its habitat was the skin surface of the common clawed frog that produces peptide antibiotics.

To sum up, integrated morphological and cytophysiological research on the vegetative and cyst-like cells of strain FM3 during the course of transition to the dormant state and exit from it that was conducted in the present work revealed a number of peculiar physiological and ultrastructural features. Under different experimental conditions strain FM3 was found to form three different CLC morphotypes that

Table 3. Antagonistic activity of strain FM3 against some test cultures

Test culture	Vegetative cell *
Gram-negative bacteria	
<i>Escherichia coli</i> K12	+/—
<i>Escherichia coli</i> B	+
<i>Erwinia amylovora</i> B34	+
<i>Alcaligenes rughlandii</i> B 1333	+
Gram-positive bacteria	
StN	+
StS	+/—
<i>Micrococcus luteus</i> B 1819	+
<i>Microbacterium</i> str. S3	+
<i>Bacillus sphaericus</i> B 506-2	+
<i>Bacillus weihenstephanensis</i> KBAB4	+

* A culture grown in the liquid medium, immediately after isolating the strain from the frog skin surface.

Table 4. Antimicrobial activity of the vegetative cells of permanently (for 3 years) maintained strain FM3 and of the cells of the culture obtained by germinating dormant forms (CLCs)

Test culture	Presence of a lysis area	
	18 hs after CLC germination	vegetative cells of the permanently maintained strain
<i>Escherichia coli</i> K12	—	—
<i>Escherichia coli</i> B	+ ~4 mm lysis area	+ ~2 mm lysis area
<i>Erw. amylovora</i> B34	—	—
<i>Erwinia carotovora</i> B15	—	—
<i>Alcaligenes ruchlandii</i> B 1333	—	—
<i>Alcaligenes faecalis</i> BKM1518	—	—
<i>Pseudomonas aeruginosa</i> PAO1	—	—
<i>Pseudomonas caryophyllii</i> BKMB1296	—	—
StS	—	—
<i>Micrococcus luteus</i> B 1819	+ ~2 mm lysis area	+ ~2 mm lysis area
<i>Micrococcus roseus</i> B1236	—	—
<i>Bacillus sphaericus</i> B 506-2	—	—
<i>B. weihenstephanensis</i> KBA 134	—	—
<i>Bacillus subtilis</i>	+ ~2 mm lysis area	—
<i>Arthrobacter</i> sp. B52	+ ~1 mm lysis area	+ ~1 mm lysis area
<i>Deinococcus radiodurans</i>	+ ~5 mm lysis area	—

Table 5. Sensitivity to antibiotics detected using DI-PLS-50-01 indicator disks

Antibiotic	Antibiotic characteristics	Resistance	
		vegetative cells	CLCs, first transfer
Bacitracin, 0.04 U	Polypeptide antibiotic	+	+
Ceftriaxone, 200 mg/mL	Third-generation cephalosporin antibiotic with a wide action spectrum. Produces a bactericidal effect by suppressing cell wall synthesis in microorganisms	+/-	—
Polymyxin POL, 300 U	Belongs to cyclic polypeptide antibiotics	—	—
Meropenem MPN, 10 µg	Synthetic carbapenem antibiotic	+/-	+/-
Ceftazidime CAZ, 30 µg	Third generation cephalosporin antibiotic	—	+/-
Furazolidone FRN, 300 µg	Synthetic nitrofurantoin derivative	+/-	+/-
Imipenem IM, 10 µg	Tienamycine derivative. Belongs to the carbapenem group, inhibits bacterial cell wall synthesis	+/-	—
Trimethoprim/sulfamethoxazole TS 1.25/23/75 µg	Combined antimicrobial preparation consisting of sulfamethoxazole and trimethoprim	+	+

differ in their ultrastructural organization as well as in their abundance. This fact points to a high adaptability of the tested strain and, in all likelihood, of other representatives of the genus *Stenotrophomonas*, with regard to unfavorable environmental conditions. The experimental conditions used for obtaining the CLCs of strain FM3 maximally approximated the possible conditions under which bacteria survive in hospitals.

The data obtained in this work contributes to our knowledge of the bacteria of the genus *Stenotrophomonas* and provides new information concerning the special features of the lifestyle of the bacterial microbiota on the skin surface of amphibians.

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