ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 5, pp. 529–541. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © V.N. Khmelenina, N.E. Suzina, Yu.A. Trotsenko, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 5, pp. 515–527.

REVIEW

Surface Layers of Methanotrophic Bacteria

V. N. Khmelenina[a], N. E. Suzina[a, b], and Yu. A. Trotsenko[a, b, 1]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Russia*
[b] *Pushchino State Institute of Natural Sciences, Russia*

Received April 11, 2013

Abstract—Structural and functional characteristics of the regular glycoprotein layers in prokaryotes are analyzed with a special emphasis on aerobic methanotrophic bacteria. S-Layers are present at the surfaces of *Methylococcus, Methylothermus,* and *Methylomicrobium* cells. Different *Methylomicrobium* species either synthesize S-layers with planar (*p*2, *p*4) symmetry or form cup-shaped or conical structures with hexagonal (*p*6) symmetry. A unique, copper-binding polypeptide 'CorA'/MopE (27/45 kDa), which is coexpressed with the diheme periplasmic cytochrome *c* peroxidase 'CorB'/Mca (80 kDa) was found in *Methylomicrobium album* BG8, *Methylomicrobium alcaliphilum* 20Z, and *Methylococcus capsulatus* Bath. This tandem of the surface proteins is functionally analogous to a new siderophore: methanobactin. Importantly, no 'CorA'/MopE homologue was found in methanotrophs not forming S-layers. The role of surface proteins in copper metabolism and initial methane oxidation is discussed.

Keywords: methanotrophic bacteria, surface layers and proteins, structure and functions, surfaceome

DOI: 10.1134/S0026261713050068

The common feature of many prokaryotes is the presence of regular glycoprotein layers at the external surface of their cell wall (CW), which are referred to as S-layers [1–4]. These structures of oblique, tetra- or hexagonal symmetry are intensively studied by various methods but still remain the most enigmatic components of microbial cells as regards their functions and origin [5–7]. This review presents the state-of-the-art and prospects of investigation of the structural and functional organization of S-layers in different groups of prokaryotes. Special attention is paid to the organization of S-layers and surface proteins involved in copper metabolism in aerobic methanotrophs.

GENERAL CHARACTERIZATION OF PROKARYOTIC S-LAYERS

S-Layer proteins and genes. With few exceptions, S-layers are formed by homopolypeptides or glycoproteins. Their sequences in different prokaryotes have a low homology and their molecular masses (MM) are in the range of 25 to 200 kDa. As a rule, S-layer proteins are characterized by a high content of hydrophobic and acidic amino acids and lysine, which are responsible for the total negative charge and pI value in the range of 4–6, but few or none sulfur-containing amino acids [7, 8]. The proteins of the S-layers of lactobacilli with pI values between 9.4 and 10.4 are an exception. The S-layers of thermophilic prokaryotes, as opposed to mesophilic ones, have a slightly higher content of hydrophobic and basic amino acids [8, 9].

Glycoproteins form crystal lattices of oblique (*p*1, *p*2), tetragonal (*p*4), or hexagonal (*p*3, *p*6) symmetry at microbial cell surface. Structural units of the S-layer are 3–30 nm in size and may consist of 1, 2, 3, 4, or 6 identical (glyco)protein monomers depending on the type of symmetry (Fig. 1a). The lattices formed by S-layer proteins have pores of 2–8 nm in diameter and 5–10 nm in depth.

In bacteria and archaea, S-layer proteins make up to 15% of the total cell protein content. Calculations have shown that ~5×10^5 S-protein molecules are needed to completely cover the surface of one bacillary cell [9]. Consequently, bacteria with a doubling time of 20 min must synthesize about 500 S-layer protein molecules per second, which demonstrates the efficiency of the promoters of the respective genes. The genes encoding S-layer proteins have been identified in 400 species of prokaryotes, with more than 40 genes being cloned and sequenced [10, 11]. It is notable that phylogenetically divergent prokaryotes have no homology between S-layer proteins.

The S-layer genes are usually transcribed as monocistronic units and are expressed from one or more promoters [12]. The promoter of the S-protein of *Lactobacillus acidophilus* is almost twofold more efficient than the lactate dehydrogenase promoter, which is considered to be one of the strongest bacterial promoters. In most cases, S-protein gene transcripts contain the elongated 5' untranslated regions, which form a hairpin-like structure preventing rapid degradation of the S-protein mRNA by RNase [13–15]. At the same time, S-protein mRNA has a longer half-life

[1] Corresponding author; e-mail: trotsenko@ibpm.pushchino.ru

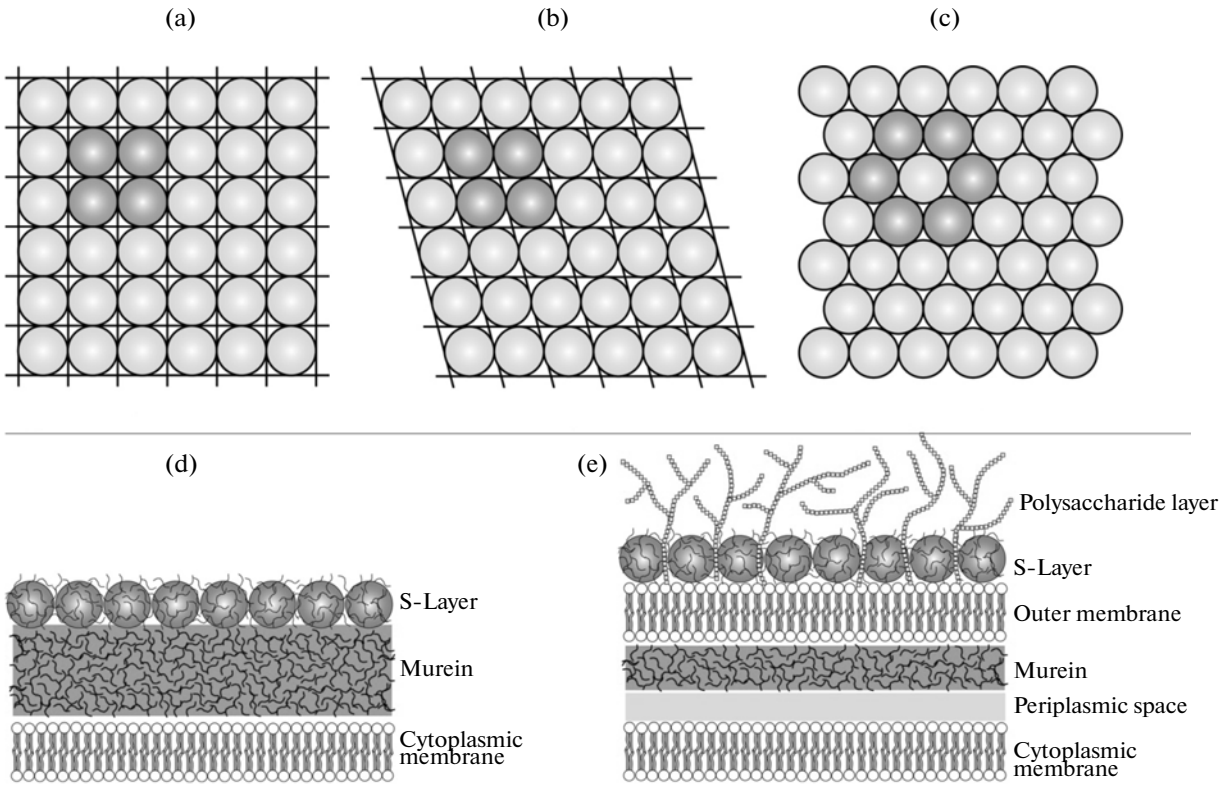

Fig. 1. The schemes of packing of S-layers having tetragonal (a), oblique (b) and hexagonal (c) symmetry and the arrangement of protein subunits at the cell surface of gram-positive (d) and gram-negative (e) bacteria.

(10–22 min) than the mRNA of most bacterial proteins (2–4 min). Such a long lifetime of S-protein mRNA may create problems for the cells during the abrupt changes in environmental conditions inhibiting bacterial growth. Nevertheless, S-proteins are usually absent in the cultivation medium, i.e., there is a strict regulation and coordination of their synthesis and cell growth.

Regulation of the synthesis of S-layer protein has been most thoroughly studied in *Thermus thermophilus*, where the respective protein SlpA repressed the transcription of the *slpA* gene; the mechanism of such repression has not, however, been elucidated. The C-terminal fragment of the SlpA protein binds to the untranslated N-terminal sequences of mRNA; accumulation of this protein in the cytoplasm of a thermophile can stop its own translation in case of sudden cessation of cell growth. *Campylobacter fetus* possesses up to 8 different gene homologues that can be expressed at different stages of infection, which allows the pathogen to alter the CW immune properties [16].

As a rule, the carbohydrate part of the glycoprotein makes up to 10% of the S-layer mass and may be rather diverse. The S-layer carbohydrate of *Clostridium thermohydrosulfuricum* consists of rhamnose and mannose, while that of *Desulfotomaculum nigrificans* consists of glucose, mannose, galactose, and rhamnose, and that of *Sulfobacillus thermosulfidooxidans* consists of mannose, glucose, xylose, galactose and glucosamine [8, 9]. The S-layer of *Bacillus stearothermophilus* has two types of carbohydrate chains: one of them contains rhamnose and the other one contains glucose, *N*-acetyl glucosamine, and di-*N*-acetylmannuronic acid [17]. The S-layer glycoprotein of *Clostridium symbiosum* contains *N*-acetylgalactosamine, *N*-acetylmannosamine, and a glucosamine derivative [18]. The glycoprotein of *Halobacterium halobium* and *H. salinarium* has *N*- and *O*-bound glycan chains containing galactose, galacturonic, glucuronic, and 3-*O*-methylgalacturonic acids, as well as *N*-acetyl glucosamine [19]. Apart from glycosylation, posttranslational modifications of S-proteins include the attachment of lipids, phosphate and sulfate groups, or proteolytic processing [20]. The study of S-layer proteins by the method of circular dichroism showed the low contents of α-helix (2–14%) and β-helix (20–35%) but a high level of aperiodic structures [21].

Cell surface association of S-layers. In gram-positive bacteria, S-proteins are associated with the sec-

ondary CW polymers such as teichoic, teichuronic or lipoteichoic acids and lipoglycans, which, in turn, are covalently or noncovalently bound to peptidoglycan (murein) (Fig. 1b) [22]. The specific domain named SLH-motif (S-layer homologous) is responsible for direct association of S-layers to the peptidoglycan carbohydrate [23, 24]. It is interesting that the SLH-motif is homologous to the regions of the C-terminal part of some exoenzymes associated with the cell surface: polysaccharide-hydrolyzing cellulase and xylanase [25]. However, the cell wall "anchoring" of S-proteins by SLH sequences is not characteristic of all gram-positive bacteria, since the S-proteins of *B. stearothermophilus* [26], *Lactobacillus brevis* [27], and *Corynebacterium glutamicum* [28] contain no SLH-motifs.

In gram-negative bacteria, S-layers adjoin the outer membrane lipid bilayer (Fig. 1c); at the same time, the attachment of S-layers may be mediated by: (1) divalent cations interacting with the charged components of lipopolysaccharides [27–29]; (2) (non)specific bonds between the N-terminal region of the S-layer (or carbohydrates of glycosylated S-proteins) and peculiar forms of lipopolysaccharides [30–32]; and (3) hydrophobic interactions with fatty acids [28, 33, 34].

In archaea, S-layers are in direct contact with the cytoplasmic membrane (CM), being anchored by the hydrophobic regions of protein projections [35]. Obviously, S-layers serve as immobilizing structures for subjacent membrane lipids and proteins, indirectly influencing the CM properties. The calculations performed for *Staphylothermus marinus* showed that up to 5% of membrane lipids could be immobilized during the interaction with the S-layer [36].

The influence of S-layers on the physicochemical properties of subjacent CW components of gram-positive bacteria has been proved by the studies of artificial S-layer/membrane complexes [37]. Purified S-layer proteins were recrystallized at the surface of homogenous lipid mono- and bilayers and, as a result, the membranes became less fluid and elastic but more resistant to heat and hydrostatic pressure. It is quite probable that in gram-negative bacteria with the high protein content in the outer membrane their interaction with S-proteins also considerably changes the physicochemical properties of the membranes [38].

The basic models of three-dimensional organization of the best studied S-layer proteins at the cell surface of gram-positive and gram-negative bacteria and the modern methods of in vivo visualization of S-layers in their environment have been summarized in the recent review [39].

Potential functions of S-layers. The presence of S-layers in prokaryotes of most phylogenetic groups suggests the common functional purpose of these structures, although the attempts to elucidate their role have usually resulted only in recitation of their most probable, useful, or significant functions. The specific role of S-layers has been shown for the physiological groups of microorganisms inhabiting certain econiches; however, there is obviously no function in common for all prokaryotes [6]. The capacity of S-layer proteins for self-organization into paracrystal structures in vivo or in vitro with the minimum expenses of metabolic energy leads to the suggestion that S-layers are ancient forms of CW that have developed into matrix proteins and porins [3, 6]. This hypothesis is favored by the data that S-layers perform the morphogenetic function in archaea and in representatives of the most ancient bacterial phyla: *Thermotoga, Aquifex*, and *Thermus* [9, 40]. Insufficient understanding of the ecophysiological role of S-layers is partially due to the preferential study of isolated structures or proteins, the interaction of which with other CW components or with the environment of the cells has remained out of sight of researchers for a long time. This situation was additionally complicated by specifics of the respective experiments, compared to the study of isolated individual proteins [23, 37].

The key role of S-layers in maintenance of cell shape and structural integrity is most evident in archaea, where S-layers are the CW only rigid component [35, 41]. Though spontaneous mutants lacking S-layers do not occur among the *Archaea*, the cultivation of *Haloferax volcanii* on a magnesium-free medium resulted in the loss of S-layers, with the cells losing their inherent globular shape [42].

In gram-positive bacteria, the lattice protein structure of S-layer may be to a certain extent considered as an equivalent of periplasmic space [9]. Detailed study of microbial CW ultrastructure using cryo-electron tomography revealed that the significance of this compartment, which is obvious for gram-negative bacteria, is underestimated in gram-positive bacteria and archaea. Thus, *B. subtilis* and *S. aureus* lacking S-layers have a more bulky peptidoglycan compared to the species possessing S-layers and even form the periplasmic space [43, 44]. The thickness of the peptidoglycan–teichoic acid complex in *B. subtilis* and *S. aureus* is up to 33 and 19 nm, respectively [43, 44]. On the contrary, the bacilli and clostridia possessing S-layers have a much thinner peptidoglycan (3–6 nm) [45]. The folding, export, degradation, and storage of exoproteins may occur in the periplasmic space formed by S-layers. For example, bacillary S-layers have been shown to contain the exoenzyme-binding sites. In *Staphylothermus marinus*, the S-layer is located at a distance of 70 nm from CM, contacting it with specific "stems", in the center of which there are two copies of the thermostable protease that endures heating up to 95°C as a free protein and up to 125°C as a component of S-layer [46]. S-Layer-associated exoamylase was found in *B. stearothermophilus* [17].

S-Layers have a "porous" structure and, hence, perform the function of a biofilter regulating the entry of necessary nutrients and the release of metabolites [5, 9]. S-layers also act as ion exchangers: they bind

organic nutrients or toxic metals and retain them close to the cell surface [47].

Protection from immune response should be mentioned as one of the specific functions of S-layers. The S-layers with pores of 2–4 nm in diameter protect the cells from the attack of lytic enzymes, the action of surfactants and various toxic reagents, or the penetration of intracellular parasites, such as *Bdellovibrio bacteriovorus* [2, 47].

The special function of S-layers is to provide for cell adhesion [9]. The thermophilic bacilli with S-layers have a much higher adsorption capacity on positively charged or hydrophobic surfaces compared to S-layer-defective mutants. It has been shown for many prokaryotes that S-layers, independently or in combination with other surface components, e.g., LPS, can intensify autoagglutination of the cells [2].

The ability of prokaryotes to form S-layers and the peculiar features of organization of these structures prompted their application as taxonomic markers, at least for some groups of microorganisms. In particular, S-layer morphology correlates with the taxonomic position of cyanobacteria, although individual strains are characterized by specific and even unique details of S-layer organization [48]. At the same time, S-layers of different species of *Bacillaceae* differ considerably in the molecular mass and degree of glycosylation of proteins, the pore size of the crystal lattice, and other parameters [5, 41]. Moreover, some bacteria lose S-layers when cultivated under laboratory conditions [49, 50]. Bacteria with more than one S-layer are known [4, 49]. *Bacillus anthracis* and *Brevibacillus brevis* [51], representatives of *Aquaspirillaceae* [52, 53], and some archaea [40] have a double S-layer. *B. stearothermophilus*, along with the S-layer, has an additional protein layer under the inner surface of the peptidoglycan layer; both protein layers were retained after treatment with lysozyme [49].

The orderly microstructure and the ability for self-assembly in vitro in solutions and on solid carriers (silicon plates, metals, polymers) or on the interphase boundary (lipid membranes, liposomes) are a basis of diverse applications of S-layers in nanobiotechnologies [54]. S-Layers are used for the construction of epitope carriers in vaccines or of ultrafiltration membranes with the same-sized pores, as well as in highly sensitive biosensors by way of fixation of antibodies or other molecules in the nodes of the crystal lattice. Available information about the composition and genetic organization of S-layers reflects the new possibilities of their application. The gene-engineering approaches may be used to incorporate specific functional domains into S-layer proteins, with maintenance of the ability of chimeric proteins for self-assembly. These approaches resulted in development of novel types of affine structures, micro carriers, protein membranes, diagnostic devices, and vaccines. Special reviews are recommended for more detailed acquaintance with the potential fields of application of S-layers [55, 56].

PHENOMENOLOGY OF S-LAYERS OF AEROBIC METHANOTROPHS

Properties of the surface layers and proteins of methanotrophs. Aerobic methanotrophs are represented by 20 genera of the classes *Alpha-* and *Gammaproteobacteria*, as well as by the genus *Methylacidiphilum* of the phylum *Verrucomicrobia*. By now, S-layers have been revealed in all species and strains of methanotrophs from the genera *Methylomicrobium, Methylococcus*, and *Methylothermus* (*Gammaproteobacteria*). S-Layers have been described in *Mc. capsulatus* (Texas and 874) and *Methylothermus thermalis* MYTH, *Methylomicrobium album* BG8, *Mm. alcaliphilum* (6 strains), '*Mm. modestohalophilum*' 10S, *Mm. buryatense* (6 strains), *Mm. kenyense* AMO1 and *Mm. japanense* NI [57, 58]. These methanotrophs, except for the mesophilic nonhalophilic neutrophile *Mm. album* BG8, can grow at enhanced salinity (*Methylomicrobium*) or temperature (*Methylococcus* and *Methylothermus*). S-Layers have not been found in methanotrophs of the genus *Methylobacter*, including the halophilic species of this genus. One should note the absence of S-layers in the representative group of aerobic methylobacteria (>40 genera) growing on methanol or other oxidized or substituted methane derivatives.

Though surface layers with periodic striation are present in the alphaproteobacterial methanotroph *Methylosinus trichosporium* OB3b, they are formed in the late stationary growth phase, and their nature and type of symmetry have not been determined [59]. Very unusual surface structures have been found in some representatives of the genus *Methylocystis* [57, 59, 60]. These are tubes of 40 or 70 nm in diameter and 0.03–1.2 or 2.5 μm in length with radial location relative to the cell surface (Fig. 2a). The tubular structures are formed by helically twisted fibrils consisting of protein globules (Fig. 2b). Their chaotic arrangement at the cell surface does not correspond to the formal definition of S-layers, and their functional purpose has not been determined.

On the contrary, in the thermotolerant methanotroph *Mc. capsulatus* Texas, S-layers of the *p*4 symmetry with a lateral lattice parameter of 60 Å are located under the capsule [59]. Tetragonal S-layers of another strain of this species, *Mc. capsulatus* 874, were found under suboptimal conditions of culture growth but were lost (absent) in the cells growing at the maximum rate in the medium with completely balanced mineral composition and gas phase components [61].

In contrast to the above prokaryotes, including *Mc. capsulatus*, which have S-layers as lattices of planar (2D) geometry, methanotrophs of the genus *Methylomicrobium* form more complex spatially organized structures. In particular, the S-layer of the mesophilic

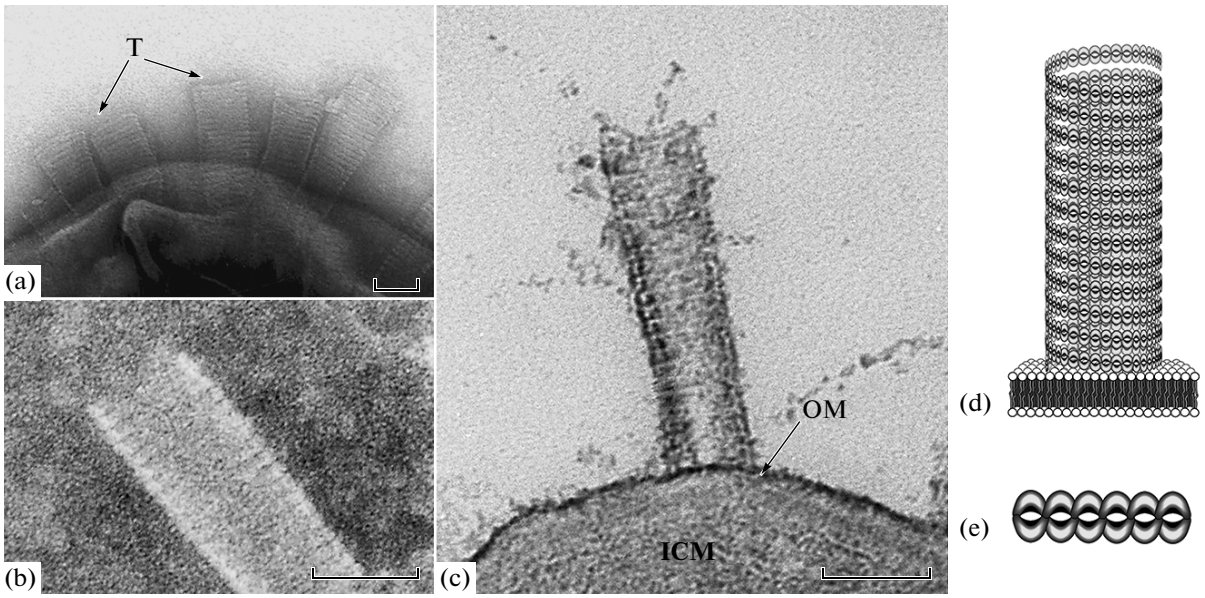

Fig. 2. The ultrastructure and supramolecular organization of tubular surface formations of *Methylocystis echinoides* 2. Negatively stained preparation (a, b); ultrathin section fragment (c); tentative organization of protein subunits as a helical band (d); schematic representation of the packing of subunits as a double flattened helix (e). Tubular structure (T); outer membrane (OM); intracytoplasmic membranes (ICM). Scale bar is 50 nm.

nonhalophile *Mm. album* BG8 is formed by a monolayer of goblet-shaped structures (45–55 nm in diameter and about 60 nm in depth) arranged in the *p*6 symmetry. There is a protein globule in the center of each "cup" with the walls formed by fibrillar proteins [62, 63]. In the three strains of *Mm. alcaliphilum* (20Z, 1S, and 2S) isolated from the soda lakes of Tyva and Kulunda steppe (Russia), S-layers are also represented by hexagonally arranged cup-like structures of 36 nm in height and 33 nm in diameter, with a packing density of 900 U/μm^2 [64–66]. Figure 3 schematically shows the morphological unit of the S-layer of strain 20Z.

In six strains of *Mm. buryatense* (4G, 5G, 6G, 7G, 5B, and 3S), cup-like structures are 40 nm in height and 29 nm in diameter but are more densely packed (1225 U/μm^2). Another S-layer morphotype has been described in *Mm. kenyense* AM1 and the five strains of *Mm. alcaliphilum* (FM3, E3, 4S, 4S1, and 5S). Ultrathin sections of these methanotrophs show S-layers as a monolayer of "upturned" hollow cones, 18 nm in height and 13 nm in diameter, also packed at the cell surface in the *p*6-symmetry [66, 67].

It should be noted that methanotrophs of the genus *Methylomicrobium* have one more type of supramolecular organization of S-layers. Thus, *Mm. alcaliphilum* ML1 (isolated from the Mono Lake, United States) and the neutrophilic halotolerant *Mm. modestohalophilum* 10S isolated from salt wedge estuaries of the Crimea form two-dimensionally organized S-layers with protein subunits arranged in oblique (*p*2) symmetry [66, 68]. Consequently, the species or strains of the genus *Methylomicrobium* have different S-layer morphology.

It is notable that the above morphotypes of S-layers of methanotrophs, including goblet-, cup-, or cone-shaped structures, have been described previously in photo- and chemoautotrophic bacteria. For example, the photosynthetic bacteria *Pelodyction clathratiforme* and *Chromatium buderi* [69, 70] have cup-shaped S-layers with cups of 35 nm in diameter and in height, which rest upon a 15-nm long and 5-nm thick stalk, and the latter is based on a thin electron-dense layer located above the CW lipoprotein layer. The S-layer in the *p*6-symmetry consisting of "upturned" hollow cones of 25 nm in height and 13 nm in diameter is formed by *Chromatium okenii* and *C. weissei* [71]. The finer protein particles (5 nm in diameter) form a net at the cell surface of *Chromatium gracile* and marine nitrifying bacteria [72]. Such type of S-layer organization is predominant in marine and freshwater purple sulfur bacteria [70]. Among heterotrophs, three-dimensional S-layers similar in shape to those in *Mm. alcaliphilum* 20Z were found in *Flexibacter polymorphus* [73]. The cup-shaped structures of this marine bacterium are 25 nm in diameter and 24 nm in depth.

Similar to most prokaryotes, the proteins of S-layers of methanotrophs are glycosylated, which is confirmed by their especially sharp contours in case of prefixation of the cells with ruthenium red [65]. The protein content in the S-layer of strains 20Z and 10S was 80 and 96%, respectively, and the carbohydrate component in *Mm. alcaliphilum* 20Z consisted mainly

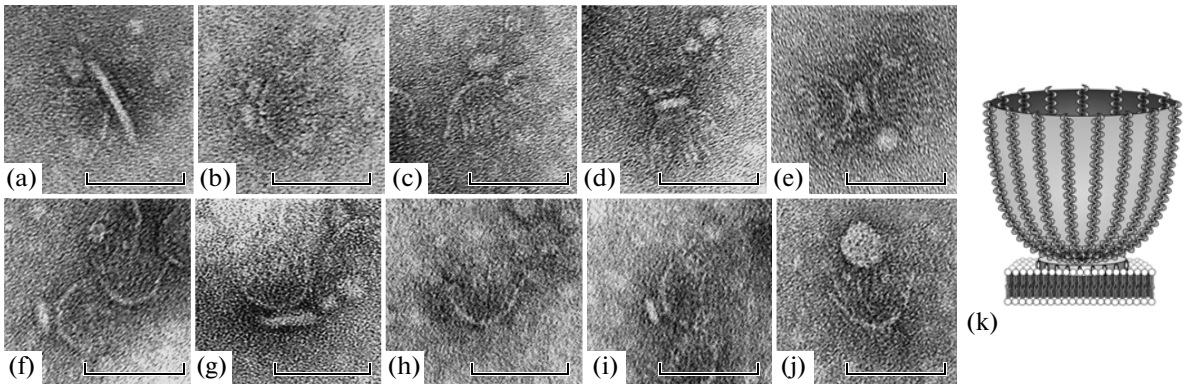

Fig. 3. The ultrastructure and supramolecular organization of cup-shaped surface formations in *Methylomicrobium alcaliphilum* 20Z. Electron microscopic images of different profiles of isolated, negatively stained, cup-shaped structures (a–j) and schematic representation of the packing of protein subunits as the bundles radially diverging from the "cup" base (k). Scale bar is 50 nm.

of glucose (92%), while in the strain 10S it consisted of glucose (76%) and galactose (14%).

In *Mm. alcaliphilum* 20Z, S-layers were detected during cell cultivation on methane in the presence of NaCl under alkaline or neutral conditions, but disappeared during cell growth on methanol or in a salt-free medium under methane [74]. Interestingly, methanol-grown cells of *Mm. buryatense* 5B did not lose S-layers, but cup-shaped structures in this case were less contrasted after pretreatment of the cells with ruthenium red [75].

The presence of S-layers in halophilic and thermophilic/tolerant methanotrophs is in agreement with the function of these structures as a molecular framework that provides for additional CW rigidity and prevents cell lysis during salinity and temperature fluctuations. In principle, one more function may be suggested for the S-layers of moderately thermophilic methanotrophs: methane binding under conditions of limited solubility of the gases. This function is also important at high growth rates of salt-dependent morphotype I methanotrophs, when CH_4 and O_2 uptakes increase. This assumption is indirectly confirmed by the fact of enhanced methane solubility in the matrix of S-layers of the strain 20Z [76].

ASSOCIATION OF S-LAYERS WITH THE SURFACE PROTEINS OF METHANOTROPHS

The composition and functions of surface proteins were most actively studied in *Mc. capsulatus* Bath, the first model methanotroph for which the complete genome sequence was annotated [77]. The chromosome of this thermotolerant organism, consisting of a circular DNA molecule of 3.304 Mbp, contains 3120 predicted encoding sequences, as well as 51 insertion elements from various families. Genomic analysis revealed the multicopy genes responsible for the primary oxidation and assimilation of methane carbon, transport, amino acid biosynthesis, and energy metabolism. The high content of duplicate genes and the presence of sterols and *trans*-fatty acid isomers in the outer membrane contribute to the adaptation of *Mc. capsulatus* Bath to the fluctuating environmental conditions [78, 79].

Proteomic approaches were used to demonstrate that *Mc. capsulatus* Bath has the components of surfaceome (a complex of cell surface-associated proteins) different from those of other gram-negative bacteria [80, 81]. Most of the outer membrane proteins have a β-cylinder structure and belong to the group of TonB-dependent receptors accelerating the transport of high-molecular compounds (e.g., vitamin B_{12}). An ample quantity of lipoproteins has been found, such as thermally regulated proteins MopG, MopI, MopF (Methylococcus outer membrane proteins) and other proteins of the OmpA family [81].

In the pre-genome epoch, the outer membrane-bound proteins MopA, MopB, MopC, MopD, and MopE with molecular masses of 27, 40, 46, 59, and 66 kDa, respectively, have been described in *Mc. capsulatus* Bath [82]. It confirmed experimentally that MopC and MopD exist in vivo as a heterodimer with a total molecular mass of 95 kDa and presumably form a porin for copper ions. MopE (66 kDa) proved to be the major one of the outer membrane proteins; in its C-terminal region, between amino acids 291 and 306, there is a conservative region characteristic of membrane proteins of the OmpA family involved in substrate transport [83]. The MopE protein contains a signaling sequence, which is cleaved after alanine-204. The C-terminal sequence of the MopE protein shows a high homology with the surface 27-kDa protein 'CorA' (copper repressible), which has been previously characterized in *Mm. album* BG8 [63] and was recently found in *Mm. alcaliphilum* 20Z [84]. MopE was expressed at a low concentration of copper ions in the medium, and the mature protein (45 kDa) was found in the culture liquid of *Mc. capsulatus* Bath.

The MopE protein has a specific site for the copper ion binding, which is formed by histidine residues His-132 and His-203 and kynurenine (the product of tryptophan-130 oxidation) [85]. However, when obtained by heterologous expression in *E. coli*, the MopE protein contained no kynurenine and did not bind copper, which was indicative of the presence of a specific mechanism of tryptophan-130 oxidation. Analogous conservative amino acids are also present in the 'CorA' proteins from *Mm. alcaliphilum* 20Z and *Mm. album* BG8 [84]. Analysis of the sequenced genomes revealed that the 'CorA' homologues were unique for the methanotrophs forming S-layers, because the respective genes have not been found in the genomes of other bacteria.

Electron microscopic studies of *Mm. alcaliphilum* 20Z with the antibodies against gold-labeled 'CorA' protein showed that 'CorA' was localized in the base of the cup-shaped structures of S-layers [84]. The mutant with insertion in the '*corA*' gene did not grow in the presence of CH_4, while during its growth on methanol the cup-shaped structures of its S-layer either were chaotically arranged close to the CW surface or formed aggregates in the intercellular space as a bilayer of "cups," with reciprocal orientation of their bases. Consequently, the presence of the 'CorA' protein is necessary for growth on methane and for attachment of the S-layer to the outer membrane. On the contrary, no viable mutant in the 'CorA' protein could be obtained in *Mm. album* BG8 [86].

It should be noted that the designation 'CorA' (copper repressible) proposed for the copper-repressed protein of *Mm. album* BG8 [63] is not quite correct, because this abbreviation (CorA, cobalt resistant) had been previously assigned to an extensive family of magnesium transporters functioning in bacteria and archaea, as well as in animals and humans [87, 88]. The CorA magnesium transporter gene homologues are also present in the genomes of methanotrophs *Mm. alcaliphilum* 20Z, *Mm. album* BG8, *Ms. trichosporium* OB3b, *M. silvestris* BL2, and *Mb. tundripaludum* SV96, with the exception of *Mc. capsulatus* Bath [84]. It is notable that 'CorA' and MopE of methanotrophs show no more than 7% identity of amino acid sequences with the magnesium transporters.

In *Mm. album* BG8 and *Mm. alcaliphilum* 20Z, the '*corA*' gene is located in the same operon with the '*corB*' gene encoding the periplasmic diheme cytochrome *c* peroxidase (CCP) [84, 86]. In *Mc. capsulatus* Bath, the *mopE* gene was also coexpressed with the *mca* gene encoding CCP, which is similar to *c*-type cytochromes (30% identity) and contains two heme-binding motifs [89]. The Mca and 'CorB' proteins have a 49–52% identity. In spite of the minor similarity of their sequences to those of other proteins of the bacterial CCP family, as well as the higher protein molecular mass (78 kDa compared to 35.5 kDa), their differences do not affect the functional area. This fact gave grounds to ascribe Mca and similar proteins to a novel group of bacterial peroxidases, characterized by the presence of additional elements of the secondary structure.

The classical bacterial CCP are periplasmic two-domain proteins. The C-terminal domain contains a heme with high redox potential and the N-terminal domain contains a heme with low redox potential. CCPs have two calcium binding sites. Site I located in the monomer between the two domains has a high affinity and is always occupied with a Ca^{2+} ion. On the contrary, the low-affinity site II is formed during dimerization of the enzyme [90]. Some bacteria were shown to have a subgroup of CCPs containing three heme-binding motifs.

It is supposed that CCPs protect bacterial cells from exogenous or endogenous hydrogen peroxide [91] by catalyzing its conversion into water using two electrons of redox proteins (small redox proteins, SRP):

$$2SRP + H_2O_2 + 2H^+ \longrightarrow 2SRP\ \text{oxidized} + 2H_2O.$$

Such proteins are monoheme cytochromes, cupredoxin, azurin, and pseudoazurin [86]. It is supposed that water photooxidation may be a source of exogenous H_2O_2 for bacteria and the respiratory chain may be a source of endogenous H_2O_2. Periplasmic CCPs have not been found in all gram-negative bacteria; their physiological role is not quite clear, especially in the presence of other peroxide-degrading enzymes in the cells: catalase and non-heme peroxidases, including alkyl hydroperoxide reductase, which reduces organic hydroperoxides to respective alcohols [92].

The genes encoding diheme CCP have been found in the genomes of morphotype I methanotrophs of the genera *Methylocaldum, Methylomicrobium, Methylococcus*, and *Methylobacter*, including those forming no S-layers, while type II methanotrophs (*Methylocystis* and *Methylosinus*) have only open reading frames with a 7% identity of the product with the 'CorB' protein. Acidophilic methanotrophs *Methylocella silvestris* BL2 and *Methylacidiphilum infernorum* V4 were shown to have no diheme CCP homologues [84].

The recombinant protein 'CorB' from *Mm. alcaliphilum* 20Z has a peroxide-degrading activity. Immunocytochemical analysis with gold-labeled antibodies showed the localization of 'CorB' in the periplasm [84]. On the contrary, in *Mc. capsulatus* Bath, the Mca protein was found at the external surface of CW, which does not quite conform to the periplasmic localization of other members of the family of diheme CCPs [89, 93].

The cells of the mutant *Mm. alcaliphilum* defective in the '*corB*' gene did not lose S-layers, more actively oxidized methane, and had a high growth rate on methane in the alkaline medium (pH 11), where wild-type cells did not grow [84]. Such phenotype of the 'CorB' mutant confirms involvement of diheme CCP in the regulation of the level of H_2O_2 (the key interme-

diate of methane monooxygenation). Methane oxidation by the MMO complex includes the stage of H_2O_2 formation, which is subsequently degraded in the active center, releasing the radicals directly participating in catalysis [94]. Obviously, the diheme CCP catalyzing H_2O_2 transformation to water controls the level of reactive oxygen species (ROS), e.g., during limitation by methane. In the CCP mutant, the level of radicals and the rate of CH_4 oxidation increased.

One more function of diheme CCP has been suggested for methanotrophs: tryptophan oxidation to kynurenine in the surface proteins MopE or 'CorA' [81]. This assumption is based on the similarity of the CCP structure with the diheme fragments of the MauG family characterized in methylamine-growing methylobacteria [95, 96]. The MauG protein is necessary for the biosynthesis of the functional methylamine dehydrogenase (MADH) and catalyzes the modification of tryptophan tryptophylquinone, the cofactor of MADH [95].

Based on the above, we proposed a hypothesis explaining the coexpression of 'CorA' and 'CorB' in the methanotrophs with S-layers. Diheme CCP ('CorB') modifies the 'CorA' (or MopE) protein by oxidizing tryptophan-130. It contributes to the binding and transport of Cu^{2+} ions to pMMO, probably with the involvement of other carrier proteins. In case of copper deficiency, the level of 'CorA' and 'CorB' expression increases, intensifying the cation transport to pMMO and providing the reaction of methane oxidation, whereas 'CorB' is a regulator of the H_2O_2 level. In the 'CorB'-lacking mutant, near the MMO active center (in the periplasm and, probably, ICM), the concentrations of H_2O_2 and, as a consequence, of oxygen radicals, increase, which is accompanied by a higher rate of methane monooxygenation [84]. In this case, the oxidation of tryptophan-130 to kynurenine (the necessary condition for Cu binding in the 'CorA' protein) is performed nonenzymatically due to the action of high ROS concentrations [89]. However, this hypothesis requires experimental confirmation.

It should be noted that 57 proteins containing the heme-binding motif were identified in the genome of *Mc. capsulatus* Bath; five of them were members of the cytochrome c_{553} family and four proteins have been pre-annotated as CCP [77, 93, 97]. However, only one CCP has been characterized: the enzyme is a homodimer with a subunit of 35.8 kDa, similar to most of the known proteins of this family [98].

The homeostasis of copper ions in methanotrophs involves a rather complicated system of integral outer membrane proteins, because several cell surface-bound cytochromes *c* have been revealed, with the level of their expression in *Mc. capsulatus* Bath regulated by the concentration of this cation. In addition, the copper level regulation in methanotrophs, including *Mc. capsulatus* Bath and *Mm. album* BG8, also involves a chromopeptide methanobactin (1.2 kDa), which is functionally analogous to Fe-siderophores pyoverdine and azotobactin [93, 100, 101]. Methanobactin (Mb) consisting of 7 amino acid residues and other functional groups forms a compact pyramid, in the base of which there is a chromophore, 4-thionyl-5-hydroxyimidazole, which binds copper ions in the stoichiometric ratio of 1 : 1 [98]. Cu-Mb shows an oxidase activity and catalyzes the dismutation of O_2^- to H_2O_2 and H_2O_2 reduction to H_2O [99]. Cu-Mb is therefore a redox-active molecule capable of superoxide and hydroperoxide detoxification without formation of hydroxyl radicals [102, 103]. It is supposed that Mb is involved in the regulation of pMMO activity in the periplasm and in the copper-dependent expression of soluble and particulate MMO forms in the cytoplasm [101].

Intriguingly, the alpha- and gamma-proteobacterial methanotrophs differ in the levels of extracellular Mb: *Ms. trichosporium* OB3b excretes more Mb into the medium than *Mc. capsulatus* Bath or *Mm. album* BG8 [104]. In addition, the Mb of *Mm. album* BG8 has a lower affinity to copper than the Mb of *Ms. trichosporium* OB3b. The methanobactins of methanotrophs also differ in molecular mass and spectral properties (UV absorption, fluorescence, and EPR spectra) [104]. The Mb from *Ms. trichosporium* OB3b can even remove Cu(I) from the Mb of gamma-proteobacterial methanotrophs. Consequently, this siderophore may be a factor regulating the composition of natural methanotrophic communities under conditions of copper deficiency. The available data demonstrate that methanobactins form a structurally diverse group of molecules [105].

In the absence of Cu(II) or Cu(I), Mb binds Ag(I), Au(III), Co(II), Cd(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(VI), or Zn(II), but not Ba(II), Ca(II), La(II), Mg(II), and Sr(II). The binding constant for these metals is lower than for Cu(II), and copper in Mb can replace other metals, except for Ag(I) and Au(III). The growth and activity of Mb-synthesizing methanotrophs may be accompanied by in situ solubilization or immobilization of many metals, determining their potential ecophysiological significance [106].

It is supposed that methanotrophs synthesize Mb from the peptides formed on ribosomes, and the gene encoding the peptide precursor for Mb was identified in the genome of *Ms. trichosporium* OB3b. Since Mb-like compounds were recently found in yeast mitochondria, these molecules are a more universal biological phenomenon than the peculiarity inherent in methanotrophs only [107].

The mechanisms of Cu-Mb absorption by methanotrophs are unknown. By analogy with the transport of other siderophores, Cu-Mb can be recognized by TonB receptors of the outer membrane and incorporated via the periplasmic binding proteins TonB-ExbB-ExbD and ABC transporters integrated into the

inner membrane, with at least one of them being specific to Cu-Mb [108].

It would be reasonable to suggest that Mb and the 'CorA'/'CorB' system have analogous functions: H_2O_2 detoxification and copper transport to MMO. Based on the experimental data it has been supposed that S-layers of methanotrophs screen the Cu-Mb transport into the cells [84]. As a consequence, it is necessary to realize a compensatory mechanism to maintain cation homeostasis and regulation of the H_2O_2 level. The 'CorA'/'CprB' tandem acts as such a mechanism. At the same time, while the important ecophysiological role of S-layers in methanotrophs is doubtless further investigation is required [84].

In conclusion, it should be noted that the cell envelope of aerobic methanotrophs is a brilliant example of multifunctionality of this structure performing the interaction between these bacteria and the environment. However, in spite of the obvious progress in the study of the surfaceome of *Mc. capsulatus* Bath as a model organism, it is necessary to answer a number of fundamental questions concerning the structural organization of CW in various methanotrophs and the functions of associated proteins. It is necessary to determine the gene sequences encoding the protein components of S-layers and to understand the following: (1) what are the peculiar features and mechanisms of interaction between Mb and CW components in the methanotrophs synthesizing S-layers; (2) what is the role of S-layers in the thermal and osmotic adaptation of these bacteria; and (3) why S-layers are absent in aerobic methylobacteria not growing on methane.

Thus, further systemic genoproteomic comparative research of the proteins of the surfaceome of different methanotrophs, including the assessment of strength of the promoters of the respective genes, are of undoubted interest due to the possibility of constructing new systems of heterologous expression of target peptides exposed at the surface of recombinant strains. It is especially promising due to nonpathogenicity of methanotrophs, their low protease activity, and the ability to reach high cell density during the cultivation on simple mineral media.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (project no. 10-04-01224-a) and the Federal Target Program "Scientific and Scientific–Pedagogical Personnel of Innovative Russia."

We are grateful to A.V. Machulin for his assistance in graphical drawing and to S.N. Dedysh and A.S. Reshetnikov for their valuable advice.

REFERENCES

1. Houwink, A.L., A macromolecular monolayer in the cell wall of *Spirillum* spec., *Biochim. Biophys. Acta*, 1953, vol. 10, pp. 360–366.
2. Baumeister, W. and Lembcke, G., Structural features of archaebacterial cell envelopes, *J. Bioenerg. Biomembr.*, 1992, vol. 24, pp. 567–575.
3. Messner, P. and Sleytr, U.B., Crystalline bacterial cell surface layers, *Adv. Microb. Physiol.*, 1992, vol. 3, pp. 213–275.
4. Sleytr, U.B. and Beveridge, T.J., Bacterial S-layers, *Trends Microbiol.*, 1999, vol. 7, pp. 253–260.
5. Engelhardt, H., Are S-layers exoskeletons? The basic function of protein surface layers revisited, *J. Struct. Biol.*, 2007, vol. 160, no. 2, pp. 115–124.
6. Messner, P., Schäffer, C., Egelseer, E.M., and Sleytr, U.B., Occurrence, structure, chemistry, genetics, morphogenesis, and function of S-layers, in *Prokaryotic Cell Wall Compounds—Structure and Biochemistry*, König, H., Claus, H., and Varma, A., Eds., Springer, 2010, pp. 53–109.
7. Sleytr, U.B., Huber, C., Ilk, N., Pum, D., Schuster, B., and Egelseer, E.M., S-Layers as a tool kit for nanobiotechnological applications, *FEMS Microbiol. Lett.*, 2007, vol. 267, pp. 131–144.
8. Severina, L.O., Bacterial S-layers, *Microbiology* (Moscow), 1995, vol. 64, no. 6, pp. 725–733.
9. Sleytr, U.B. and Sara, M., Bacterial and archaeal S-layer proteins: structure-function relationships and their biotechnological applications, *Trends Biotechnol.*, 1997, vol. 15, pp. 20–26.
10. Messner, P., Steiner, K., Zarschler, K., and Schaffer, C., S-Layer nanoglycobiology of bacteria, *Carbohydr. Res.*, 2008, vol. 343, no. 12, pp. 1934–1951.
11. Jarrell, K.F., Jones, G.M., Kandiba, L., Nair, D.B., and Eichler, J., S-Layer glycoproteins and flagellins: reporters of archaeal posttranslational modifications, *Archaea*, 2010, pp. 948–956.
12. Fernández, L.A. and Berenguer, J., Secretion and assembly of regular surface structures in Gram-negative bacteria, *FEMS Microbiol. Rev.*, 2000, vol. 24, no. 1, pp. 21–44.
13. Boot, H.J., Kolen, C.P., and Pouwels, P.H., Identification, cloning, and nucleotide sequence of a silent S-layer protein gene of *Lactobacillus acidophilus* ATCC 4356 which has extensive similarity with the S-layer protein gene of this species, *J. Bacteriol.*, 1999, vol. 177, pp. 7222–7230.
14. Palva, A., Molecular biology of the *Lactobacillus brevis* S-layer gene (*slp*A), *FEMS Microbiol. Rev.*, 1997, vol. 20, pp. 83–88.
15. Fernández-Herrero, L.A., Olabarria, G., and Berenguer, J., Surface proteins and a novel transcription factor regulate the expression of the S-layer gene in *Thermus thermophilus* HB8, *Mol. Microbiol.*, 1997, vol. 24, no. 1, pp. 61–72.
16. Thompson, S.A., *Campylobacter* surface-layers (S-layers) and immune evasion, *Ann. Periodontol.*, 2002, vol. 7, no. 1, pp. 43–53.
17. Messner, P., Bock, K., Christian, R., Schulz, G., and Sleytr, U.B., Crystalline bacterial cell surface layers, *Adv. Microb. Physiol.*, 1992, vol. 3, pp. 213–275.
18. Messner, P., Bock, K., Christian, R., Schulz, G., and Sleytr, U.B., Characterization of the surface layer gly-

coprotein of *Clostridium symbiosum* HB25, *J. Bacteriol.*, 1990, vol. 172, pp. 2576–2583.

19. Paul, G., Lompeich, F., and Wieland, F., Asparaginyl-*N*-acetylgalactosamine. Linkage unit of halobacterial glycosaminoglycan, *J. Biol. Chem.*, 1986, vol. 261, pp. 1020–1024.
20. Claus, H., Akça, E., Deaerdemaeker, T., Evrard, C., Declercq, J.-P., Harris, J.R., Schlott, B., and König, H., Molecular organization of selected prokaryotic S-layer proteins, *Can. J. Microbiol.*, 2005, vol. 51, pp. 731–743.
21. Phipps, B., Huber, R., and Baumeister, W., The cell envelope of the hyperthermophilic archaebacterium *Pyrobaculum organothrophum* consists of two regularly arrayed protein layers: three-dimensional structure of the outer layer, *Mol. Microbiol.*, 1991, vol. 5, pp. 253–265.
22. Navarre, W.W. and Schneewind, O., Surface protein of gram-positive bacteria and mechanisms of their targeting to the cell envelope, *Microbiol. Mol. Biol. Rev.*, 1999, vol. 63, pp. 174–182.
23. Engelhardt, H. and Peters, J.J., Structural research on surface layers—A focus on stability, surface layer homology domains, and surface layer-cell wall interactions, *J. Struct. Biol.*, 1998, vol. 124, pp. 276–302.
24. Sara, M., Conserved anchoring mechanisms between crystalline cell surface S-layer proteins and secondary cell wall polymers in Gram-positive bacteria, *Trends Microbiol.*, 2001, vol. 9, pp. 47–49.
25. Leibovitz, E., Lemaire, M., and Miras, I., Occurence and function of a common domain in S-layer and other exocellular proteins, *FEMS Microbiol. Rev.*, 1997, vol. 20, pp. 127–133.
26. Kuen, B., Koch, A., Asenbauer, E., Sara, M., and Lubitz, W., Molecular characterization of the second S-layer gene *sbsB* of *Bacillus stearothermophilus* PV72 expressed by oxidative stress, *J. Bacteriol.*, 1997, vol. 179, pp. 1664–1670.
27. Callegari, L., Riboli, B., Sanders, W., Coconelli, P., Kok, J., Venema, G., and Morelli, L., The S-layer gene of *Lactobacillus helveticus* CNRZ 892: cloning, sequencing and heterologous expression, *Microbiology* (UK), 1998, vol. 144, pp. 719–726.
28. Chami, M., Bayan, N., and Peyret, J.L., The S-layer protein of *Corynebacterium glutamicum* is anchored to the cell wall by its C-terminal hydrophobic domain, *Mol. Microbiol.*, 1997, vol. 23, pp. 483–492.
29. Walker, S.G., Karunaratne, D.N., Ravenscroft, N., and Smit, J., Characterization of mutants of *Caulobacter crescentus* defective in surface attachment of the paracystalline surface layer, *J. Bacteriol.*, 1994, vol. 176, pp. 6321–6323.
30. Dworkin, J., Tummuru, M.K., and Blaser, M.J., A lipopolysaccharide-binding domain of the *Campylobacter fetus* S-layer protein resides within the conserved N terminus of a familiy of silent and divergent homologs, *J. Bacteriol.*, 1995, vol. 177, pp. 1734–1741.
31. Nomellini, J.F., Kupcu, S., Sleytr, U.B., and Smit, J., Factors controlling in vitro recrystallization of the *Caulobacter crescentus* paracrystalline S-layer, *J. Bacteriol.*, 1997, vol. 179, pp. 6349–6354.
32. Ford, M.J., Nomellini, J.F., and Smit, J., S-Layer anchoring and localization of an S-layer-associated protease in *Caulobacter crescentus*, *J. Bacteriol.*, 2007, vol. 189, pp. 2226–2237.
33. Peters, J., Peters, M., Lottspeich, F., Schäfer, W., and Baumeister, W., Nucleotide sequence analysis of the gene encoding the *Deinococcus radiodurans* surface protein, derived amino acid sequence, and complementary protein chemical studies, *J. Bacteriol.*, 1987, vol. 169, pp. 5216–5223.
34. Hansmeier, N., Bartels, F.W., Ros, R., Anselmetti, D., Tauch, A., Pühler, A., and Kalinowski, J., Classification of hyper-variable *Corynebacterium glutamicum* surface-layer proteins by sequence analyses and atomic force microscopy, *J. Biotechnol.*, 2004, vol. 112, pp. 177–193.
35. Baumeister, W. and Lembcke, G., Structural features of archaebacterial cell envelopes, *J. Bioenerg. Biomembr.*, 1992, vol. 24, pp. 567–575.
36. Stetefeld, J., Jenny, M., Schulthess, T., Landwehr, R., Engel, J., and Kammerer, R.A., Crystal structure of a naturally occuring parallel right-handed coiled coil tetramer, *Nat. Struct. Biol.,* 2000, vol. 7, pp. 772–776.
37. Sleytr, U.B., Sára, M., Pum, D., and Schuster, B., Characterization and use of crystalline bacterial cell surface layers, *Prog. Surf. Sci.*, 2001, vol. 68, pp. 231–278.
38. Jensen, M.O. and Mouritsen, O.G., Lipids do influence protein function—the hydrophobic matching hypothesis revisited, *Biochim. Biophys. Acta*, 2004, vol. 1666, pp. 205–226.
39. Pavkov-Keller, T., Howorka, S., and Keller, W., The structure of bacterial S-layer proteins, *Progress Mol. Biol*,, 2011, vol. 103, pp. 73–130.
40. Lasa, I., de Grado, M., de Pedro, M.A., and Berenguer, J., Development of *Thermus-Escherichia* shuttle vectors and their use for expression of the *Clostridium thermocellum celA* gene in *Thermus thermophilus, J. Bacteriol.*, 1992, vol. 174, no. 20, pp. 6424–6431.
41. Howorka, S., Sara, M., Wang, Y., Kueni, B., Sleytr, U.B., Lubitz, W., and Bayley, H., Surface-accessible residues in the monomeric and assembled forms of a bacterial surface layer protein, *J. Biol. Chem.*, 2000, vol. 275, pp. 37876–37886.
42. Eichler, J., Post-translational modification of the S-layer glycoprotein occurs following translocation across the plasma membrane of the haloarchaeon *Haloferax volcanii, Eur. J. Biochem.*, 2001, vol. 268, pp. 4366–4374.
43. Matias, V.R.F. and Beveridge, T.J., Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space, *Mol. Microbiol.*, 2005, vol. 56, pp. 240–251.
44. Matias, V.R.F. and Beveridge, T.J., Native cell wall organization shown by cryo-electron microscopy confirms the existence of a periplasmic space in *Staphylococcus aureus, J. Bacteriol.*, 2006, vol. 188, pp. 1011–1021.
45. Beveridge, T.J. and Graham, L.L., Surface layers of bacteria, *Microbiol. Rev.*, 1991, vol. 55, pp. 684–705.
46. Peters, J., Baumeister, W., and Lupas, A., Hyperthermostable surface layer protein tetrabrachion from the

archaebacterium *Staphylothermus marinus*: evidence for the presence of a right-handed coiled coil derived from the primary structure, *J. Mol. Biol.*, 1996, vol. 257, no. 5, pp. 1031–1041.

47. Koval, S.F., The effect of S-layers and cell surface hydrophobicity on prey selection by bacterivorous protozoa, *FEMS Microbiol. Rev.*, 1997, vol. 20, pp. 138–142.
48. Smarda, J., Smajs, D., Komrska, J., and Krzyzánek, V., S-Layers on cell walls of cyanobacteria, *Micron*, 2002, vol. 33, no. 3, pp. 257–277.
49. Beveridge, T.J., Pouwels, P., Sara, M., Kotiranta, A., and Launatmaa, K., Functions of S-layers, *FEMS Microbiol. Rev.,* 1997, vol. 20, pp. 99–119.
50. Fjellbirkeland, A., Kleivdal, H., Joergensen, C., Thestrup, H., and Jensen, H.B., Outer membrane proteins of *Methylococcus capsulatus* (Bath), *Arch. Microbiol.*, 1997, vol. 168, pp. 128–133.
51. Couture-Tosi, E., Delacroix, H., Mignot, T., Mesnage, S., Chami, M., Fouet, A., and Mosser, G., Structural analysis and evidence for dynamic emergence of *Bacillus anthracis* S-layer networks, *J. Bacteriol.*, 2002, vol. 184, pp. 6448–6456.
52. Austin, J.W., Engel, A., Murray, R.G.E., and Aebi, U., Structural analysis of the S-layer of *Lampropedia hyaline, J. Ultrastruct. Mol. Struct. Res.*, 1989, vol. 102, pp. 255–264.
53. Smith, S.H. and Murray, R.G.E., The structure and associations of the double S-layer on the cell wall of *Aquaspirillum sinuosum, Can. J. Microbiol.*, 1990, vol. 36, pp. 327–335.
54. Jarrell, K.F., Jones, G.M., Kandiba, L., Nair, D.B., and Eichler, J., S-Layer glycoproteins and flagellins: reporters of archaeal posttranslational modifications, *Archaea*, 2010, pp. 948–956.
55. Ilk, N., Vollenkle, C., and Edelseer, E.M., Molecular characterization of the S-layer gene *sbpA* of *Bacillus sphaericus* CCM2177 and production of functional S-layer fusion protein with ability to recrystallize in a defended orientation while presented the fused allergen, *Appl. Environ. Microbiol.*, 2002, vol. 68, pp. 3251–3260.
56. Debabov, V.G., Bacterial and archaeal S-layers as the subject of nanobiotechnology, *Mol. Biol.* (Moscow), 2004, vol. 38, pp. 482–493.
57. Gal'chenko, V.F., *Metanotrofnye bakterii* (Methanotrophic Bacteria), Moscow: GEOS, 2001.
58. Trotsenko, Yu.A. and Khmelenina, V.N., *Ekstremofil'nye methanotrofy* (Extremophilic Methanotrophs), Galchenko, V.F., Ed., Pushchino: ONTI PSC RAS, 2008.
59. Suzina, N.E. and Fikhte, B.A., *Ul'trastrukturnaya organizatsiya methanotrofnykh bakterii* (Ultrastructural Organization of Methanotrophic Bacteria), Pushchino: ONTI NTsBI AS USSR, 1986.
60. Haubold, R., Two different types of surface structures of methane utilizing bacteria, *Z. Allg. Microbiol.*, 1978, vol. 18, pp. 511–515.
61. Tyurin, V.S., Gorskaya, L.A., Kaftanova, A.S., Loginova, T.M., and Mikhailov, A.M., Some features of the ultrastructure of *Methylococcus capsulatus* grown under different conditions, *Prikl. Biokhim. Mikrobiol.,* 1985, vol. 21, no. 6, pp. 770–775.
62. Jeffries, P. and Wilkinson, J.F., Electron microscopy of the cell wall complex of *Methylomonas albus, Arch. Microbiol.*, 1978, vol. 119, no. 2, pp. 227–229.
63. Berson, O. and Lidstrom, M., Cloning and characterization of *corA*, a gene encoding a copper-repressible polypeptide in the type I methanotroph, *Methylomicrobium albus* BG8, *FEMS Microbiol. Lett.*, 1997, vol. 148, pp. 169–174.
64. Khmelenina, V.I., Kalyuzhnaya, M.G., Starostina, N.G., Suzina, N.E., and Trotsenko, Y.A., Isolation and characterization of halotolerant alkaliphilic methanotrophic bacteria from Tuva soda lakes, *Curr. Microbiol.*, 1997, vol. 35, pp. 257–261.
65. Khmelenina, V.N., Kalyuzhnaya, M., Sakharovski, V.G., Suzina, N.E., Trotsenko, Y.A., and Gottschalk, G., Osmoadaptation in halophilic and alkaliphilic methanotrophs, *Arch. Microbiol.*, 1999, vol. 172, no. 5, pp. 321–329.
66. Khmelenina, V.N., Shchukin, V.N., Reshetnikov, A.S., Mustakhimov, I.I., Suzina, N.E., Eshinimaev, B.Ts., and Trotsenko, Yu.A., Structural and functional features of methanotrophs from hypersaline and alkaline lakes, *Microbiology* (Moscow), 2010, vol. 79, no. 4, pp. 472–482.
67. Sorokin, D.Y., Jones, B.E., and Kuenen, J.G., A novel obligately methylotrophic, methane-oxidizing *Methylomicrobium* species from a highly alkaline environment, *Extremophiles,* 2000, vol. 4, pp. 145–155.
68. Kalyuzhnaya, M.G., Khmelenina, V.N., Starostina, N.G., Baranova, S.V., Suzina, N.E., and Trotsenko, Yu.A., A new moderately halophilic methanotroph of the genus *Methylobacter, Microbiology* (Moscow), 1998, vol. 67, no. 4, pp. 438–444.
69. Pfenning, N. and Cohen-Basire, G., Some properties of the green bacterium *Pelodyction clathratiforme, Arch. Microbiol.*, 1967, vol. 59, pp. 226–236.
70. Remsen, C.C., Watson, S.W., and Trueper, H.G., Macromolecular subunits in the wall of marine photosynthetic bacteria, *J. Bacteriol.*, 1970, vol. 103, no. 1, pp. 255–258.
71. Hageage, D.J. and Gherna, R.L., Surface structure of *Chromatium okenii* and *Chromatium weissei, J. Bacteriol.*, 1971, vol. 106, no. 2, pp. 687–690.
72. Watson, S.W. and Remsen, C.C., Macromolecular subunits in the walls of marine nitrifying bacteria, *Science*, 1969, vol. 163, pp. 685–686.
73. Ridgway, H.F., Wagner, R.M., Dawsey, W.T., and Lewin, R.A., Fine structure of the cell envelope of *Flexibacter polymorphus, Can. J. Microbiol.*, 1975, vol. 21, no. 11, pp. 1733–1750.
74. Kalyuzhnaya, M.G., Reshetnikov, A.S., Khmelenina, V.N., Suzina, N.E., and Trotsenko, Y.A., The S-layers of haloalkaliphilic methanotroph *Methylomicrobium alcaliphilus* 20Z: isolation and structure-functional characterization, Abstr. Int. Symp. "Modern Problems of Microbial Biochemistry and Biotechnology," Pushchino, 2000, p. 66.
75. Eshinimaev, B.Ts., Khmelenina, V.N., Sacharovski, V.G., Suzina, N.E., and Trotsenko, Yu.A., Physiological, biochemical, and cytological characteristics of

a haloalkalitolerant methanotroph grown on methanol, *Microbiology* (Moscow), 2002, vol. 71, no. 5, pp. 512–518.

76. Kalyuzhnaya, M., Khmelenina, V.N., Eshinimaev, B., Suzina, N., Nikitin, D.N., Solonin, A., Lin Ji-Ring, McDonald, I.R., Murrell, J.C., and Trotsenko, Y.A., Taxonomic characterization of new alkaliphilic and alkalitolerant methanotrophs from soda lakes of the Southeastern Transbaikal region and description of *Methylomicrobium buryatense* sp. nov., *Syst. Appl. Microbiol.*, 2001, vol. 24, no. 2, pp. 166–176.
77. Ward, N., Larsen, O., Sakwa, J., Bruseth, L., Khouri, H., Durkin, A.S., Dimitrov, G., Jiang, L., Scanlan, D., Kang, K.H., Lewis, M., Nelson, K.E., Methe, B., Wu, M., Heidelberg, J.F., Paulsen, I.T., Fouts, D., Ravel, J., Tettelin, H., Ren, Q., Read, T., DeBoy, R.T., Seshadri, R., Salzberg, S.L., Jensen, H.B., Birkeland, N.K., Nelson, W.C., Dodson, R.J., Grindhaug, S.H., Holt, I., Eidhammer, I., Jonasen, I., Vanaken, S., Utterback, T., Feldblyum, T.V., Fraser, C.M., Lillehaug, J.R., and Eisen, J.A., Genomic insights into methanotrophy: the complete genome sequence of *Methylococcus capsulatus* (Bath), *PLoS Biol.,* 2004, vol. 2, pp. 1616–1628.
78. Jahnke, L.L., The effect of growth temperature on the methyl sterol and phospholipid fatty acid composition of *Methylococcus capsulatus* (Bath), *FEMS Microbiol. Lett.*, 1992, vol. 93, pp. 209–212.
79. Loffler, C., Eberlein, C., Mausezahl, I., Kappelmeyer, U., and Heipieper, H.J., Physiological evidence for the presence of a *cis–trans* isomerase of unsaturated fatty acids in *Methylococcus capsulatus* Bath to adapt to the presence of toxic organic compounds, *FEMS Microbiol. Lett.*, 2010, vol. 308, pp. 68–75.
80. Scott, J.R.H.-C.S., Surface proteins of gram-positive bacteria and how they get there, *Annu. Rev. Microbiol.*, 2006, vol. 60, pp. 397–402.
81. Karlsen, O.A., Larsen, O., and Jensen, H.B., The copper responding surfaceome of *Methylococccus capsulatus* Bath, *FEMS Microbiol. Lett.*, 2011, vol. 323, no. 2, pp. 97–104.
82. Fjellbirkeland, A., Kleivdal, H., Joergensen, C., Thestrup, H., and Jensen, H.B., Outer membrane proteins of *Methylococcus capsulatus* (Bath), *Arch. Microbiol.*, 1997, vol. 168, pp. 128–133.
83. Fjellbirkeland, A., Kruger, P.G., Bemanian, V., Hogh, B.T., Murrell, C.J., and Jensen, H.B., The C-terminal part of the surface-associated protein MopE of the methanotroph *Methylococcus capsulatus* (Bath) is secreted into the growth medium, *Arch. Microbiol.*, 2001, vol. 176, pp. 197–203.
84. Shchukin, V.N., Khmelenina, V.N., Eshinimaev, B.Ts., Suzina, N.E., and Trotsenko, Yu.A., Primary characterization of dominant cell surface proteins of halotolerant methanotroph *Methylomicrobium alcaliphilum* 20Z, *Microbiology* (Moscow), 2011, vol. 80, no. 5, pp. 608–618.
85. Helland, R., Fjellbirkeland, A., Karlsen, O.A., Ve, T., Lillehaug, J., and Jensen, H.B., An oxidized tryptophan facilitates copper binding in *Methylococcus capsulatus-secreted* protein MopE, *J. Biol. Chem.*, 2008, vol. 283, no. 20, pp. 897–904.
86. Karlsen, O.A., Larsen, O., and Jensen, H.B., Identification of a bacterial di-heme cytochrome *c* peroxidase from *Methylomicrobium album* BG8, *Microbiology*, 2010, vol. 10, pp. 2682–2690.
87. Silver, S., Active transport of magnesium in *Escherichia coli, Proc. Natl. Acad. Sci. USA*, 1969, vol. 62, pp. 764–771.
88. Niegowski, D. and Eshaghi, S., The CorA family: structure and function revisited, *Cell Mol. Life Sci.*, 2007, vol. 64, pp. 2564–2574.
89. Karlsen, O.A., Kindingstad, L., Angelska, S.M., Bruseth, L.J., Straume, D., Puntervoll, P., Fjellbirkeland, A., Lillehaug, J.R., and Jensen, H.B., Identification of a copper-repressible C-type heme protein of *Methylococcus capsulatus* (Bath). A member of a novel group of the bacterial di-heme cytochrome *c* peroxidase family of proteins, *FEBS Lett.*, 2005, vol. 272, pp. 6324–6335.
90. Fulop, V., Ridout, C.J., Greenwood, C., and Hajdu, J., Crystal structure of the di-haem cytochrome *c* peroxidase from *Pseudomonas aeruginosa, Structure*, 1995, vol. 3, pp. 1225–1233.
91. Minard, K.I. and McAlister-Henn, L., Antioxidant function of cytosolic sources of NADPH in yeast, *Free Radic. Biol. Med.*, 2001, vol. 31, pp. 832–843.
92. Kelly, D.J., The physiology and metabolism of *Campylobacter jejuni* and *Helicobacter pylori, J. Appl. Microbiol.*, 2001, vol. 90, pp. 16–24.
93. Karlsen, O.A., Lillehaug, J.R., and Jensen, H.B., The presence of multiple *c*-type cytochromes at the surface of the methanotrophic bacterium *Methylococcus capsulatus* (Bath) is regulated by copper, *Mol. Microbiol.*, 2008, vol. 70, no. 1, pp. 15–26.
94. Choi, D.W., Kunz, R.C., Boyd, E.S., Semrau, J.D., Antholine, W.E., and Han, J.I., The membrane-associated methane monooxygenase (pMMO) and pMMO-NADH:quinone oxidoreductase complex from *Methylococcus capsulatus* (Bath), *J. Bacteriol.*, 2003, vol. 185, pp. 5755–5764.
95. Chistoserdov, A.Y., Chistoserdova, L.V., McIntire, W.S., and Lidstrom, M.E., Genetic organization of the *mau* gene cluster in *Methylobacterium extorquens* AM1: complete nucleotide sequence and generation and characteristics of *mau* mutants, *J. Bacteriol.*, 1994, vol. 176, pp. 4052–4065.
96. Wang, Y., Li, X., Jones, L.H., Pearson, A.R., Wilmot, C.M., and Davidson, V.L., MauG-dependent in vitro biosynthesis of tryptophan tryptophylquinone in methylamine dehydrogenase, *J. Am. Chem. Soc.*, 2005, vol. 127, pp. 8258–8259.
97. Bergmann, D.J., Zahn, J.A., and DiSpirito, A.A., High molecular-mass multi-*c*-heme cytochromes from *Methylococcus capsulatus* (Bath), *J. Bacteriol.*, 1999, vol. 181, pp. 991–997.
98. Zahn, J.A., Bergman, D.J., Kunz, J.M., and DiSpirito, A.A., Membrane-associated quinoprotein formaldehyde dehydrogenase from *Methylococcus capsulatus* (Bath), *J. Bacteriol.*, 2001, vol. 183, pp. 6832–6840.
99. Zahn, J.A. and DiSpirito, A.A., Membrane-associated methane monooxygenase from *Methylococcus capsu-*

latus (Bath), *J. Bacteriol.*, 1996, vol. 178, pp. 1018–1029.

100. Kim, H.J., Graham, D.W., and DiSpirito, A.A., Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria, *Science*, 2004, vol. 305, pp. 1612–1615.
101. Choi, D.W., Antholine, W.E., and Do, Y.S., Effect of methanobactin on the activity and electron paramagnetic resonance spectra of the membrane-associated methane monooxygenase in *Methylococcus capsulatus* Bath, *Microbiology* (UK), 2005, vol. 151, pp. 3417–3426.
102. Choi, D.W., Semrau, J.D., and Antholine, W.E., Oxidase, superoxide dismutase, and hydrogen peroxide reductase activities of methanobactin from types I and II methanotrophs, *J. Inorg. Biochem.*, 2008, vol. 102, pp. 1571–1580.
103. Kim, H.J., Galeva, N., Larive, C.K., Alterman, M., and Graham, D.W., Purification and physical-chemical properties of methanobactin: a chalkophore from *Methylosinus trichosporium* OB3b, *Biochemistry*, 2005, vol. 44, pp. 5140–5148.
104. Choi, D.W., Bandow, N.L., and McEllistrem, M.T., Spectral and thermodynamic properties of methanobactin from gamma-proteobacterial methane oxidizing bacteria: a case for copper competition on a molecular level, *J. Inorg. Biochem.*, 2010, vol. 104, pp. 1240–1247.
105. Semrau, J.D., DiSpirito, A.A., and Yoon, S., Methanotrophs and copper, *FEMS Microbiol. Rev.*, 2010, vol. 34, pp. 496–531.
106. Choi, D.W., Do, Y.S., Zea, C.J., McEllistrem, M.T., Lee, S.W., Semrau, J.D., Pohl, N.L., Kisting, C.J., Scardino, L.L., Hartsel, S.C., Boyd, E.S., Geesey, G.G., Riedel, T.P., Shafe, P.H., Kranski, K.A., Tritsch, J.R., Antholine, W.E., and DiSpirito, A.A., Spectral and thermodynamic properties of Ag(I), Au(III), Cd(II), Co(II), Fe(III), Hg(II), Mn(II), Ni(II), Pb(II), U(IV), and Zn(II) binding by methanobactin from *Methylosinus trichosporium* OB3b, *J. Inorg. Biochem.*, 2006, vol. 100, no. 12, pp. 2150–2161.
107. Krentz, B.D., Mulheron, H.J., Semrau, J.D., Dispirito, A.A., Bandow, N.L., Haft, D.H., Vuilleumier, S., Murrell, J.C., McEllistrem, M.T., Hartsel, S.C., and Gallagher, W.H., A comparison of methanobactins from *Methylosinus trichosporium* OB3b and *Methylocystis* strain Sb2 predicts methanobactins are synthesized from diverse peptide precursors modified to create a common core for binding and reducing copper ions, *Biochemistry*, 2010, vol. 49, no. 7, pp. 10117–10130.
108. Schauer, K., Gouget, B., Carrière, M., Labigne, A., and de Reuse, H., Novel nickel transport mechanism across the bacterial outer membrane energized by the TonB/ExbB/ExbD machinery, *Mol. Microbiol.*, 2007, vol. 63, no. 4, pp. 1054–1068.

Translated by E. Makeeva