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Primary Structure of Selected Archaeal Mesophilic and Extremely Thermophilic Outer Surface Layer Proteins

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Summary

The archaea are recognized as a separate third domain of life together with the bacteria and eucarya. The archaea include the methanogens, extreme halophiles, thermoplasmas, sulfate reducers and sulfur metabolizing thermophiles, which thrive in different habitats such as anaerobic niches, salt lakes, and marine hydrothermals systems and continental solfataras. Many of these habitats represent extreme environments in respect to temperature, osmotic pressure and pH-values and remind on the conditions of the early earth. The cell envelope structures were one of the first biochemical characteristics of archaea studied in detail. The most common archaeal cell envelope is composed of a single crystalline protein or glycoprotein surface layer (S-layer), which is associated with the outside of the cytoplasmic membrane. The S-layers are directly exposed to the extreme environment and can not be stabilized by cellular components. Therefore, from comparative studies of mesophilic and extremely thermophilic S-layer proteins hints can be obtained about the molecular mechanisms of protein stabilization at high temperatures. First crystallization experiments of surface layer proteins under microgravity conditions were successful. Here, we report on the biochemical features of selected mesophilic and extremely archaeal S-layer (glyco-) proteins.

Keywords : Archaea ; cell walls ; S-layer ; (glyco-)proteins ; protein structure

INTRODUCTION

During evolution cellular organisms developed cell walls which improved the rigidity and integrity of their cells and probably enabled them to colonize new biotopes. Regular crystalline surface layers (S-layers) are widespread among prokaryotes and it seems that they are probably the earliest cell wall structures (BAUMEISTER and LEMBCKE, 1992; BEVERIDGE and GRAHAM, 1991; ENGEL-HARDT and PETERS, 1998; KANDLER and KÖNIG, 1985, 1993; KÖNIG and MESSNER, 1997; MESSNER and SLEYTR, 1992; SUMPER and WIELAND, 1995). The crystallization on the cell surface is an entropy driven process and requires no enzymatic acitivity. Two dimensional crystalline protein or glycoprotein arrays (S-layers) represent the outermost cell wall layer in about 350 different so far investigated species of the prokaryotic domains bacteria and archaea. S-layers consist of single (glyco-)protein species with molecular masses ranging from about 40 to 170 kDa, which form lattices of oblique, tetragonal or hexagonal architecture. Depending on the growth conditions some microorganisms can also produce different surface proteins (BAUMEISTER and LEMBCKE, 1992; BEVERIDGE and KOVAL, 1993; BOOT and POUWELS, 1996; EGELSEER et al., 2001; KÖNIG and MESSNER, 1997; MESSNER and SLEYTR, 1992). Structural differences might be found as a consequence of post-translational modifications such as glycosylation, transfer of phosphate and sulfate groups or proteolytic processing of the proteins (SÁRA and SLEYTR, 2000; SCHÄFER and MESSNER, 2001; SLEYTR, 1997). With a few exceptions, which include lac-tobacilli and the archaea *Methanothermus fervidus* and *Methanothermus sociabilis* (BRÖCKL et al., 1991), S-layer proteins have weakly acidic isoelectric points (SÁRA and SLEYTR, 2000; SLEYTR, 1997).

Depending on their location on the cell surface and their stability S-layers fulfill quite different functions. They form protective coats, maintain the shape and direct cell division, function as molecular sieves and attachment sites for extracellular enzymes and, represent virulence factors (BEVERIDGE et al., 1997, SLEYTR and BEVERIDGE, 1999). Representing the outermost cell envelope layer they are directly exposed to the often extreme conditions of their environment. As a consequence, intrinsic resistances against environmental stresses like high salt, acidity and temperature should be attributed to archaeal S-layers. The molecular mechanisms for this high stability are

only poorly understood. For example, members of the genus *Metbanococcus* living in mesophilic, thermophilic or extremely thermophilic environments represent an ideal model system for comparative analyses of their S-layers as a focus for thermal adaption (AKÇA et al., 2002). It became obvious that the diversity of the archaea is also reflected in a remarkable diversity of cell envelope types (Table 1). It was the aim of this study to compare the biochemical characteristics of selected archaeal S-layer proteins living under different environmental conditions. The knowledge of the threedimensional structure is a prerequisite to understand the molecular mechanisms of protein stabilization under extreme conditions. Therefore, we decided to propose this proteins for microgravity crystallization experiments.

Table 1. *Diversity of archaeal cell envelope types.*

Selected Archaeal Genera	Cell Envelope Types	References (selection)
Extreme Halophiles		
Haloarcula	S-layer	WAKAI et al., 1997
Halobacterium	S-layer	LECHNER and SUMPER, 1987
Halococcus	heteropolysaccharide	SCHLEIFER et al., 1982
Haloferax	S-layer	SUMPER et al., 1990
Natronococcus	glutaminylglycan	NIEMETZ et al., 1997
Methanogens		
Methano bacterium	pseudomurein/	KANDLER and KÖNIG, 1978
	gene of S-layer protein	AKÇA et al., 2002
Methanobrevibacter	pseudomurein	KANDLER and KÖNIG, 1978
Methanococcus	S-layer	AKÇA et al., 2002
Methanocorpusculum	S-layer	ZELLNER et al., 1987
Methanoculleus	S-layer	BAYLEY and KOVAL, 1994
Methanogenium	S-layer	KANDLER and KÖNIG, 1978
Methanomicrobium	S-layer	KANDLER and KÖNIG, 1978
Methanolacinia	S-layer	ZELLNER et al., 1989
Methanolobus	S-layer	KÖNIG and STETTER, 1982
Methanoplanus	S-layer	WTILDGRUBER et al., 1982
Methanopyrus	pseudomurein	Kurr et al., 1991
Methanosaeta	sheath	PATEL AND SPROTT, 1990
Methanothrix	sheath	KANDLER and KÖNIG, 1985
Methanosarcina	methanochondroitin/	KREISL and KANDLER, 1986
	S-layer	MAYERHOFER et al., 1998
Methanosphaera	pseudomurein	KÖNIG, 1986
Methanospirillum	sheath	SOUTHAM et al., 1993
Methanothermus	pseudomurein/	STETTER et al., 1981
	S-layer	BRÖCKL et al., 1991
Sulfate reducers		
Archaeoglobus	S-layer	STETTER, 1992
Extremely thermophilic sulfur metabolizers		
Acidianus	S-layer	BAUMEISTER et al., 1991
Desulfurococcus	S-layer	WILDHABER et al., 1987
Hyperthermus	S-layer	ZILLIG et al., 1990
Pyrodictium	S-layer	STETTER et al., 1983
Pyrobaculum	S-layer	PHIPPS et al., 1991
Pyrococcus	S-layer	FIALA and STETTER, 1986
Staphylothermus	S-layer	PETERS et al., 1995
Sulfolobus	S-layer	DEATHERAGE et al., 1983
Phermoproteus	S-layer	MESSNER et al., 1986
Thermoplasmas		
Phermoplasma	Glycocalyx	YANG and HAUG, 1979

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COMPARISON OF S-LAYERS OF MESOPHILIC, THERMOPHILIC AND EXTREMELY THERMOPHILIC ARCHAEA

S-layer proteins of *Methanococcus vannielii* (T_{opt} = 37 °C), *Methanococcus thermolithotrophicus* (T_{opt} = 65 °C) and *Methanococcus jannaschii* (T_{opt} = 85 °C) with the prominent bands of 60 kDa, 82 kDa and 80 kDa, respectively, on SDS-PAGE were investigated. (Table 2; AKÇA et al., 2002). Despite the high overall homology of the nucleotide sequences of the genes of the methanococcal S-layer proteins the deduced amino acid composition displayed some noteworthy differences (Table 3). In the mesophilic (*Methanococcus voltae, Methanococcus vannielii*) and thermophilic (*Methanococcus thermolithotrophicus*) methanococci e.g. the nonpolar amino acid alanine is the most abundant amino acid, whereas in the extreme thermophile *Methanococcus jannaschii* it is the acidic amino acid aspartic acid. In the latter species lysine residues mainly localized in non-conserved positions are also found in significant higher amounts compared to the mesophilic ones (Table 3). The occurrence of the amino acids cysteine and histidine is characteristic for the S-layer protein of *Methanococcus jannaschii* and they are not present in the other methanococci. Although no glycan residues could be detected by periodate-Schiff staining (PAS) in the S-layer proteins of methanococci, two potential N-glycosylation sites were found in the mesophilic methanogen *Methanococcus voltae,* five in the thermophilic methanogen *Methanococcus thermolithotrophicus* and eight in the extreme thermophilic species *Methanococcus jannaschii,* while sequon structures (N-glycosylation sites) were missing in *Methanococcus vannielii.* The sequon structures are located nearly at the same positions in the methanococcal S-layer proteins. A unique feature of the S-layer sequences investigated in this study is a putative Ca^{++}-binding site in *Methanococcus jannaschii* predicted by the amino acid sequence DLDSGDEVDILDY.

The overall amino composition (Table 4) of archaeal S-layer proteins is characterized by the predominance of nonpolar amino acids (exception: *Methanosarcina mazei),* followed by polar and acid amino acids and a lower content of basic ones. An increase of charged residues is a common feature of the thermophilic and extremely thermophilic species compared to their mesophilic counterparts. Generally, the amount of glutamine and asparagine decreases in the thermophiles compared to the mesophiles (DAS and GERSTEIN, 2000). An exception are the S-layer glycoproteins of *Methanothermus fervidus* and *Mt. sociabilis,* which have a high content of asparagine (BRÖCKL et al., 1991). The S-layer proteins of the two mesophilic methanococci have more nonpolar residues resulting in a higher degree of hydropathic-ity than the corresponding (extremely) thermophilic methanococcal proteins. In this respect, the S-layer protein of the extreme thermophile *Staphylothermus marinus* revealed the highest hydropathicity value and that of the extreme halophile *Halobacterium halobium* the lowest one.

The calculated acidic isolelectric point (4.27) of the S-layer polypeptide from *Methanococcus jannaschii* (BULT et al., 1996) is in agreement with the results of the preparative isoelectric focusing. Also the S-layer proteins of *Methanococcus vannielii* and *Methanococcus thermolithotrophicus* focused at a similar acidic pH. In contrast, the calculated pIs of the S-layer proteins of *Methanothermus fervidus, Methanobacterium thermoautotrophicus* (gene *MTH719*) and *Methanosarcina mazei* are in the range of 8.47 and 8.9 (Table 4).

A prediction of the deduced secondary structure indicated a higher content of helical structures in the S-layer proteins of the mesophilic species *(Methanococcus voltae, Methanococcus vannielii)* than in *Methanococcus thermolithotrophicus* and *Methanococcus jannaschii,* which in turn exhibit more loops (Table 5). Despite differences in the growth temperature similarities exist between the S-layer proteins of *Methanococcus* and *Pyrococcus* and between those of *Archaeoglobus fulgidus, Methanothermus fervidus, Methanobacterium thermoautotrophicus* (gene *MTH719*) and *Methanosarcina mazei.* Since halobacteria are adapted to high salt, similarities between the S-layer proteins of methanococci and halobacteria could not be expected.

The presumptive leader peptides (ca. 30 amino acids) of the S-layer proteins (e.g. methanococci; AKÇA et al., 2002) showed the typical characteristics of a signal sequence with a positively charged N-region, a hydrophobic core, a polar C-region and an alanine residue at the peptide cleavage site (NIELSEN et al., 1997). Furthermore, the alignments revealed a notable degree of homology between the S-layer genes of the mesophilic up to the extremely thermophilic methanococci (Table 6) especially at the N- and C-termini. Surprisingly, the S-layer genes of the methanococci shared a significant homology with the presumptive S-layer genes of the extremely thermophilic heterotrophs *Pyrococcus abyssi* and *Pyrococcus hori-kosbi.*

The S-layer proteins of *Methanosarcina mazei* (mesophilic) and the gram-positive methanogens *Methanobacterium thermoautotrophicum* (thermophilic), *Methanothermus fervidus* (extremely thermophilic) and *Methanothermus sociabilis* (extremely thermophilic) possess a significant degree of similarity. They have the conserved sequence I-Q-[E/A]-A-I-D in common. The S-layer proteins of these species and that of the sulfate-

reducing *Archaeoglobus fulgidus* (extremely thermophilic) also shared high similarities in the amino acid sequence. No relationship of the latter species and the halobacteria was found with the methanococci or with the extremely thermophilic sulfur-dependent species *Staphylothermus marinus.*

Table 2. *S-layer genes and proteins from selected mesophilic and thermophilic archaea used for sequence comparisons.*

Species	Growth optimum (°C)	Accession No. (gene designation)	Size (aa)	Molecular mass (Daltons)	N-glycosylation sites [1)]	Glycosylation detected[2)]
Archaeoglobus fulgidus	85	AF0275 (*slgb*-1)	[914]	[99221]	[4]	+
		AF1413 (*slgb*-2)	[2425]	[266571]	[45]	
Haloarcula japonica	37	D87290 (*csg*)	890	89814	6	+
Halobacterium halobium	37	J02767 (*csg*_HALHA)	852	89814	7	+
Haloferax volcanii	37	M62816 (*csg*_HALVA)	827	85189	13	+
Methanobacterium thermoautotrophicum	65	AAB85224 (MTH719)	[574]	[61922]	[25]	n.d
		AAB85221 (MTH716)	[1755]	[191961]	[72]	
		AAB85988 (MTH1513)	[1408]	[152737]	[38]	-
Methanococcus jannaschii	85	AJ311636[3)](*slmj1*)	558	60547	8	-
Methanococcus thermolithotrophicus	65	AJ308554[3)] (*slmt1*)	559	59225	5	-
Methanococcus vannielii	37	AJ308553[3)] (*slmv1*)	550	57546	n.f.	-
Methanococcus voltae	37	M59200 (*sla*)	565	59707	2	
Methanosarcina mazei	37	X77929 (*slgB*)	[652]	[68878]	[11]	n.d.
Methanothermus fervidus	85	X58297 (*slgA*)	593	65481		
Methanothermus sociabilis	85	X5S296 (*slgA*)	593	65481	19	+
Pyrococcus abyssi	97	NT01PA0829 (PAB1861)	[604]	[65985]	n.f.	n.d.
Pyrococcus horikoshii	95	NT01PH1418 (PH1395)	[236]	[26128]	n.f.	n.d.
Staphylothermus marinus	92	U57967	1524	166287	61	+

[1)]predicted by *PROSITE;* [2)]PAS-staining, + positive, - negative; [3)]deposited with the EMBL nucleotide sequence data base; [ ]: open reading frame of a presumptive coding region of an S-layer gene; n.d. not determined; n.f. not found.

Table 3. *Amino acids (aa) composition of selected archaeal S-layer proteins (mol%).*

aa	*Mc. voltae*	*Mc. vannielii*	*Mc. thermolithotrophicus*	*Mc. jannaschi*	*Pc. abysii*	*Pc. horikoshi*	*Ms. mazei*	*Mb. thermoautotrophicum MTH719*	*Mt. fervidus*	*Ag. fulgidus AF1413*	*St. marinus*	*Ha. japonica*	*Hb. halobium*	*Hf. volcanii*
Ala	14.0	16.0	12.2	9.9	8.3	6.8	6.7	6.1	4.4	5.1	5.2	7.8	7.2	8.1
Arg	0.5	1.1	1.3	0.7	0.8	1.7	1.5	3.7	2.9	4.0	2.2	2.1	2.3	1.8
Asn	4.6	5.6	5.0	5.6	4.6	2.5	9.8	11.8	12.0	7.1	10.1	5.7	6.6	5.2
Asp	11.3	10.4	11.6	14.0	7.5	7.6	3.5	3.7	3.9	6.1	5.8	13.8	13.4	10.9
Cys	-	-	-	0.4	0.2	0.4	1.1	0.7	1.3	1.2	0.5	0.0	0.0	0.0
Gln	1.4	2.5	1.6	0.5	2.0	1.7	1.8	2.4	1.0	2.0	2.0	2.6	3.2	2.7

Glu	7.3	4.4	6.3	6.5	7.5	10.6	1.7	2.4	5.2	6.8	3.3	9.7	8.2	8.1
Gly	7.4	8.0	7.5	7.2	8.6	9.3	10.1	9.9	9.8	9.4	6.6	8.9	9.7	10.3
His	-	-	-	0.5	0.3	0.4	1.1	0.9	0.2	1.4	0.8	0.4	0.2	0.2
Ile	4.6	4.9	6.4	4.8	7.8	7.6	7.1	8.7	13.5	6.8	8.5	5.5	4.9	6.0
Leu	8.8	7.6	7.9	8.6	6.6	8.5	4.6	7.5	4.7	5.9	10.8	5.6	5.6	6.4
Lys	8.1	6.9	8.8	10.0	9.6	10.2	5.1	3.3	7.1	3.8	2.8	0.7	1.9	0.7
Met	2.1	2.0	2.0	2.7	1.0	0.4	1.4	1.0	1.5	1.4	1.1	0.2	1.1	0.8
Phe	2.5	3.3	1.8	1.8	3.8	2.5	2.1	2.8	2.7	3.6	3.6	4.0	2.2	1.8
Pro	1.6	2.2	1.6	2.3	3.1	2.5	4.1	3.3	3.2	3.5	3.3	3.3	2.5	2.3
Ser	5.5	5.1	6.3	4.1	4.5	3.4	12.4	6.6	6.2	10.0	7.2	8.5	9.0	10.5
Thr	6.7	5.3	5.2	5.2	7.6	8.9	13.2	10.8	7.9	6.8	11.2	11.0	10.6	13.7
Trp	0.5	0.9	0.5	0.7	1.3	1.3	2.0	0.9	1.3	1.2	0.9	0.2	0.2	0.4
Tyr	3.4	2.9	2.9	4.1	4.8	5.5	5.1	5.2	5.7	5.7	4.5	2.0	2.7	1.9
Val	9.6	10.5	11.1	10.4	10.1	8.1	5.5	8.2	5.4	8.2	9.6	7.9	8.5	8.1

- not found; genus abreviations: cf. Table 6; aa - amino acids

Table 4. *Characteristics of the amino acid composition of selected archaeal S-layer proteins.*

Species	Characteristics of amino acid composition						
	Nonpolar[a]	Polar[a]	Acidic[a]	Basic[a]	Aliphatic Index[b]	Hydropathicity[b]	pI[b]
Mc. voltae	48.5	24.2	18.6	8.6	94.16	-0.091	4.15
Mc. vannielii	52.5	24.3	14.8	8.0	95.51	0.061	4.25
Mc. thermolithotrophicus	48.5	23.5	17.9	10.1	100.14	-0.079	4.30
Mc. jannaschii	45.0	23.3	20.5	11.2	92.42	-0.296	4.27
Ms. mazei	40.2	46.8	5.2	7.7	68.22	-0.315	8.9
Mb. thermoautotrophicum MTH719	46.5	39.4	6.1	7.9	93.03	-0.086	8.75
Mt. fervidus	43.7	36.9	9.1	10.2	91.10	-0.236	8.47
Ag. fulgidus AF1413	43.9	34.0	12.9	9.2	72.28	-0.331	4.68
St. marinus	47.6	37.5	9.1	5.8	108.39	0.169	4.45
Hb. halobium	40.6	33.4	21.6	4.4	72.86	-0.543	3.60
Hf. haloferax	43.0	35.2	19.0	2.7	80.17	-0.275	3.44
Ha. japonica	43.0	30.2	23.5	3.2	73.94	-0.462	3.40
Pc. abysii	48.3	26.0	15.0	10.7	93.74	-0.119	4.68
Pc. horikoshii	45.3	24.1	18.2	12.3	92.92	-0.308	4.62

[a] mol (%) calculated after Karlson (1994); [b] calculated with ProtPARAM Tool; genus abbreviation cf. Table 6

Table 5. *Predicted secondary structures of archaeal S-layer proteins.*

Species	% predicted[a]		
	Helix	Sheet	Loop
Mc. voltae	36.1	27.1	36.3
Mc. vannielii	45.1	19.3	35.6
Mc. thermolithotrophicus	26.7	27.5	45.8
Mc. jannaschii	22.3	25.3	51.4
Pc. abyssi	25.0	33.8	41.2
Pc. horikoshii	21.2	32.6	46.2
Ms. mazei	4.9	39.4	55.7
Mb. thermoautotrophicum MTH719	14.3	38.9	46.9
Mt. fervidus	10.1	40.0	49.9
Ag. fulgidus AF1413	10.6	40.2	49.2
St. marinus	14.5	45.5	40.0
Ha. japonica	5.7	29.8	64.5

Hb. halobium	7.0	30.6	62.3
Hf. volcanii	8.2	34.5	57.3

[a] predicted with PHD program; genus abbreviation cf. Table 6

Thermal stabilization of S-layer proteins has been attributed to posttranslational modifications (e.g. glycosylation), covalent cross-linking or salt-bridging (ENGEL-HARDT and PETERS, 1998). However, the limited number of crystal structures from thermophiles and extreme ther-mophiles has hampered detailed structural comparisons with mesophiles. HANEY et al., (1999) compared the sequences of 115 proteins (S-layer proteins were not included) of *Methanococcus jannaschii* with their homologues from mesophilic *Methanococcus* species. The properties mostly correlated with the proteins of the thermophiles included higher residue volume and residue hydrophobic -ity, more charged amino acids (especially glutamic acid, lysine and arginine) and fewer uncharged polar residues (serine, threonine, asparagine and glutamine). In a similar study a high number of proteins from mesophilic and (extremely) thermophilic *Bacillus* and *Methanococcus* species were compared (MCDONALD et al., 1999). The authors found an increase of isoleucine, glutamic acid, lysine, arginine and a decrease in methionine, asparagine, glutamine, serine and threonine in the thermophilic methanococcal proteins. In recent studies the complete genome sequences of mesophiles and thermophiles were analysed (CAMBILLAU and CLAVERIE, 2000; DAS and GER-STEIN, 2000; CHAKRAVARTY and VARADARAJAN, 2000). A large difference between the proportions of charged *versus* polar (noncharged) amino acids was found to be a common signature of all hyperthermophilic organisms. As pointed out by CAMBILLAU and CLAVERIE (2000) and DAS and GERSTEIN (2000) the equal increase of oppositely charged residues in hyperthermophiles results from a thermodynamic advantage because of the increased stability of coulombic interactions with temperature. Also HANEY et al. (1999) discussed ionic interactions as a mechanism for thermostabilization of proteins. Electrostatic interactions are important for S-layer stability in extreme halophiles (SUMPER, 1993) as well in other bacteria (SÁRA and SLEYTR, 1987, 2000). Similarly the histones from mesophilic *(Methanobacterium formicicum),* thermophilic *(Methanobacterium thermoautotrophicum)* and extremely thermophilic *(Methanothermus fervidus)* archaea which have similar amino acid sequences, but very different thermodynamic stabilities (LI et al., 2000; TABASSUM et al., 1992) are stabilized by buried intramolecular arginine-aspartate interactions and intramolecular salt bridges on the surface of histone dimers. This tendency holds also for the S-layer proteins of methanococci (AKÇA et al., 2002). In our study we found an increase of charged residues and a reduction of polar residues in the S-layer proteins of the thermophilic and hyperthermophilic species as compared to their mesophilic counterparts. As the overall hydrophobicity was even higher in the mesophilic strains it seems not to play a major role for adaptation to higher temperatures in the case of *Methanococcus thermolithotrophicus* and *Methanococcus jannaschii.* Similarly, an increase of solvent accessible surfaces in hyperthermophilic proteins was observed in the study of CAMBILLAU and CLAVERIE (2000).

Thus, the increase in charged amino acids, especially of lysine, as found in thhhhe S-layer proteins of *Methanococcus thermolithotrophicus* and *Methanococcus jannaschii* could contribute to their increased thermal stability. Similarly, an increase in charged residues can be observed in the S-layer proteins of *Methanosarcina mazei* (mesophilic) > *Methanobacterium thermoautotrophicum* (thermophilic) > *Methanothermus fervidus* (extremely thermophilic) and *Archaeoglobus fulgidus* (extremely thermophilic). Interestingly, the S-layer glycoprotein of *Methanothermus fervidus* contains high amounts of as-paragine (BRÖCKL et al., 1991) and a basic isoelectric point as proved by isoelectric focusing.

A significant feature of the S-layer protein from *Methanococcus jannaschii* (AKÇA et al., 2002) and other extreme thermophiles (Table 3) is the occurrence of cysteine which has been detected in only few S-layer proteins (SÁRA and SLEYTR, 2000). Intra- or intermolecular disulfide-bridges may be another factor involved in the thermal stability of thermophilic surface proteins.

Based on data from the literature (THOMM, 1996) we propose signal sequences for transcription and translation of the S-layer genes of methanococci (AKÇA et al., 2002; Table *7).* The promotor sequence (TATA-box, boxA; DNA-dependent RNA-polymerase binding region) and the BRE-box (transcription factor B recognition element) correspond largely to the proposed consensus sequences of methanogenic archaea (THOMM, 1996). In contrast to *Methanococcus jannaschii,* several tandem promotors have been described for the S-layer gene of *Methanococcus voltae* (KANSY et al. 1994). The proposed ribosome binding site 5'-AGGAGAU-3', usually located 3-9 nucleotides in front of the translation start point (DALGAARD and GARRET, 1993), was found to be complementary to a region at the 3' terminus of the 16 S rRNA of *Methanococcus jannaschii.* Translation of *Methanococcus jannaschii* and *Methanococcus thermolithotrophicus* is supposed to terminate with 8 and 3 stop codons, respectively. A series of stop codons is a common feature of methanogenic archaea (DALGAARD and

GARRETT 1993). The data from the literature (BuLT et al. 1996) show that in the case of *Methanococcus jannaschii* the stop codons are followed by a poly A/poly T sequence (nucleotides 1729-1877), which probably leads to the formation of a hair-pin and termination of transcription.

Table 6. *Comparison of the deduced amino acid sequence of archaeal S-layer proteins*[1)].

Species with S-layers genes	*Mc. vol.*	*Mc. van.*	*Mc. lit.*	*Mc. jan.*	*Pc. aby.*	*Pc. hor.*	*Ms. max.*	*Mb. the.*	*Mt. fer.*	*Ag- ful.*	*St. the.*	*Ha. jap.*	*Hb. ha.*	*Hf. vol.*
Mc. voltae		44 (59)	48 (61)	38 (54)	23 (38)	28 (44)	-	-	-	-	-	-	-	-
Mc. vannielii	47 (60)		49 (62)	44 (59)	24 (38)	31 (45)	-	-	-	-	-	-	-	-
Mc. thermolitho- trophicus	50 (63)	49 (61)		53 (69)	26 (41)	29 (41)	-	-	-	-	-	-	-	-
Mc. jannaschii	40 (56)	44 (60)	53 (69)		25 (37)	23 (49)	-	-	-	-	-	-	-	-
Pc. abyssi	24 (39)	25 (39)	26 (41)	26 (40)		79 (81)	-	-	-	-	-	-	-	-
Pc. horikoshii	27 (41)	32 (45)	29 (41)	29 (46)	79 (87)		-	-	-	-	-	-	-	-
Ms. mazei	-	-	-	-	-	-		28 (43)	30 (40)	28 (42)	-	-	-	-
Mb. thermoauto- trophicum MTH719	-	-	-	-	-	-	26 (43)		30 (40)	27 (41)	-	-	-	-
Mt. fervidus	-	-	-	-	-	-	26 (36)	28 (38)		37 (59)	-	-	-	-
Ag. fulgidus AF1413	-	-	-	-	-	-	26 (41)	34 (50)	25 (40)		-	-	-	-
St marinus	-	-	-	-	-	-	-	-	-	-		-	-	-
Ha. japonica	-	-	-	-	-	-	-	-	-	-	-		52 (66)	40 (56)
Hb. halobium	-	-	-	-	-	-	-	-	-	-	-	46 (59)		83 (83)
Hf. volcanii	-	-	-	-	-	-	-	-	-	-	-	38 (53)	37 (53)	

[1)]Alignments of amino acid sequences (BLAST 2.0). Data are given as numbers of identity or similarity values (brackets) of the aligned regions. - no significant homology found by BLAST 2.0; Genus abbreviation: *Ag. Archaeoglobus; Ha. Haloarcula; Hb. Halobacterium; Hf. Haloferax; Mb. Methanobacterium, Mc. Methanococcus, Ms. Methanosarcina, Mt. Methanothermus; Pc. Pyro-coccus, St. Staphylothermus.*

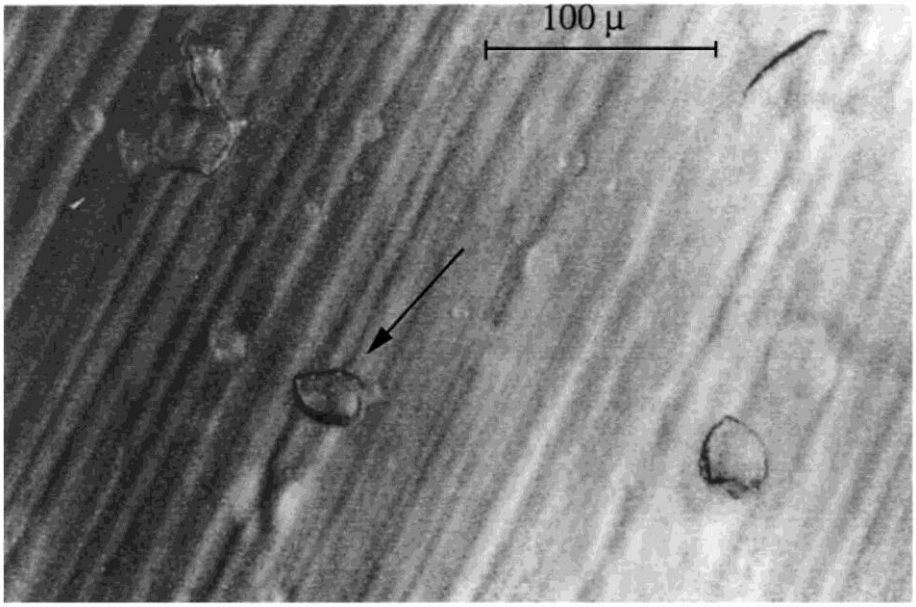
Table 7*. Presumptive signal sequences for transcription and translation of the S-layer genes of methanococci.*

Gene recognition site	Sequence	Position	Consensus-Sequence[1)]
Promotor			
a. BRE-Box:			
Mj (MJ0822)	-CGTAA-	-33 - -29	-CGAAA-
Mt(AJ308554)	-GGAAA-	-35- -31	
Mva (AJ308553)	-GGAAA-	-40- -36	
b. TATA-Box:			
Mj	-TTTATATA-	-26- -19	-AA/TT TATATA-
Mt	-TATATATA-	-28 - -21	
Mv	-TATAATAA-	-32 - -25	
c. Transcription start:			
Mj	-ATAC-	1	A/TTGC
Mt	-ATCC-	1	
Mva	-ATAC-	1	
Shine-Dalgarno-Sequence:			
Mj	-AGGTGAT-	33-39	
Mt	-AGGGTGA-	64-70	

Mva	-AGGTGAA-	60-66
Translation start:		
Mj	-ATG-	45
Mt	-ATG-	77
Mv	-ATG-	72

[1)]according to Thomm (1996); *Mj - Methanococcus jannaschii; Mt - Methanococcus thermolithotrophicus, Mva - Methanococcus vannielii;* Accession no. of the corresponding genes: *Mj* (MJ0822), *Mt* (AJ308554), *Mva (AJ308553).*

Fig. 1. *Micrograph of some crystals inside a HD-reactor. Arrow: crystal used for the determination of the cell parameters mentioned in the text.*



In general, only minor structural differences were observed in the primary and secondary structures of the S-layer proteins of mesophilic and extremely thermophilic methanococci. One important point to consider is that the ancestor of *Methanococcales* was probably a ther-mophile (KESWANI et al., *1996).* This pattern supports the hypothesis that mesophily is a modern adaptation and thermophilic structures are still conserved in mesophilic proteins, especially S-layer proteins. An exchange of an amino acid in mesophilic proteins may then be simply the result of a relaxation of selection against this amino acid which may be of importance in the extremely thermophilic counterparts (MCDONALD et al., 1999).

CRYSTALLIZATION UNDER MICROGRAVITY CONDITIONS

Methanothermus fervidus has been isolated from Icelandic hot solfatara field (STETTER et al., 1981). It possesses an S-layer outside of the pseudomurein sacculus. The gene (slgA) encoding the S-layer glycoprotein has been sequenced (BRÖCKL et al., 1991) and the chemical structure of the heterosaccharide has been elucidated (KÄRCHER et al., 1993). The mature peptide is predicted to consist of 593 amino acids resulting in a molecular mass of 65 kDa (BRÖCKL et al., 1991). With mass spectrometry (MALDI) a molecular mass of 83 kDa was determined for the mature glycoprotein indicating that the glycan moiety accounts for 22%.

Methanothermus fervidus grows optimally at 85 °C and the S-layers may serve a models to elucidate the molecular strategies for survival at high temperatures. For X-ray analysis it is essential to obtain crystals with high quality. Crystal growth under microgravity conditions is advantageous, since it overcomes certain difficulties. The first crystallization experiments were conducted under microgravity conditions (EVRARD et al., 1999) using the Advanced Protein Crystallization Facilities developed by Dornier. The crystals were succesfully grown during the flight STS-95 of the space shuttle Discovery. Hanging drop reactors (HD-80) were used. One of the crystals with dimensions of 30 × 20 × 5 µm were selected for X-ray analysis (Fig. 1). The diffraction experiments were performed at the EMBL DESY synchroton facility in Hamburg. Diffraction spots were

observed to a resolution of 4.2 Å. Cell parameters were: a = 232.6, b = 55.9, c = 144.3 Å, β = 129°. The crystal system is monoclinic and has the space group C2. Meanwhile, we also observed crystals from S-layer proteins of other prokaryotic species with dimensions of up to 224 µm (unpublished results).

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