Genetic characterization, heterologous expression and application of S-layer proteins from the bacterial isolates *Lysinibacillus sphaericus* JG-B53 and *Lysinibacillus sphaericus* JG-A12

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Lederer FL, Günther TJ, Raff J, Pollmann K. 2011. *E. coli* filament formation induced by heterologous S-layer expression. Bioeng Bugs 2(3):178-181.

Lederer FL, Günther TJ, Weinert U, Raff J, Pollmann K. 2012. Development of functionalised polyelectrolyte capsules using filamentous *Escherichia coli* cells. Microb Cell Fact 11:163.

Tagungsbeiträge

Lederer F, Günther T, Raff J, Pollmann K. 2010. Heterologous expression of a surface layer-like protein in *E. coli* causes a drastic morphological change of the cell. **Vortrag** auf der Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM) in Hannover.

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Lederer F, Günther T, Raff J, Pollmann K. 2011. Recombinant S-layer production induces disordered cell division in *E. coli* filaments. **Poster** auf der Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM) in Karlsruhe.

Lederer F, Günther T, Raff J, Pollmann K. 2011. Recombinant S-layer production induces disordered cell division in *E. coli* filaments. **Poster** auf der Jahrestagung der Federation of European Microbiological Societies (FEMS) in Genf, Schweiz.

Lederer FL, Kutschke S, Pollmann K. 2012. Recombinant production of genetically modified S-layer proteins in different expression systems. **Poster** auf der Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM) in Tübingen.

Patente

Pollmann K, Lederer F, Raff J. 2011. P0904 - *E. coli*-Sekretionssystem auf der Basis von S-Layer-Proteinen. DE102009032645B3 - 17.03.2011; EP 22700033 A1 - 05.01.2011

Pollmann K, Raff J, Lederer F. 2011. P0903 - Mikroröhren, umfassend Bestandteile der äußeren Membran von *E. coli* Zellen und rekombinant exprimierte S-Layer-Proteine, Verfahren zu ihrer Herstellung und Verwendung. DE102009032645B3 - 17.03.2011; EP 22700033 A1 - 05.01.2011

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Abbreviations

A.	*Aquaspirillum*	N	amino terminus
A	Adenine, Attachment	NB	Nutrient broth
aa	Amino acids	NGS	Next Generation Sequencing
AFM	Atomic force microscopy	Nt	nucleotide
APS	Ammonium persulfate	NTD	N-terminal domain
B.	*Bacillus*	OD	Optical density
BDT	Big Dye Terminator	OM	Outer membrane
bp	Base pairs	OMV	Outer membrane vesicle
BSA	Bovine serum albumine	ONPG	*o*-nitrophenyl-*ß*-D-galactopyranoside
C	Cytosine, carboxy terminus, cytoplasm	ORF	Open reading frame
CD	Central domain	P	Pellet, periplasm
cDNA	complementary DNA	PAGE	Polyacrylamide gelelectrophoresis
CTD	C-terminal domain	PAH	Poly(allyamine hydrochloride)
cyt	cytosol	PBS	Phosphate buffered saline
D	Dimensional, Discussion	PCR	Polymerase chain reaction
Da	Dalton	PE	polyelectrolyte
DAPI	4',6-diamidino-2-phenylindole	PEI	Poly(ethylenimine)
dATP	Deoxyadenosin triphosphate	PF reads	purified filtered sequence reads
DEPC	diethylpyrocarbonate	PG	Peptidoglycan
DNA	Deoxyribonucleic acid	pI	Isoelectric point
dNTP	Deoxyribonucleoside triphosphate	pos	position
ds	double strand	Pp	Periplasm
DTT	dithiothreitol	PSS	sodium poly (styrenesulfonate)
E	Experiments and Results	PVDF	Polyvinylidene fluoride
E.	*Escherichia*	R	reverse
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid	RNA	Ribonucleic acid
EDX	Energy dispersive X-ray	RCA	Radio corporation of america

	spectroscopy		
fts	Filamenting temperature sensitive	rpm	rotation per minute
F	forward	S	supernatant
G	Guanine	s	second
g	gravitation	SAP	Shrimp alkaline phosphatase
GFP	Green fluorescent protein	SCWP	Secondary cell wall polymer
h	hour	SEM	Scanning electron microscopy
HBSS	Hank's buffered salt solution	SDS	Sodium dodecyl sulfate
HGT	Horizontal gene transfer	S-layer	Surface layer
I	Introduction	SLH	S-layer homologous
IgG	Immunoglobulin G	SllB	S-layer like protein B
IM	Inner membrane	SP	Signal peptide
IPTG	Isopropyl-ß-D-thiogalactopyranoside	sp	species
IR	Infrared	SRP	Signal recognition protein
JG	Johanngeorgenstadt	T	Thymine, time
L.	*Lysinibacillus*	TAT	Twin-arginine transport
L	Ladder	TBE	Tris-borate-EDTA
LB	Luria Bertani	TBS	Tris buffered saline
LDF	Linear discriminant function	TE	Tris-EDTA
LIC	Ligation independent cloning	TEM	Transmission electron microscopy
LPS	Lipopolysaccaride	TEMED	Tetramethylethylendiamine
M	Marker, molar, Materials and Methods	Tris	tris(hydroxymethyl)aminomethane
Mbp	Million bases pairs	trunc.	truncated
MCS	Multiple cloning site	u	unit
MES	2-(*N*-morpholino)ethanesulfonic acid	v/v	volume per volume
min	minute	Vol.	volume
mRNA	messenger RNA	w/v	mass per volume
MW	Molecular weight	wh	whole

Abbreviations for amino acids					
A	Ala	Alanine	M	Met	Methionine
C	Cys	Cysteine	N	Asn	Asparagine
D	Asp	Asparatic acid	P	Pro	Proline
E	Glu	Glutamic acid	Q	Gln	Glutamine
F	Phe	Phenylalanine	R	Arg	Arginine
G	Gly	Glycine	S	Ser	Serine
H	His	Histidine	T	Thr	Threonine
I	Ile	Isoleucine	V	Val	Valine
K	Lys	Lysine	W	Trp	Tryptophan
L	Leu	Leucine	Y	Tyr	Tyrosine

1 Introduction

1.1 Survival of microorganisms in heavy metal polluted environments

The largest environmental sources of heavy metals are probably volcanic emissions, forest fires, deep-sea vents, and geysers (Janssen et al., 2010). Another source of heavy metals that affect the environment is mining with its following processing steps. The uranium mining in eastern Germany, which started in the beginning of the 19th century, produced up to 3000 waste piles and 20 tailings that were contaminated with heavy metals. The area of 168 km^2 is classified as more or less contaminated (Beleites, 1992). Radionuclides were mobilised as result of mining and processing activity. Their mobility is influenced by the interaction with ions, minerals and microorganisms (Merroun, 2006). From the uranium mining waste pile Haberland that is located near Johanngeorgenstadt several soil samples were taken from the acidic sediment (pH 4.5) (Selenska-Pobell et al., 1999). Bacteria that were recovered from these samples were analysed regarding their interactions with uranium and other heavy metals (Merroun et al., 2005; Raff and Selenska-Pobell, 2003).

Generally, cell surface properties and diverse metabolic activities influence the interaction of bacteria with metal ions in their environment (Douglas and Beveridge, 1998). Bacteria, living in extreme environments, may interact efficiently with these inorganic contaminants (heavy metals) through different mechanisms such as intracellular accumulation (Merroun et al., 2003), precipitation (Jroundi et al., 2007; Nedelkova et al., 2007), or biosorption at the cell surface (Merroun et al., 2005) (Figure I1). Biosorption and biomineralisation are natural mechanisms that are widely used for bioremediation (Merroun et al., 2011). Biosorption is effected by the bioavailability of metal binding sites (Macaskie, 1990). In contrast, biomineralisation mechanisms are less limited and are regarded as a promising technology for metal removal from highly diluted solutions that takes place under aerobic conditions (Merroun et al., 2011). The metal immobilisation results from sorption of metal ions or complexes to cell components or exopolymers (Leung et al., 2001) or from precipitation as insoluble organic or inorganic compounds (Boswell et al., 2001; Renninger et al., 2001). On the other hand, bacterial mobilisation of radionuclides and metals is caused by autotrophic or heterotrophic leaching as well as chelation by microbial metabolites and siderophores, and methylation (Leung et al., 2001; Merroun et al., 2005).

Remediation of heavy metal polluted environments is essential to protect living organisms from their toxic influences. Usually remediation is a cost efficient process which may

generate subsequent secondary environmental pollutions. Therefore bioremediation of toxic metal contaminated sites by using bacteria is getting more and more attractive as an alternative technology because of its efficient, affordable and environmentally friendly advantages (He et al., 2011).

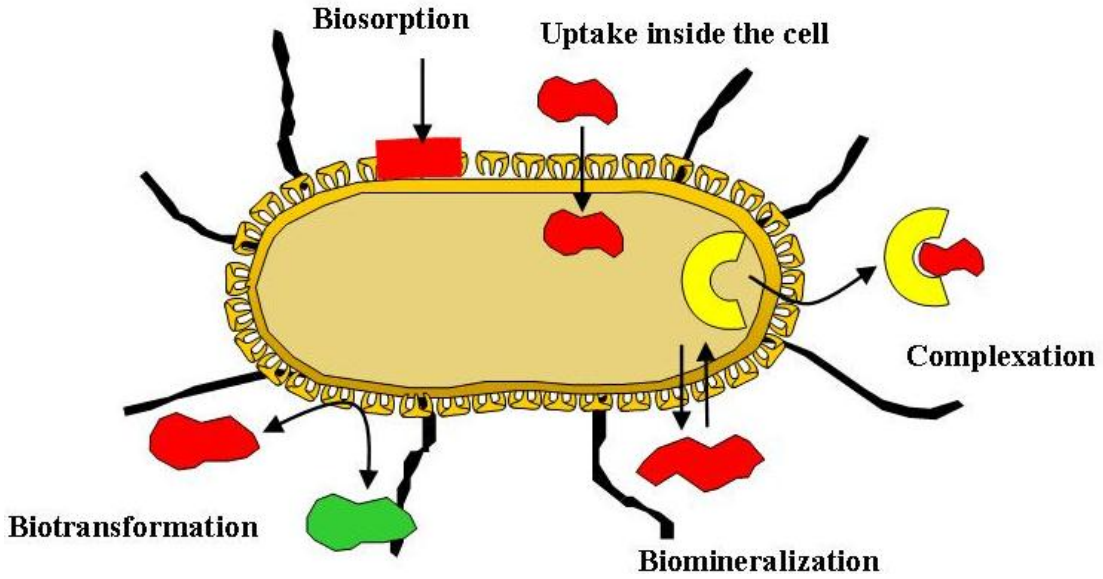

Figure I1. Schematic illustration of microbial interactions with radionuclides.
(Allocated by J. Raff)

The Gram positive bacteria that were isolated from the uranium mining waste pile Haberland were in most cases members of the order of Bacillales and assigned to the families *Bacillaceae* and *Paenibacillaceae* by 16S-analyses (Selenska-Pobell et al., 1999). Most of these isolated rod-shaped endospore producers' possess surface layer (S-layer) proteins as outermost cell envelope. Generally, these S-layers were found to contribute to heavy metal tolerance of the cells. The strains *Lysinibacillus sphaericus* JG-A12 and *Bacillus* sp. JG-B53 that were investigated in the present work were assigned to the genus *Bacillus*. Members of the genus *Bacillus* are facultative anaerobic and in most cases saprophytic, using a range of naturally occurring substrates (Maiden et al., 1992).

Bacteria are able to respond quickly to changing environmental conditions. Horizontal gene transfer is a method that equips microorganisms with a multiplicity of genes that support the microbial survival and proliferation (Martinez et al., 2006). The genes were transferred with mobile genetic elements like plasmids, insertion sequences, phages, transposons and integrons between microorganisms (Canchaya et al., 2003; Frost et al., 2005; Mahillon and Chandler, 1998; Nemergut et al., 2004; Pearson et al., 1996). Genes encoding proteins for the

development of the cell envelope were the second most transferred genes. The bacterial cell envelopes belong to the most important cell attributes that interact directly with the environment and it is essential to equip cells with multiple cell surfaces genes that enable rapid response to changing environmental conditions (Nakamura et al., 2004). So, in some cases, different S-layer variants were found to be encoded by the same bacterial strain. These copies enable the organism to select between different versions, thus offering the possibility to react adequate to different stressors (Jakava-Viljanen et al., 2002; Kuen et al., 1997; Mignot et al., 2001; Mignot et al., 2002). For example, increasing oxygen pressure causes the expression of another S-layer variant instead of the wild-type S-layer of *Geobacillus stearothermophilus* (formally *Bacillus stearothermophilus*) during controlled growth in a fermenter (Sára and Sleytr, 1994). One strategy to alter microbial surface properties is the programmed DNA-rearrangement which affects the variation of protein expression (Borst and Greaves, 1987). DNA rearrangements are induced by different mechanisms. *Lactobacillus acidophilus* ATCC 4356 for example exhibits two S-layer protein genes, the actively transcribed *slpA* gene and the silent *slpB* gene, which are located in a distance of 3 kb from each other at the chromosome in a reverse orientation relative to each other. Through inversion of a chromosomal segment the *slpA* gene is interchanged with the *slpB* gene. This chromosomal rearrangement results in the placement of the formerly silent gene behind the promoter (Boot et al., 1996a; Boot and Pouwels, 1996c). Coevally the regulation of the protein expression of different S-layer protein genes is an efficient method to deal with changing environmental conditions.

Up to now S-layer protein genes have been found within more than 539 species of all important taxa of bacteria and archaea and some species like *Paenibacillus* sp. JDR-2 encode more than 50 different S-layer proteins within their genome (NCBI database).

1.2 Surface layer proteins – the outer cell envelope

Surface layer proteins, so called S-layer proteins, are distributed in almost all phylogenetic branches of bacteria and archaea (Engelhardt and Peters, 1998). These proteins are one typical characteristic of nearly all archaea and many bacteria use S-layer proteins as additional cell envelope (Sleytr and Beveridge, 1999; Sleytr and Messner, 1988). These proteins are probably the basic and oldest form of bacterial cell envelope. Houwink discovered these protein structures while analysing *Spirillum serpens* using the electron microscope (Houwink, 1953). S-layer proteins are characterised by high stability and resistance to adverse conditions

like extreme pH, high temperatures, exogenous proteases, mechanical stress, attacks from phages and predation from foreign organisms (Engelhardt and Peters, 1998).

The monomolecular protein layer is a result of secretion and subsequent crystallisation of single protein molecules (Boot and Pouwels, 1996c) which are glycosylated or phosphorylated in some cases (Messner and Sleytr, 1992). The identical protein or glycoprotein subunits of S-layer proteins form two-dimensional paracrystalline structures which cover the whole cell within all stages of bacterial growth (Bahl et al., 1997; Pum and Sleytr, 1994; Pum et al., 1993; Sleytr and Sára, 1997). Purified S-layer proteins recrystallise to characteristic sheets and tubes in 2D structures (Sleytr and Messner, 1983; Sleytr et al., 1997a). The bacterial S-layer proteins form morphological units with centre-to-centre distances which vary between 2.5-35 nm. The lattice symmetry of S-layer proteins of archaea is often hexagonal (p3, p6), while bacteria seem to exhibit preferentially oblique (p1, p2) or tetragonal (p4) lattices (König, 1988; Messner and Sleytr, 1992; Sleytr et al., 1996) (Figure I2). S-layer protein lattices of bacteria contain pores of identical size between 2-8 nm and layer thicknesses between 5-25 nm (Sleytr et al., 2001).

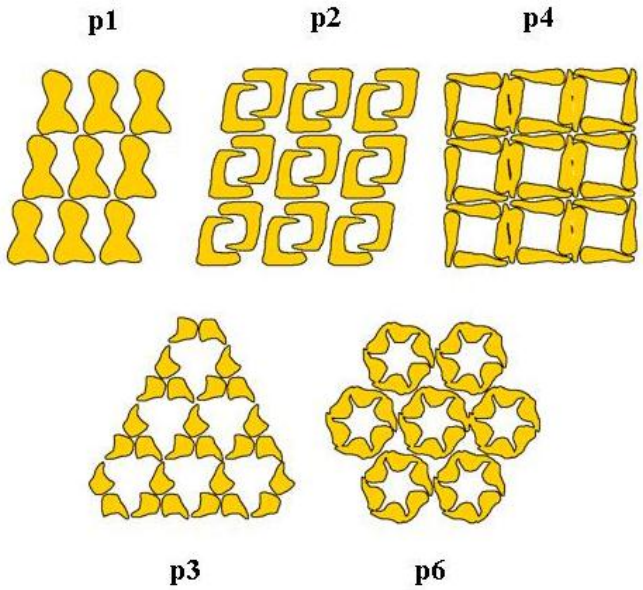

Figure I2. Schematic illustration of possible S-layer lattice symmetries.
(Allocated by J. Raff)

S-layer proteins possess characteristic amino acid compositions with 10 mol% lysine, 8-12 mol% threonine, 15 mol% glutamic acid and aspartic acid and 40-60 mol% hydrophobic amino acids (Engelhardt, 1988; Messner and Sleytr, 1992; Sára and Sleytr, 2000; Sleytr, 1997b). However, low content or absence of cysteine and methionine is characteristic for

bacterial S-layer proteins while S-layer proteins of archaea typically contain sulphur-containing amino acids (Akca et al., 2002; Claus et al., 2002). Therefore in case of archaea the S-layer protein subunits are supposed to be linked by covalent bonding (Beveridge and Graham, 1991; König, 1988). On the other hand, S-layer protein subunits of bacteria are linked by weak, non-covalent bonding forces like, e.g. salt-bridging, ionic bonding and hydrogen-bonding (Beveridge and Graham, 1991; König, 1988; Messner and Sleytr, 1992). About 40 % of the S-layer amino acids are organised as ß-sheets and 10-20 % as α-helices (Claus et al., 2005). The S-layer envelope is characterised by an uncharged, in most cases plane outer face with variable structure and amino acid composition as well as by a negatively charged structured inner face with conserved amino acid composition (Engelhardt, 1988). S-layer proteins are weakly acidic in most cases, but some are basic like those of *Methanothermus* (pI = 8.4) and lactobacilli (pI > 9.5) (Sleytr, 1997b). The molecular mass of S-layer proteins ranges between 40-200 kDa (Sleytr, 1997b). Up to 15 % of the total proteins produced by the cells are S-layer proteins, thus being the major protein species in S-layer expressing organisms (Kuen et al., 1994). Posttranslational modifications like glycosylation, phosphorylation, sulphurylation, lipid transfer and proteolytic cleavage of N- and C-terminal fragments control sizes and molecular features of translated prokaryotic S-layer proteins (Boot and Pouwels, 1996c; Eichler, 2003). S-layer proteins are secreted by either conserved general pathway SEC or the ATP binding cassette transporter (Fernández and Berenguer, 2000; Kawai et al., 1998; Sára and Sleytr, 2000). Signal peptides which are essential for S-layer protein secretion are in average 30 aa in length and exhibit a positively charged N-terminus, a hydrophobic core and a C-terminal recognition site for cleavage specific signal peptidases (Bendtsen et al., 2004).

The linking between S-layer subunits of bacteria and the underlying cell envelope is generally effected by non-covalent bonds (Pavkov et al., 2008). The cell anchoring of S-layer proteins is mediated in many S-layer expressing organism by S-layer homologous (SLH) domains (Lemaire et al., 1995; Lupas et al., 1994). The SLH domain has a conserved sequence of about 55 aa and is located at the N terminus or C terminus of S-layer proteins and several cell envelope proteins (Engelhardt and Peters, 1998). Generally, SLH motif exhibiting S-layer proteins possess 1-3 SLH domains. The amino acid composition of SLH domains exhibit strong similarities to carbohydrate-binding proteins such as lectins (Jarosch et al., 2000). SLH domains bind not directly to the peptidoglycan but to wall-associated polymers (Ilk et al., 1999; Mesnage et al., 1999; Ries et al., 1997; Sára et al., 1996). Many S-layer expressing organism possess S-layer proteins with functional S-layer homologous domains that recognise

pyruvylated SCWPs (Cava et al., 2004; Mader et al., 2004; Mesnage et al., 2000) as proper anchoring structures (Brechtel and Bahl, 1999; Chauvaux et al., 1999; Huber et al., 2005; Ilk et al., 1999). As result of their location in the N-terminal part of S-layer proteins and in the C-terminal part of cell-associated exoproteins and enzymes of Gram positive and Gram negative bacteria SLH motifs were divided into three main groups with specific properties: I: S-layer proteins II: extracellular enzymes and protein involved in polysaccharide degradation, III: outer membrane proteins including Omp α (Engelhardt and Peters, 1998). SLH domains were found in cell surface proteins of many Gram negative and Gram positive bacteria such as *Bacillaceae*, but not all S-layer proteins possess SLH domains (Archibald et al., 1993), such in the case of *Geobacillus stearothermophilus* (Claus et al., 2005). The binding mechanism between the S-layer proteins SbsC of *Geobacillus stearothermophilus* strain ATCC 12980 that possess no SLH domains and SCWPs occurs between a highly conserved positively charged N-terminal region of the S-layer protein and the negatively charged SCWPs (Pavkov et al., 2008; Schäffer et al., 1999).

The ubiquitous occurrence of S-layer proteins in the biosphere points to a broad spectrum of functions which are defined for S-layer proteins (Sára and Sleytr, 2000). Remarkable S-layer characteristics are their strong resistance to extreme environmental conditions such as high ionic strength, low pH and high temperatures (Claus et al., 2002; Engelhardt and Peters, 1998) suggesting that they contribute to the stabilisation and protection of the cells (Claus et al., 2005). In particular, archaea need the surface layer proteins as universal attribute for shape forming and stabilisation (Wildhaber and Baumeister, 1987). The occurrence of S-layer proteins in pathogenic organisms suggests their function as virulence factor (Blaser et al., 1987; Kay and Trust, 1991). The S-layer proteins of for instance *Aeromonas salmonidica*, *Campylobacter fetus* and *Bacillus anthracis* play a protective role against humoral and cellular immune defence and support the pathogenicity of these microorganisms (Etienne-Toumelin et al., 1995; Mesnage et al., 1997). However, *Lactobacillus acidophilus* strains which are essential for eupepsia exhibit S-layer proteins that mediate the adhesion to mammalian gut epithelial cells (Schneitz et al., 1993). Other S-layer proteins, for example of *Geobacillus stearothermophilus*, work as adhesion sites for cell-associated exoenzymes (Sára and Sleytr, 2000). Furthermore, S-layer work as molecular sieve, molecule and ion trap and have in particular the ability to bind selectively heavy metal ions. The uranium mining waste pile soil isolate *Lysinibacillus sphaericus* JG-A12 is able to selectively bind high amounts of uranium, thus protecting the inner of the cell from toxic uranium effects (Merroun et al., 2005; Raff, 2002).

S-layer lattices form with their strict modular construction the basis for many applications (Ilk et al., 2002; Schäffer and Messner, 2004; Sleytr et al., 1999; Sleytr et al., 2001). They can potentially be used as ultrafiltration membranes (Sára and Sleytr, 1987), drug microcontainers (Schuster et al., 2008), filter materials (Raff et al., 2003) or patterning structures in nanotechnology (Fahmy et al., 2006). These applications require an efficient, inexpensive and reproducible synthesis of S-layer proteins, ideally permitted by heterologous expression.

In the present work the strains *Lysinibacillus sphaericus* JG-A12 and *Bacillus* sp. JG-B53 were used. The uranium mining waste pile isolate *Lysinibacillus sphaericus* JG-A12 exhibits the S-layer protein SlfB, which covers the cells with subunits of square lattice symmetry (p4) and possesses a lattice constant of 12.5 nm (Raff, 2002). SlfB is composed of 1238 aa and possesses a molecular weight of 129.4 kDa and a theoretical isoelectric point of 5.23. SlfB is phosphorylated but not glycosylated and exhibits three N-terminal located SLH domains. Cells of *Lysinibacillus sphaericus* JG-A12 are able to bind selectively and reversible high amounts of metals such as uranium, lead, copper, aluminium, gallium and cadmium. However, the purified, recrystallised S-layer proteins of *L. sphaericus* bind high amounts of uranium in a strain-specific way (Pollmann et al., 2005; Raff, 2002).

Bacillus sp. JG-B53 is an isolate from the uranium mining waste pile Haberland which expresses an S-layer protein with square lattice symmetry and a predicted molecular weight, determined by SDS-PAGE, of 150 kDa. Similar to *L. sphaericus* JG-A12, *Bacillus* sp. JG-B53 cells bind selectively and reversible high amounts of heavy metals. In comparison to SlfB, the purification of *Bacillus* sp. JG-B53 S-layer proteins was found to be more efficient than the purification of SlfB and the purified B53 S-layer proteins exhibit excellent recrystallisation characteristics at multiple surfaces (personal communication with J. Raff).

1.3 Heterologous protein expression in *Escherichia coli*

The heterologous expression of bacterial surface layer (S-layer) proteins has failed in many cases (Boot et al., 1993; Bowditch et al., 1989; Kuen et al., 1995). The cost-efficient and large scale production of recombinant proteins is of great interest because of the high application potential of bacterial S-layers (Raff et al., 2003; Sára et al., 2005). The S-layer protein SbsA of the *Geobacillus stearothermophilus* has been successfully expressed in *E. coli*. Following expression, sheet-like intracellular structures have been monitored, indicating the self-assembly of recombinant S-layer proteins in the cytosol (Kuen et al., 1995). Expression of the S-layer protein SlfB of the *Lysinibacillus sphaericus* JG-A12 has also been successful

(Pollmann and Matys, 2007), but structural changes in S-layer proteins have been found after subsequent purification.

The silent plasmid-located S-layer protein gene *sllB* of *Lysinibacillus sphaericus* JG-A12 has been successfully expressed in *E. coli* BL21(DE3) (Lederer, 2008). *E. coli* BL21(DE3) growing at room temperature and expressing the silent S-layer protein gene variant exhibited morphological changes. Filamentous cell structures with dimensions of 1-2 µm x 50-100 µm were formed within the exponential growth phase and in reaching the stationary growth phase *E. coli* single cells started to leave former cell filament enclosing tube-like structures (Lederer, 2008).

Escherichia coli are bacteria which naturally colonise the colon of mammalians. These rod-shaped Gram negative peritrich flagellated enterobacteria have dimensions of 1.1-1.5 µm x 2.0-6.0 µm (Orskov, 1984) and are non-sporulating and facultative anaerobe. Under aerobic conditions *E. coli* generate energy with the help of the respiratory chain and mixed acid fermentation at anaerobic conditions. The optimal growth temperature is 37 °C, thus enabling a cleavage growth rate of 20 minutes when living conditions are in the optimum. *E. coli* is used amongst others as an indicator for contamination of water with excretes. Within the colon of mammalians *Escherichia coli* produce vitamin K2 and are pathogenic in some cases. *Escherichia coli* are one of the scientifically best analysed organisms, working as tool in the molecular microbiology and biotechnology. The relatively small *E. coli* genome of 4.65 x 10^6 base pairs was one of the first completely identified genomes at all.

E. coli B 834 is a genetically modified strain that is used in basic research as a model organism in the investigation of bacterial genetics, physiology and molecular biology. *E. coli* BL21(DE3), an *E. coli* strain derived from the B 834 strain, is widely used as host for heterologous expression of proteins of interest. However, misfolding of the expressed recombinant proteins frequently occurs in *E. coli*, causing the formation of inclusion bodies and often complicating their preparation. The Sec-dependent translocation in *Escherichia coli* is the favoured transport mechanism of large proteins, which finds application in the secretion of several recombinant proteins, too. However, the secretion capacity of the *E. coli* transport machinery is limited. The excess of expressed recombinant proteins favours their accumulation in inclusion bodies (Mergulhao et al., 2005; Mergulhao and Monteiro, 2004).

Filamentous forms similar to those monitored and investigated in the present work have been described only in a few studies and occur only under special culture conditions or in genetically modified strains (Koch et al., 1987; Painbeni et al., 1997; Parker et al., 1992;

Preusser, 1959). In particular in connection with temperature sensitive mutants the observation of filamentous *Escherichia coli* cells was described several times. Investigations with these mutants identified filamenting temperature sensitive (fts) genes that are essential components of the bacterial cell division machinery (Lutkenhaus and Addinall, 1997). So, several reports described the induced filament formation of *E. coli* cells by inhibition of genes that exhibit essential properties for cell division processes (Bi and Lutkenhaus, 1990; Goehring and Beckwith, 2005; Jacobs and Shapiro, 1999; Lutkenhaus and Addinall, 1997; Romberg and Levin, 2003). Additionally, the inhibition of chromosome separation is reported to interrupt cell division processes (Kaimer et al., 2008). The construction of the filaments described in this study and the underlying mechanisms of their formation have not been investigated yet and are part of the present work.

1.4 Material design based on polyelectrolytes using biocomponents as template

Filamentous *Escherichia coli* cells, which were developed by heterologous expression of the silent surface layer like protein gene *sllB* of the uranium mining was pile isolate *Lysinibacillus sphaericus* JG-A12, were discussed as interesting biotemplate, e.g. for the production of catalytic active composites or metal microwires.

The production of polyelectrolyte capsules using cells of different organisms such as erythrocytes, bacteria and spores as biotemplates has been described several times (Balkundi et al., 2009; Franz et al., 2010; Georgieva et al., 2004). The stepwise polyelectrolyte adsorption at different materials such as cells or polymer particles is a useful way to create polymer multilayer films with defined chemical and physical properties. Decher and co-workers proposed this technique originally for the combination of linear polycations and polyanions (Decher, 1997; Decher et al., 1992). The combination of multilayer systems with proteins was described later (Lvov et al., 1995). The starting material for this method is a solid substrate with a negatively charged planar surface. The formation of the first polyelectrolyte layer is started by addition and adsorption of cationic polyelectrolytes to the substrates. The adsorption is carried out at relatively high polyelectrolyte concentrations. A number of ionic groups remain exposed to the interface towards the solution that affects the effectively reserved surface charge. Substrate rinsing in pure water is followed by incubation of the substrate in an anionic polyelectrolyte solution. Multilayer assemblies are obtained by repeating these steps. Additionally, organic molecules and biocomponents such as proteins,

particles, biopolymers and surfactants can be incorporated in these films, thus realising a multifunctionalisation of these layers (Onda et al., 1996).

In the present study the design of biofunctionalised polyelectrolyte capsules by using filamentous *E. coli* as biotemplate for the assembly of polyelectrolytes was described. The tubes were coated with bacterial S-layer proteins. The polyelectrolyte tubes were used as template for the bio-inspired synthesis of palladium nanoparticles. Nanoparticles are very attractive for the development of new materials since their properties usually differ significantly from those of the bulk material. In particular, their physical behaviour can be drastically changed and the catalytic activity can be significantly enhanced due to the altered volume/surface ratio. The development of cluster-assembled materials with discrete, size-selected nanoparticles is of great interest to enable the fine-tuning of the properties of the nanoparticles. Especially the design of bio-nanohybrid materials by the combination of biomolecules with nanoparticles is an emerging topic at the border of Biology, Material Sciences, and Nanotechnology (Ruiz-Hitzky et al., 2008).

This work investigated the potential of the use of the S-layer induced filamentous cell structures for the construction of functional metallic wires that can be used for electronic devices or new catalysts. The possibility to combine such inorganic structures with biological functions opens up new perspectives for multifunctional hybrid materials.

1.5 Aims of the thesis

During a former diploma study the heterologous expression of the silent S-layer protein gene *sllB* of the uranium mining waste pile soil isolate *Lysinibacillus sphaericus* JG-A12 in *Escherichia coli* BL21(DE3) caused the formation of filamentous *E. coli* cells accompanied by extraordinary cell stability (Lederer, 2008). Aim of the diploma study was the comparison of SllB after cloning, expression and purification with the still heterologously expressed functional S-layer protein SlfB of *L. sphaericus* JG-A12 (Pollmann and Matys, 2007). However, morphological modification of the expression strain *E. coli* BL21(DE3) were observed exclusive in SllB expressing *E. coli* cells (Lederer, 2008). The main goal of this study was to verify these results of the diploma study. The unusual cellular modification induced by heterologous expression of SllB should be analysed more detailed using different microscopic methods like AFM, TEM or light microscopy. In order to get more detailed information to the composition of the filamentous cells, they should be stained with cell component specific stains like membrane or DNA stain. The assumption, that the SllB S-layer

proteins are responsible for cellular modifications, should be verified by the coupling of S-layer protein genes with a GFP-fusion protein gene. In order to localise the recombinant proteins within the filamentous cells protein samples need to be taken and should be analysed using enzyme assays. The cell enclosing tubes should be analysed in order to localise the recombinant S-layer proteins and to identify the tube composition using different microscopic, spectroscopic and protein analytic methods. The mechanisms which might be responsible for the filamentous *E. coli* cells should be discussed in detail. The stable filamentous *Escherichia coli* cells and tubes seem to be quite interesting for diverse applications. The filamentous structures should be modified in order to prepare their usage for various applications. The design of filamentous polyelectrolyte tubes combined with native S-layer proteins and synthesised nanoparticles should be done to develop catalytic active filamentous structures. New designed structures should be analysed using different spectroscopic and microscopic methods like TEM, SEM and EDX.

The sequencing of several genomes of bacterial soil isolates which were taken from the uranium mining waste pile Haberland and the characterisation of S-layer protein genes within the genomes was a further aim of this study. The identified S-layer protein genes from *Bacillus* sp. JG-B53 should be analysed in order to characterise the genes and proteins regarding their potential for heterologous expression, their S-layer homologous domains, and analogies to other known S-layer proteins of different microorganisms. Microbial strategies that enable the bacterial survival in uranium contaminated environments should be analysed and discussed in relation to the genome data. The potential role of horizontal gene transfer for bacterial survival strategies by equipping bacteria with different gene variants should be discussed. For these analyses the genome data should be analysed with the bioinformatic program CLC bio Genomics Workbench, further gene and protein specific programs and finally verified with RNA specific methods.

2 Materials and Methods

2.1 Organisms, mutants and vectors

Table M1. Organisms.

Organism	Medium	Characteristic	Origin
***E. coli* NovaBlue GigaSingles**	LB, SOC	Plasmid production strain. Genotype: *end*Al *hsd*R17 (r_{K12}-m_{K12}+) *sup*E44 *thi-l rec*Al *gyr*A96 relAl *lac* [F' pro $A^+B^+lacI^qZ\Delta M15$::Tn*10*(Tc^R)]	Novagen
***E. coli* BL21(DE3)**	LB, SOC	Protein expression strain. Genotype: F^- *ompT* $hsdS_B$ (r_B-m_B-) *gal dcm* (DE3)	Novagen
***Lysinibacillus sphaericus* JG-A12**	NB	S-layer expressing environmental isolate	Laboratory strain collection
***Bacillus* sp. JG-B53**	NB	S-layer expressing environmental isolate	Laboratory strain collection

Table M2. Mutants.

Name	Origin	Vector	Resistance	Strain	Insert size (bp)	Primer pair	Number
SllB_1	*L. sphaericus* JG-A12 *sllB*	pET30 Ek/LIC	Kanamycin	*E. coli* BL21(DE3)	3210	Lic93f Lic_PlHis	KP31
SllB_2	*L. sphaericus* JG-A12 *sllB*	pET30 Ek/LIC	Kanamycin	*E. coli* BL21(DE3)	2599	Lic704f Lic_PlHis	KP87a
SllB2-GFP	*L. sphaericus* JG-A12 *sllB* and pGFP	pET30 Ek/LIC	Kanamycin	*E. coli* BL21(DE3)	3315	Lic704f Lic_Pl-GFP	KP115
pGFP	pGFP	pGFP	Ampicillin	*E. coli* BL21(DE3)	716	-	KP72

Table M3. Vectors.

Vector	Length (bp)	Characteristics	Origin	Resistance gen
pET-30 Ek/LIC	5439	linear	Novagen	Kanamycin
pGFP	3344	coiled	Clontech	Ampicillin

2.2 Cultivation of microorganisms

2.2.1 Cultivation of *Escherichia coli* cells

Escherichia coli cells (Table M1) were routinely grown at 37 °C or room temperature in Luria Bertani (LB) medium containing 1 % (w/v) of Bacto tryptone, 0.5 % (w/v) of yeast extract and 1 % (w/v) of NaCl (pH 7.0). *Escherichia coli* mutant cells (Table M2) were grown in LB-medium supplemented with 35 µg ml^{-1} Kanamycin or with 100 µg ml^{-1} Ampicillin.

2.2.2 Cultivation of *Bacillus* strains

The *Bacillus* strains *Lysinibacillus sphaericus* JG-A12 and *Bacillus* sp. JG-B53 (Table M1), which were isolated from the uranium mining waste pile Haberland located near the town Johanngeorgenstadt, were routinely grown at 30 °C in nutrient broth (NB) medium containing 0.5 % (w/v) of Bacto peptone and 0.3 % (w/v) of meat extract (pH 7.0).

2.3 Standard methods for nucleic acid treatment

To avoid contaminations with bacteria and DNA restriction enzymes, all heat stabile solutions and materials were treated under high pressure saturated steam at 121 °C heat for 15-20 minutes in the autoclave 2540 EL (Tuttnauer). Heat labile materials were treated with 70 % Ethanol for at least 20 minutes and dried afterwards. Solutions which were heat labile were sterile filtered with the syringe filter Filtropur S with a pore size of 0.2 µm (Sarstedt).

2.3.1 Nucleic acid analytic methods

2.3.1.1 Photometric quantitation of concentration and purity of nucleic acids

Concentration and purity of nucleic acids were determined with the NanoDrop 2000/2000c UV/Vis Spectrophotometer (Thermo Scientific). The nucleic acid concentrations were analysed by measuring the absorbance at a wavelength of 260 nm (OD_{260}) in the Micro-Volume Pedestal. An OD_{260} value of 1 was defined as a concentration of 50 µg ml^{-1} of double-stranded DNA, while an OD_{260} value of 1 was defined as a concentration of 40 µg ml^{-1} of RNA (Sambrook et al., 1989).

A sample volume of 2 µl was analysed without dilution. The purity of nucleic acid samples was evaluated by determination of the ratio of the absorbance at 260 nm to the absorbance at 280 nm. Pure DNA shows a value of 1.8, while pure RNA shows a value of 2.0 (Sambrook et

al., 1989). In addition to photometric nucleic acid quantitations the samples were analysed by agarose gel electrophoresis (2.3.1.2).

2.3.1.2 Agarose gel electrophoresis for quantitation of concentration and purity of nucleic acids

The nucleic acid samples were analysed by agarose gel electrophoresis to control the results of the photometric measurements with the NanoDrop 2000/2000c (2.3.1.1). The horizontal agarose gel electrophoresis is used for the analytic and preparative separation of DNA and RNA fragments ranging from 50 base pairs/bases to several mega base pairs/bases to control quantity and quality of nucleic acid samples. Nucleic acid fragments with sizes between 1000-4000 base pairs/bases were analysed in 1.2 % agarose gels. The gels were prepared with agarose (Invitrogen) and 0.5 % Tris-Borat-EDTA (TBE) buffer containing 44.5 mM Tris, 44.5 mM boric acid and 1 mM Na_2-EDTA in a PerfectBlue Gelsysteme Maxi M (Peqlab). The gel was loaded with 3 µl nucleic acid sample mixed with 0.3 µl Midori Green Direct (Biozym) which contains loading dye and staining solution. To control the size of nucleic acid fragments a DNA ladder was mixed with 0.5 µl Midori Green Direct. The electrophoresis worked at constant voltage of 130 V (PowerPac 300). The Midori-Green pre-stained nucleic acids were analysed with the Bio Doc Analyze System (Biometra).

2.3.2 Isolation and purification of nucleic acids

2.3.2.1 Isolation of genomic DNA of *Bacillus* sp. JG-B53 and *Lysinibacillus sphaericus* JG-A12

The DNA of *Bacillus* sp. JG-B53 and *L. sphaericus* JG-A12 was purified using the MasterPure Gram positive DNA Purification Kit (Epicentre). The purification started by harvesting 1 ml of an overnight Gram positive bacterial cell culture by centrifugation at 5,000 x g for 5 minutes. The cell pellet was solved in 150 µl TE buffer containing 10 mM Tris-HCl and 1 mM EDTA at pH 8.0. In order to pre-lyse the bacteria 1 µl Ready-Lyse Lysozyme was added to the cell sample and the mixture was incubated for 30 minutes at 37 °C. Lysozyme cleaves *ß*-1.4-glycosidic bonds between the alternating amino sugars *N*-acetylglucosamine and *N*-acetylmuramic acid of the peptidoglycan lattice of the Gram positive bacterial cell wall. Afterwards 150 µl of the Gram Positive Cell Lysis Solution supplemented with 1 µl Proteinase K (50 µg μl^{-1}) was added to the sample, mixed thoroughly and incubated at 65 °C for 15 minutes at 600 rpm for total lysis of the cells digestion all proteins. The resulting sample product was placed on ice for 5 minutes. Protein precipitation

was started by the addition of 175 µl of MPC Protein Precipitation Reagent to 300 µl of the lysed sample. The sample was mixed for 10 seconds and the debris was collected by centrifugation at 4 °C at 12,000 x g for 10 minutes in the microcentrifuge 5415R (Eppendorf). The supernatant, which contained the nucleic acids, was transferred to a sterile microcentrifuge tube, while the pellet containing the remaining cell debris was discarded. To remove RNA of the nucleic acid sample 1 µl RNase A (5 µg μl^{-1}) was added to the sample, mixed thoroughly and incubated at 37 °C for 30 minutes. For precipitation of the genomic DNA 500 µl isopropanol was added to the recovered supernatant which was mixed with the sample by inverting the tubes 40 times. The precipitated DNA was collected by centrifugation at 4 °C at 12,000 x g for 10 minutes in the microcentrifuge. The remaining supernatant was removed and the DNA pellet was washed twice with 70 % ethanol. Finally the genomic DNA pellet was dried with the vacuum centrifuge Concentrator 5301 (Eppendorf) and solubilised in 25 µl ultra pure water (LiChrosolv, Merck Millipore).The quantitation of purity and concentration of the DNA was analysed with the NanoDrop 2000/2000c (2.3.1.1) and agarose gel electrophoresis (2.3.1.2).

2.3.2.2 Isolation of total RNA of *Bacillus* sp. JG-B53

Analyses with RNA need more intense treatments of solutions and materials to ensure RNase free working. All materials and surfaces were treated with RNase away solution (Roth), all solutions were prepared with 0.1 % DEPC (diethyl pyrocarbonate, Roth) water and all steps were performed on ice. Total RNA of *Bacillus* sp. JG-B53 was isolated from a bacterial culture in the mid-exponential growth phase. Ten millilitres of the bacterial suspension were harvested by centrifugation at 5,000 x g for 5 min. Afterwards the cell pellet was resuspended in 100 µl TE buffer containing 10 mM Tris-HCl and 1 mM EDTA at pH 8.0. After addition of 6 µl Lysozym (50 mg ml^{-1}) the Gram positive bacteria were incubated at 30 °C for 30 minutes to pre-lyse the cells. Afterwards the total RNA-isolation was performed with the InnuPrep RNA Mini Kit (Analytic Jena). The isolated RNA was dissolved in 30 µl RNase free water and treated with DNase I (Biozym) to remove remaining DNA. The OD_{260} value was determined spectrophotometrically for the total RNA concentration and purity with the NanoDrop 2000/2000c UV/Vis Spectrophotometer (Thermo Scientific) (2.3.1.1). The total RNA purification was analysed additionally with agarose gel electrophoresis (2.3.1.2).

2.3.2.3 Plasmid mini preparation of *E. coli*

E. coli clones (Table M2) containing plasmids with the correct insert length were identified by colony screening (2.5.6). One colony of bacteria was used to spike 5 ml LB medium

supplemented with the appropriate antibiotic and was incubated at 37 °C at 250 rpm over night. The plasmid mini preparation of 2 ml of the overnight culture was performed with the Wizard® Plus SV Minipreps DNA purification system (Promega). The purified plasmid DNA was eluted from the cleaning column by the addition of 30 µl ultra pure water (LiChrosolv). The purified plasmids were analysed with agarose gel electrophoresis (2.3.1.2), polymerase chain reaction (2.3.5.1), DNA sequencing (2.3.5.3) and were transformed to the protein expression strain *E. coli* BL21(DE3) (2.5.5).

2.3.2.4 DNA purification by Ethanol-Acetate precipitation

The DNA precipitation started with the addition of 0.1 vol. 3 M sodium acetate (pH 4.6) and 2.5 vol. 99.8 % ethanol to the DNA sample. The sample was inverted 4 times and afterwards incubated for 15 minutes in the dark. The high amounts of monovalent cat ions effect that the DNA molecules exceed their solubility product. The DNA was collected as a pellet by centrifugation at 4 °C and 12,000 x g for 20 minutes in the microcentrifuge 5415R (Eppendorf). The supernatant was removed and the pellet was washed with 3.5 vol. 70 % ethanol without destructing the DNA pellet. The sample was collected by centrifugation at 4 °C and 12,000 x g for 15 minutes, the supernatant was removed and the DNA pellet was dried with the vacuum centrifuge Concentrator 5301 (Eppendorf) and solubilised in 25 µl ultra pure water (LiChrosolv).

2.3.3 Enzymatic modification of DNA

2.3.3.1 Cleavage with restriction enzymes

Sequence specific cleavage of DNA with restriction enzymes created linear vectors with defined ends. The cleavage of plasmids and PCR products started by the combination of 10xFastDigest buffer, FastDigest enzymes (Fermentas), ultra pure water (LiChrosolv) and purified DNA product. The mix was incubated at 37 °C for 20 minutes to 6 hours and was stopped at 80 °C for 5 minutes. Cleaved plasmid DNA was treated with a dephosphorylation step to avoid self-ligation of the vector DNA (2.3.3.2).

2.3.3.2 Dephosphorylation of DNA fragments (Sambrook et al., 1989)

Linear vector DNA was dephosphorylated to avoid self-ligation of the DNA. Therefore the 5'-DNA ends were dephosphorylated by the direct addition of 3 U Shrimp Alkaline Phosphatase (SAP) (Boehringer) and SAP buffer to the cleaved DNA and incubated at 37 °C for 2 hours. The advantage of SAP is the possibility to inactivate its enzymatic activity

completely by the incubation at 65 °C for 15 minutes. The cleaved, dephosphorylated DNA product was purified by ethanol-acetate precipitation (2.3.2.4).

2.3.3.3 Ligation of DNA fragments

The ligation of cleaved purified PCR products and cleaved, dephosphorylated and purified plasmid DNA was performed using the T4-DNA Ligase (Fermentas). The ligation contained 50 ng linear plasmid and adequate amounts of insert in a ratio of 1:3 of vector and insert. The reaction mix was completed by the addition of 0.1 U T4-DNA ligase, 1 x T4-DNA ligase buffer and ultra pure water to a final volume of 20 µl. The ligation reaction was incubated at 16 °C over night, checked by agarose gel electrophoresis (2.3.1.2) and different amount of ligation products were transformed into competent *E. coli* cells (2.5.5).

2.3.4 Reverse transcription of total RNA

The reverse transcription of mRNA to cDNA was performed using the innuScript Reverse Transcriptase (Analytic Jena) and started by the combination of 3 µg RNA with 13 µl RNase free water and 1 µl reverse gene specific primer (100 ng μl^{-1}). The used primer pairs that were designed specifically for the amplification of *Bacillus* sp. JG-B53 putative S-layer protein genes and the 16S primer pair, which was used in positive and negative PCR control reactions, are presented in table M6. Each sample was incubated at 65°C for 5 minutes followed by a 10 minutes lasting cooling step at room temperature to allow the primers annealing to the RNA. Afterwards 1x Reverse Transcriptase buffer, 1 mM dNTP mix and 1.25 U of Reverse Transcriptase (Analytic Jena) were combined with the RNA-primer mix and mixed gently. The samples were incubated for 5 minutes at 42 °C and another 55 minutes at primer specific temperatures (up to 55°C) using the T3 thermocycler (Biometra). The reactions were stopped at 70 °C for 15 minutes. The resulting cDNA samples were placed on ice until their usage in PCR reactions. The PCR amplifications were performed as described previously (2.3.5.1) using the primer pairs that are shown in table M6. As positive control cDNA was amplified with 16S primers while as negative control in order to check DNA contaminations RNA was used as template and incubated with 16S primers. As another positive control PCR was performed using S-layer specific primers and genomic *Bacillus* sp. JG-B53 DNA as nucleic acid template (2.3.5.1).

2.3.5 Amplification and analyses of DNA fragments

2.3.5.1 In vitro amplification of DNA fragments by Polymerase chain reaction (PCR)

The selective amplification of DNA fragments was performed with the *Pfu* DNA polymerase (Fermentas) which was isolated from the hyperthermophilic archaeum *Pyrococcus furiosus*. The enzyme catalyses the template-dependent polymerisation of nucleotides into double-stranded DNA in the 5'→3' direction and exhibits additional 3'→5' exonuclease activity that enables the polymerase to correct nucleotide incorporation errors. The resulting error rate of *Pfu* DNA polymerase is $2.6x10^{-6}$ (Fermentas). The PCR reaction mix was composed as described in table M4 and the used primers are listed in table M6. The polymerase chain reaction was performed in the T3 thermocycler (Biometra) with the program described in table M5. The amplification of DNA fragments was analysed with agarose gel electrophoresis (2.3.1.2).

Table M4. PCR components.

Component	**Concentration/amount**
Template DNA	5-200 ng
Primer (each)	0.5 µM
dNTP mix	200 µM
$MgSO_4$	0.5-2.5 mM
10 x *Pfu* DNA polymerase buffer	1 µl
***Pfu* DNA polymerase**	0.2 µl
Ultra pure water (LiChrosolv)	*ad* 20 µl

Table M5. PCR program.

Step	**Temperature**	**Time**	**Repeat**
Initial denaturising	95 °C	2 min	
Denaturizing	95 °C	1 min	30 x
Annealing	50-60 °C	1 min	
Elongation	72 °C	2 min/kbp fragment	
Final elongation	72 °C	10-20 min	
Storage	4 °C	∞	

Table M6. PCR oligo-nucleotides.

Gene	Name	Sequence 5'-3'	Application
sllB	Lic93f	gacgacgacaagatgGCAGGATTCTCAGA TGTAGCA	Cloning in LIC site of pET-30 Ek/LIC
	Lic704f	gacgacgacaagatgATCAACAACACAA CTGTTGAA	
	Lic_PlHis	gaggagaagcccggtttaTGGAGTTGGCTT TACTGTAATA	
	Lic_Pl	gaggagaagcccggtTGGAGTTGGCTTTA CTGTAATA	
gfp	Lic_GFP_BamHI_ F	AAAggatccATGAGTAAAGGAGAAGAAC TT	Cloning in MCS of pET-30 Ek/LIC
	Lic_GFP_EagI_R	TTTcggccgCTATTTGTATAGTTCATCCA	
	T7 f	TAATACGACTCACTATAGGG	Sequencing of inserts in pET-30 Ek/LIC
	T7 r	CTAGTTATTGCTCAGCGGT	
B53_*slp1*	B53_600_1F	ATTCGCTTCATTCTTACACC	Reverse transcription, PCR + Sequencing
	B53_600_1R	GTAGTGATTTGTGCTGCTTT	
B53_*slp3*	B53_600_3F	CTGTCACATTCTCTCCATTT	Reverse transcription, PCR + Sequencing
	B53_600_3R	GCCCTTCGGAATAATAACT	
B53_*slp6*	B53_wh_6F	ACATTACCCTTCACCGAC	Reverse transcription, PCR + Sequencing
	B53_wh_6R	CTTTTCCCCTTTTGCTCC	
B53_*slp8*	B53_wh_8F	GGCGAATATACCAGTAGA	Reverse transcription, PCR + Sequencing
	B53_wh_8R	GGAAGCGATCAAGCATAA	
16S	7f	AAGAGTTTGATCNTGGCTCAG	Sequencing of 16 S DNA
	1513r	TACGGYTACCTTGTTACGACTT	

2.3.5.2 Purification of PCR products

PCR products, which were used in following sequencing or cloning steps, were purified with the Quick Step^{TM} 2 PCR Purification Kit (EdgeBio). A minimal sample volume of 20 µl was mixed with 4 µl purification resin and incubated at room temperature for 3 minutes. A special

purification column, which was stored in buffer, was centrifuged in the microcentrifuge 5415R (Eppendorf) at 700 x g for 3 minutes to remove spare buffer. The column was placed in a new microcentrifuge tube and the sample was placed in the middle of the column. The pure PCR product was collected by centrifugation at 700 x g for 2 minutes in the microcentrifuge tube. The column was removed.

2.3.5.3 Sequencing of DNA

The sequencing of PCR products was performed using the method which was described by Sanger (Sanger et al., 1977). The purified PCR products were used as template DNA for the sequencing reaction in the T3 thermocycler (Biometra). For the sequencing reaction mix 2-4 µl purified PCR product, 1 µl sequencing primer (3.2 µM), 1 x BDT buffer and 1.5 µl BDT-mix (BigDye® Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems) were combined with ultra pure water (LiChrosolv) to a final volume of 10 µl. The sequencing reaction was performed with the program described in table M7. The finished sequencing reaction was purified by ethanol-acetate precipitation (2.3.5.4). The analysis of the sequencing reaction was performed with the ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and the data were evaluated with the DNA Sequencing software (Applied Biosystems).

Table M7: Sequencing program.

Step	Temperature	Time	Repeat
Initial denaturising	96 °C	2 min	
Denaturising	96 °C	30 s	25 x
Annealing	50 °C	15 s	
Elongation	55°C or 60 °C	2 min/kbp fragment	
Storage	4 °C	∞	

2.3.5.4 Ethanol Acetate precipitation of sequencing products

The precipitation of sequencing products was performed as previously described (2.3.2.4). In the first step 0.1 vol. 125 mM Na_2-EDTA were additionally added to the sequencing product. The resulting DNA pellet was solubilised in 25 µl HiDi®-Formamid (Applied Biosystems).

2.4 Genome sequencing and bioinformatics

2.4.1 Sequencing of whole bacterial genomes

The sequencing of whole genomes of *Bacillus* sp. JG-B53, *Lysinibacillus sphaericus* JG-A12 and other in house bacteria strains, which were isolated from a uranium mining waste pile, was performed by the Next Generation Sequencing technology with the Illumina Hi Seq 2000 by AROS Applied Biotechnology A/S. The Next Generation Sequencing (NGS) technology produces with DNA amounts of at least 1.2 µg dsDNA a huge amount of data. The used Illumina Hi-Seq 2000 technology provides read lengths of 2 x 100 base pairs for the whole genome within a run time of 8 days. Therefore the fragmentation of genomic DNA was performed by nebulisation or shearing. The DNA fragments were amplified on a surface via bridge PCR. Bridge PCR is a method for *in vitro* clonal amplification where fragments are amplified upon primers attached to solid surfaces forming clonal colonies. The generated clusters were sequenced by synthesis using a technique called cyclic reversible termination. Four types of reversible dye-terminator bases were added and non-incorporated nucleotides were washed away. The DNA extended one nucleotide at a time. Using a camera, images of the fluorescently labelled nucleotides were taken. Afterwards the dye along with the terminal 3' blocker were chemically removed from the DNA fragment, to allow the next cycle (Mardis, 2008).

2.4.2 Bioinformatic analyses of whole genome sequences

Bioinformatic analyses were realised with the Genomics Workbench (CLC bio). Therefore the sequenced genome information, which were generated in a fastq format of 200 bases lengths per sequence fragment, were imported to the Genomics Workbench and assembled with all imported data. Resulting contigs, which are the assembling products with a length of 200-500,000 bases, were afterwards extracted, the open reading frames were identified and the contigs were transformed to proteins.

Sequences comparisons for S-layer proteins were obtained using the NCBI database (http://www.ncbi.nlm.nih.gov/). Using parts of these sequences within the motif search tool of the Genomics Workbench, several proteins with identical or similar sequences were checked using BLAST database (http://blast.ncbi.nlm.nih.gov/). Identified surface layer proteins were analysed with Bioedit in order to identify size and amino acid composition of the proteins. ExPASy program (http://web.expasy.org/protparam/) was used in order to calculate the theoretical isoelectric point while the signalP 4.0 program (www.cbs.dtu.dk/services/SignalP/)

was applied in order to identify the signal peptides (Petersen et al., 2011). In order to identify the S-layer homologous (SLH) domains the sequence alignment program (http://fasta.bioch.virginia.edu/fasta_www2/fasta_www.cgi?rm=lalign) was used (Huang and Miller, 1991). The analyses of the promoter regions were performed with the program (http://linux1.softberry.com/berry.phtml?topic=bprom&group=programs&subgroup=gfindb) (Solovyev and Shahmuradov, 2003). To identify inverted repeats after the Stop codon the EMBOSS program (http://emboss.bioinformatics.nl/cgi-bin/emboss/einverted) was used.

2.5 Cloning of DNA

The cloning of the plasmid-located silent S-layer protein gene *sllB* and of *sllB* gene fragments of *Lysinibacillus sphaericus* JG-A12 and the construction of the S-layer-GFP fusion protein gene was performed using the pET-30 Ek/LIC cloning Kit (Novagen). A schematic illustration of the silent S-layer protein gene *sllB* of *Lysinibacillus sphaericus* JG-A12 and the cloned gene fragments are shown in figure M1.

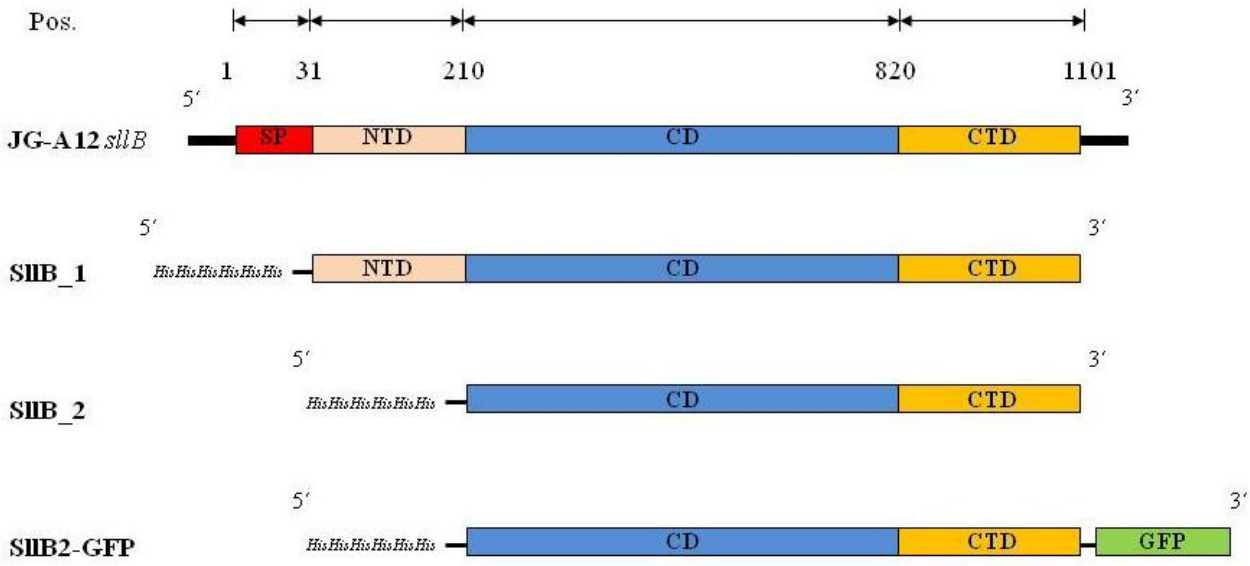

Figure M1. Comparison of the primary structures of the natural silent S-layer protein and its resultant newly designed fragments.

SP, Signal peptide; NTD, N-terminal domain; CD, central domain; CTD, C-terminal domain; GFP, green fluorescent protein; Pos., position of the amino acids in the protein.

2.5.1 The Ek/LIC vector system

The vector pET-30 Ek/LIC is a ligation-independent cloning (LIC) vector which consists of 5439 base pairs and was designed for the rapid and direct cloning of PCR products. It is

characterised by strong polypeptide expression. The vector equips fusion proteins with N-terminal cleavable His·Tag and S·Tag sequences and C-terminal His·Tag sequences for the detection and purification of proteins. The pET-30 Ek/LIC vector exhibits an origin of replication, a kanamycin resistance gene and a multiple cloning site (vector card see Attachment Figure A1). Ligation-independent cloning vectors use the 3'→5' exonuclease activity of T4 DNA Polymerase to create 13- or 14-base single-stranded overhangs in the vector. Complementary overhangs were created on PCR products by building appropriate 5' extensions into the primers. The purified PCR products were treated with LIC-qualified T4 DNA Polymerase in the presence of dATP to generate specific vector compatible overhangs. The protein expression is controlled by a T7 promoter which is activated by IPTG (Novagen user protocol TB163).

2.5.2 T4-DNA-polymerase treatment of PCR products

The creation of poly-A overhangs in PCR products which were complementary to the pET-30 Ek/LIC vector overhangs were performed with T4 DNA Polymerase (Novagen). Therefore 1 µl PCR product (0.2 pmol), 2 µl T4 DNA Polymerase buffer, 2.5 mM dATP's, 5 mM DTT and 1 unit T4 DNA Polymerase were combined with ultra pure water to a final volume of 20 µl. The sample was incubated at 22 °C for 30 minutes in the T3 thermocycler (Biometra) to create the poly-A overhangs. The reaction was stopped by incubation at 75 °C for 20 minutes in the thermocycler (Novagen user protocol TB163).

2.5.3 Ligation of pET-30 Ek/LIC vector and insert

The ligation was initiated by the combination of 1 µl Ek/LIC vector and 2 µl T4 DNA Polymerase treated PCR-product and the incubation at 22 °C for 5 minutes in the T3 thermocycler (Biometra). The addition of 6.25 mM EDTA to the ligation mix was followed by an additional 5 minutes lasting incubation step at 22 °C. The ligation mix was transformed afterwards in competent *E. coli* cells (2.5.5) (Novagen user protocol TB163).

2.5.4 Production of $CaCl_2$ competent *E. coli* cells

The production of chemical competent *E. coli* cells was performed with the Calcium Chloride method described by Jasper Rine, University of California (www.bio.com/protocoltools, 2007). Therefore freshly inoculated *E. coli* cells were used to inoculate 3 ml of LB medium and the cells were grown at 37 °C and 200 rpm one night in the incubator (Memmert). The *E. coli* preculture was used to inoculate 150 ml of LB-medium, and the cells were grown to an OD_{550}=0.45-0.55. The cell suspension was transferred to 50 ml Greiner tubes and cooled on

ice for 15 minutes. Afterwards the cells were collected by centrifugation at 550 x g for 15 minutes in a centrifuge 5804R (Eppendorf) and the supernatant was removed. The cell pellets were carefully resuspended and combined in 15 ml of a 0.1 M $MgCl_2$ solution. The cells were collected again by centrifugation at 550 x g for 15 minutes, the supernatant was removed and the cells were resuspended in 20 ml 0.1 M $CaCl_2$ solution. The cells were placed on ice for 20 minutes and subsequently collected by centrifugation at 550 x g for 10 minutes. The supernatant was removed and the cells were resuspended in 6 ml of 0.1 M $CaCl_2$ and 15 % (w/v) glycerol. The cells were divided in 50 µl aliquots to sterile microcentrifuge tubes and stored at -80 °C until using the cells for heat shock reactions (2.5.5).

2.5.5 Transformation of cloning products

Transformation is a method which enables the uptake of free soluble DNA to competent cells *via* heat shock or electroporation. Competent cells are normally very sensitive towards mechanical treatments, necessitating a careful handling. The heat shock reaction was started with a slow thawing of 50 µl $CaCl_2$ competent *E. coli* cells on ice for 3 minutes. Afterwards the cells were mixed with 2 µl ligation product or plasmid and incubated on ice for 5 minutes. The uptake of the DNA was performed by incubation at 42 °C for 30 seconds in the Thermomixer comfort (Eppendorf) which results in the short-term porosity of the bacterial cell walls. Afterwards the cells were placed on ice immediately for 2 minutes which results in the closing of the pores of the cell walls. The addition of 250 µl SOC medium [0.5 % (w/v) yeast extract, 2 % (w/v) Bacto tryptone, 20 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 20 mM $MgSO_4$, 20 mM glucose] to the transformed *E. coli* cells and the incubation of the cells at 37 °C and 250 rpm for 1 hour in the Thermomixer started the regeneration of heat shocked cells. Afterwards 3 LB agar plates supplemented with the appropriate antibiotic were uniformly inoculated with 100 µl transformed *E. coli* cells, respectively and stored at 37 °C over night in the incubator (Memmert) (Novagen user protocol TB163).

2.5.6 Colony screening

The colonies grown after transformation were checked for the presence of plasmids with the correct insert. From transformation 20 colonies with at least 1mm in diameter were chosen and picked from the agar plate using a sterile toothpick, respectively. The picked bacteria were tipped on a LB agar antibiotic plate which was separated in numbered boxes and afterwards the picked bacteria were transferred to a numbered sterile glass tube with 3 ml LB medium supplemented with the appropriated antibiotic. The cells in solution were grown at 37 °C and 250 rpm for 5 hours and the separated agar plate was incubated at 37 °C over night

in the incubator (Memmert). To identify positive clones 250 µl of the freshly grown cell culture were harvested by centrifugation at 3,400 x g for 3 minutes in the microcentrifuge 5415R (Eppendorf) and the supernatant was removed. The cells were resuspended in 50 µl ultra pure water (LiChrosolv). The cells were incubated at 99 °C for 5 minutes in the Thermomixer comfort (Eppendorf). In order to isolate the chromosomal DNA and to denaturise DNases the cell debris was collected afterwards by centrifugation at 12,000 x g for 1 minute. The supernatant was used as DNA template in a following polymerase chain reaction (2.3.5.1) with insert specific primers (Novagen user protocol TB163, modified). The polymerase chain reaction was controlled by agarose gel electrophoresis (2.3.1.2).

2.5.7 Long term storage of bacteria

The long term storage of bacteria started by the inoculation of 3 ml LB medium supplemented with the appropriate antibiotic with a single colony of the aimed bacteria. The cells were grown at the appropriate temperature at 200 rpm to an OD_{600} = 0.8. The bacteria were mixed in an amount of 0.5 ml with 1 ml sterile 50 % (w/v) glycerol in a sterile storage tube on ice. The suspension was stored at -80 °C until use.

2.6 Expression of recombinant proteins

2.6.1 Heterologous expression of S-layer variants

The expression of recombinant proteins was performed using the protein expression strain *Escherichia coli* BL21(DE3). For each *E. coli* BL21(DE3) clone separate flasks containing 100 ml LB medium supplemented with the appropriate antibiotics were inoculated with 5 ml of an LB-grown starter culture of the construct. The cultures were incubated at room temperature at 250 rpm. After 2 h of growth, recombinant protein expression was induced by the addition of 0.1 mM IPTG.

Recombinant *E. coli* cells were harvested in the mid-exponential, stationary and death phase. The cells were analysed by light microscopy (2.8.1) and atomic force microscopy (2.8.2). Recombinant *E. coli* which expressed S-layer proteins accompanied with morphological changes were analysed with Live/Dead stain (2.7.1), DAPI stain (2.7.2) and membrane stain (2.7.3) and were analysed afterwards with light and fluorescence microscopy (2.8.1). The recombinant protein expressing cells were embedded in epoxy resin and analysed with transmission electron microscopy (2.8.3).

The morphologically changed *E. coli* cells were separated in different cell protein fractions (2.6.2.2) which were analysed by SDS-PAGE (2.6.3.1) and *ß*-galactosidase assay (2.6.3.6). These results evaluated the location of recombinant S-layer proteins and the state of the cell walls regarding the porosity in the appropriate growth phase.

2.6.2 Isolation of cell components

2.6.2.1 Purification of native S-layer proteins

The cells of *Lysinibacillus sphaericus* JG-A12 were grown in NB medium (2.2.2) at 30 °C and harvested in the late exponential growth phase by centrifugation at 11,000 x g for 20 minutes. The cells were washed and after centrifugation resuspended in standard buffer [50 mM Tris-HCl, 1 mM $MgCl_2$ x 6 H_2O, 3 M NaN_3, pH 7.5]. The cells were treated with the rotating-blade blender IKA T8 (IKA Labortechnik) at maximum speed for 10 minutes on ice in order to remove bacterial flagella. Afterwards the cells were harvested by centrifugation at 6,000 x g for 10 minutes at 4 °C. The bacterial biomass was resuspended 1:1 in standard buffer accompanied by a few crystals of DNase II and RNase A. The disintegration of the cells was performed using the high-shear fluid processor (M-110S Microfluidizer processor, Microfluidics) at 4 °C and a pressure of 960 bar. The cell fragments were washed in standard buffer followed by the solubilisation of plasma membrane in standard buffer accompanied by 1 % Triton X-100 for 10 minutes at room temperature. Remaining cell wall fragments were washed in standard buffer and afterwards the peptidoglycan was lysed using standard buffer containing 0.2 mg ml^{-1} lysozyme for 6 hours at 30 °C. The S-layer protein containing fraction was washed several times in standard buffer and mixed with 6 M guanidine hydrochloride in 50 mM Tris pH 7.2 until the solution becomes clear. The solution was stirred for 2 hours at room temperature and non-protein components were collected by centrifugation at 12,400 x g for 60 minutes at 4 °C. The supernatant was dialysed against 1.5 mM Tris and 10 mM $CaCl_2$, pH 8 for 24 hours at 4 °C using dialysis tubings with a molecular weight cutoff of 50,000. The reassembled S-layer proteins were harvested by centrifugation at 12,400 x g for 1 hour and stored at 4 °C until use (Fahmy et al., 2006; Raff, 2002).

2.6.2.2 Preparation of cell protein fractions

Cells of the expression strain *E. coli* BL21(DE3) were grown at room temperature over night and 40 ml of a well-grown culture were harvested by centrifugation at 4 °C and 10,000 x g for 10 min. The proteins released to the medium, the proteins in the periplasmic protein fraction and the cytoplasmic proteins were isolated as described previously in the pET System Manual

(Novagen user protocol TB055) and were studied by protein assays. The medium protein fraction was isolated by concentration of the supernatant with Vivaspin Concentrators (Sartorius Stedim Biotech). The periplasmic protein faction was isolated using the osmotic shock protocol (Ausubel et al., 1988). The cell pellet of 40 ml well-grown culture was resuspended in 30 ml osmotic shock buffer containing 30 mM Tris-HCl and 20 % sucrose at pH 8.0. After the addition of 0.5 M EDTA (pH 8.0) to a final concentration of 1 mM EDTA the sample was stirred slowly with a magnetic stirrer for 10 minutes. The cells were stabilised within this step in respect to the special sugar concentration. The high sugar concentration in the solution affects the absorption of sugar to the cell and the leakage of water. The cells were collected by centrifugation at 4 °C and 10,000 x g for 10 minutes and the supernatant was removed. The cell pellet was resuspended afterwards in 30 ml of ice-cold 5 mM $MgSO_4$ and stirred slowly for 10 minutes on ice. This cell treatment effects the uncontrollable water penetration in the cell wall. The high pressure causes the burst of the cell walls and the periplasmic proteins were released into the buffer. The shocked cells were collected by centrifugation at 4 °C and 10,000 x g for 10 minutes and parts of the supernatant were used for the concentration with spin filters and further protein analysis methods. The rest of the supernatant was removed and the pellet of shocked cells was treated with BugBuster Protein Extraction reagent (Novagen). Therefore 5 ml of the BugBuster Protein Extraction reagent were used to resuspend 1 g of wet cell paste and the mixture was incubated on a shaking platform at a slow setting for 20 minutes. Afterwards the insoluble cell debris was collected by centrifugation at 4 °C and 16,000 x g for 20 minutes. The supernatant was transferred to a fresh microcentrifuge tube and analysed by SDS-PAGE (2.6.3.1) and *ß*-galactosidase assay (2.6.3.6).

2.6.2.3 Preparation of cell membranes

The isolation of *E. coli* membranes was performed with well grown cells from 100 ml culture which were harvested by centrifugation at 4 °C and 6,500 x g for 15 min and resuspended in 20 ml of resuspension buffer containing 100 mM NaH_2PO_4 x H_2O, 10 mM Tris base and 0.5 M NaCl at pH 8.0. The cells were disrupted by sonication (Sonifier W250-D, Branson) 5-6 times at 60 % amplitude for 30 s and the lysate was centrifuged for 15 min at 3,000 x g. Subsequently the supernatant was centrifuged at 4 °C and 12,000 x g for 20 min. After centrifugation, the supernatant included most of the membrane components and the pellet included most of the cell wall components. The supernatant was centrifuged at 4 °C and 48,000 x g for 1 h. The resulting supernatant was removed and the pellet was washed twice in 10 ml of resuspension buffer. After each washing step, the membranes were again centrifuged

at 4 °C and 48,000 x g for 1 h. Finally, the membrane pellet was transferred to 1 ml resuspension buffer and used for protein analyses.

2.6.2.4 Preparation of cell enclosing tubes

The purification of filamentous *E. coli* enclosing tubes started with the harvesting of a well grown cell culture by centrifugation at 10,000 x g for 10 minutes at 4 °C. The cell pellet was washed twice with PBS consisting of 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4 and 1.47 mM KH_2PO_4 (pH 7.4). The cells were suspended in 10 ml of 40 % sucrose and stirred at 250 rpm for 1 h at room temperature. The cells were concentrated by centrifugation at 3,000 x g for 15 min at 4 °C to form the pellet P1. The produced supernatant S1 was transferred to an empty tube. After centrifugation of the supernatant S1 at 12,000 x g for 30 min at 4 °C, the developed supernatant S2 was removed. The resulting pellet P2 and the first pellet P1 were suspended in 10 ml of 6 M urea, respectively. The pellets were incubated at room temperature with stirring for 1 h and afterwards the cells were collected by centrifugation at 3,000 x g for 15 min at 4 °C. Each supernatant was centrifuged again at 12,000 x g for 30 min at 4 °C. The tube pellets were washed twice with de-ionised water and analysed by SDS-PAGE (2.6.3.1), N-terminal sequencing of proteins (2.6.3.4) and different microscopic and spectroscopic methods (2.8).

2.6.2.5 Total lipid extraction of tubes

The purified tubes were treated with chloroform and methanol for total lipid extraction (Bligh and Dyer, 1959). Therefore 300 µl sample were combined with 375 µl chloroform and 750 µl methanol which were mixed vigorously and incubated 10 minutes to create a homogeneous single-phase system with a chloroform-methanol-water composition of 1:2:0.8, v/v. The sample was centrifuged at 14,000 rpm for 10 minutes and the supernatant was transferred to a sterile 2 ml microcentrifuge tube. A chloroform-methanol-water composition of 2:2:1.8, v/v was generated by the mixture of 375 µl chloroform and 375 µl water. The lipid-extraction mix was treated by centrifugation at 14,000 rpm for 30 minutes to separate the upper, non-lipid containing methanol-water phase of the lower lipid containing chloroform phase. Upper and lower phase were separated in microcentrifuge tubes and analysed by SDS-PAGE (2.6.3.1) and IR-spectroscopy (2.8.5).

2.6.3 Protein analysis methods

2.6.3.1 SDS-PAGE (Laemmli, 1970) mod.

The SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis) analyses were performed using the Mini-PROTEAN II electrophoresis cell (Bio-Rad) with gels in dimensions of 8.6 x 7.7 x 0.15 cm (width x length x thickness). The separation of proteins was performed with polyacrylamide concentrations of 10 % (v/v) and very large proteins were separated in gels with polyacrylamide concentrations of 7.5 % (v/v). The gels were prepared with 40 % of Acrylamid/Bis (37.5:1) (Bio-Rad), separating buffer containing 1.5 M Tris-HCl at pH 8.8, stacking buffer containing 0.5 M Tris-HCl at pH 6.8, 10 % of SDS (w/v), 10 % of ammonium peroxosulfate (APS) (v/v), TEMED and de-ionised water. The detailed composition of the SDS-PA gels is described in table M8. The SDS-gels were degassed for 30 minutes in an exsiccator before addition of SDS, TEMED and APS. The separation gel solution was transferred between 2 glass plates in the casting stand, covered with a thin N-butanol layer and polymerised for 30 minutes. Afterwards N-butanol was removed by washing the upper gel line and the staking gel solution was poured between the glass plates. Subsequently a gel comb was pushed between the glass plates and the gel was polymerised for further 30 minutes. Afterwards the gels were placed in the Mini-PROTEAN II electrophoresis cell chamber and loaded with protein samples. The protein samples were mixed with the same amount of 2 x sample buffer consisting of 6 M urea, 0.1 M Tris, 2 % of SDS (w/v), 0.2 M DTT, 1.55 M glycin and de-ionised water and incubated for 10 minutes at room temperature. The gel run was performed at 65 V for 30 minutes and further 80 minutes at 120 V in running buffer [25 mM Tris, 192 mM glycin, 0.1 % of SDS (w/v)]. The SDS-PA gels were fixed in fixing solution containing 10 % of acetic acid (w/v), 50 % ethanol (w/v) and de-ionised water for two hours. Afterwards the gels were stained in colloidal coomassie staining solution for 2-24 hours. The stock solution of colloidal coomassie staining solution consists of 2 % of *o*-H_3PO_4, 10 % of $(NH_4)_2SO_4$ and 0.1 % of coomassie brilliant blue G250 in de-ionised water and 75 ml of colloidal coomassie stock solution were combined with 25 ml of 96 % methanol (v/v). Backround staining was removed by incubation of gels in de-ionised water. Documentation of the gels was done with the Versa Doc Imaging System (Bio-Rad).

Table M8. Composition of 2 SDS-PA gels, respectively.

	Separating gel		Stacking gel
Components	**7.5 %**	**10 %**	**4 %**
Acrylamide/Bis (37.5:1)	3.75 ml	5 ml	1 ml
Separating gel buffer	5 ml	5 ml	-
Stacking gel buffer	-	-	2.5 ml
De-ionised water	11 ml	9.7 ml	6.3 ml
10 % (w/v) SDS stock	200 µl	200 µl	100 µl
TEMED	10 µl	10 µl	10 µl
10 % ammonium persulfate (APS)	100µl	100 µl	50 µl

2.6.3.2 Quantitation of proteins with Bradford assay

The quantitation of proteins was performed with the Bio-Rad Protein Assay using the method generated by Bradford (Bradford, 1976). The Protein Assay is combined of Coomassie brilliant blue G250, acetic acid and methanol. Coomassie blue stain creates in acetic solutions complexes with cationic and nonpolar hydrophobic side chains of proteins. Thereby the absorption maximum shifts from 430 nm to 595 nm. The photometric measurements were performed using the qQuant on Com2 plate reader (Biotec instruments) in amounts of 1-20 µg ml^{-1} protein within 96 well plates. The qQuant on Com2 plate reader was regulated with the KC4 program (Biotec instruments). The Bio-Rad Protein Assay solution was mixed with 3x distilled water in a 1:5 ratio and transferred in 200 µl aliquots to the wells. The quantitation of unknown protein samples was started with the measurement of a BSA calibration curve. The unknown protein samples were analysed starting with the addition of 1 µl protein sample to 200 µl Protein Assay solution. The mix was incubated at room temperature for 5 minutes and analysed by measuring the absorbance at 595 nm. The protein concentrations were calculated using the calibration curve.

2.6.3.3 Quantitation of proteins with SYPRO Ruby

The SYPRO Ruby protein gel staining is a sensitive method that is used to stain proteins after size separation within polyacrylamid gels (2.6.3.1) with fluorescence dye. The stained gels were analysed using the Versa Doc Imaging System (Bio-Rad) with excitation of the fluorescence of SYPRO Ruby in the program PD Quest. The quantitative analysis of protein bands within 2D gels was performed using the program Quantity One. In order to identify exact protein amounts SDS-gels were loaded additionally with specific BSA amounts. After

size separation of protein samples using SDS-PAGE the gel was fixed 30 minutes in fixation solution containing 10 % (v/v) ethanol and 7 % (v/v) acetic acid. Afterwards the fixation solution was removed and the gel was stained using 50 ml of SYPRO Ruby solution (Bio-Rad), which was incubated over night in the dark. Afterwards the staining solution was removed; the gel was fixed further 30 minutes, washed in distilled water and finally analysed using the Versa Doc Imaging System (Bio-Rad).

2.6.3.4 N-terminal sequencing of proteins

For N-terminal protein sequencing the proteins were separated in a 7.5 % sodium dedecyl sulfate polyacrylamide gel by SDS-PAGE and transferred to a PVDF membrane using the Western blot method (Renart, 1979; Towbin, 1979). For the transfer the Trans-Blot Semi-Dry Electrophoretic Transfer cell (Bio-Rad) was used. PVDF membrane, SDS-gel and 2 thick filter papers were equilibrated 30 minutes in Towbin buffer containing 25 mM Tris, 192 mM glycin, 20 % of methanol and 0.1 % of SDS. The semi-dry western blot sandwich was assembled with a filter paper followed by the PVDF membrane, the gel and finally the second filter paper. The reaction was performed at 14 V for 60 minutes. The blotted PVDF membrane was stained in coomassie staining solution containing 0.1 % of coomassie R-250, 40 % of methanol and 10 % of acetic acid for 10 minutes. Afterwards the membrane was decolorised in a solution of 40 % methanol and 10 % acetic acid. The membrane was dried and the remaining protein bands of interest were cut; transferred to pure tubes and analysed using an ABI 494A Procise HT sequencer (Applied Biosystems) at the HZI (Helmholtz Zentrum für Infektionsforschung) Braunschweig.

2.6.3.5 Detection of proteins with immune assay

In order to identify expressed, recombinant proteins the Western blot method with subsequent antibody detection was performed. The Western blot method was performed as previously described (2.6.3.4) using a Nitrocellulose blotting membrane (Bio-Rad). The immune assay uses the antigen-antibody bonding principle and the enzymatic detection of antibodies. The blotted and washed Nitrocellulose membrane was incubated in 50 ml of blocking solution containing TBS [20 mM Tris base; 0.5 M NaCl (pH7.5)] and 3 % (w/v) of gelatine for 1 hour at room temperature. Afterwards the membrane was washed twice in washing buffer containing TBS and 0.05 % (v/v) of Tween 20. The first polyclonal antibody, which was generated to bind the SlfB S-layer protein of *Lysinibacillus sphaericus* JG-A12, was performed by Pineda Antikoerper Service, Berlin. The first antibody solution containing 30 ml of antibody buffer solution [TBS; 0.05 % (v/v) of Tween 20, 1 % of gelatine] and 4 µl

Anti SlfB antibody was added to the membrane, incubated at 250 rpm and room temperature for 1 hour. The membrane was washed twice in washing buffer followed by the addition of the secondary antibody solution containing 20 ml antibody buffer and 7 µl of antibody conjugate goat anti rabbit IgG (Bio-Rad Immun-Blot Assay Kit). The solution was removed after incubation for 1 hour at 250 rpm and room temperature, the membrane was washed twice and equilibrated for 10 minutes in TBS. The staining of the bond secondary antibodies was performed using 1 ml of AP Color-Development-Buffer stock solution mixed with 24 ml of distilled water and supplemented with 250 µl AP-Color-Reagent A and 250 µl AP-Color-Reagent B (Immun-Blot Assay Kit, Bio-Rad). The membrane was stained for 30 seconds with the solution which was removed and the reaction was stopped by the addition of distilled water. The results were analysed using the Versa Doc Imaging System (Bio-Rad).

2.6.3.6 *ß*-galactosidase assay

The *ß*-galactosidase assay was performed with the method described by Miller (Miller, 1972)**.** *ß*-galactosidase catalyses the hydrolysis of *ß*-galactosides into monosaccharides. The quantitative analysis of the activity of *ß*-galactosidase was performed using ONPG (*o*-nitrophenyl-*ß*-D-galactopyranoside). The production of *ß*-galactosidase by *E. coli* is contingent on the presence of an activator of the *lac* operon. The addition of IPTG to *E. coli* cells activates the promoter which transcribes the cloned gene of the vector. Coevally, the *lac* operon is induced. The *ß*-galactosidase is present in the cytoplasm and hydrolyses galactopyranoside like lactose to glucose and galactose. Normally, galactose is found exclusively in the cytoplasm of cells. Therefore galactose can be used as indicator for monitoring cell damages.

The *ß*-galactosidase assay started with the combination of 50 µl cell protein fraction and 50 µl Z-buffer in a microcentrifuge tube. The Z-buffer stock solution contains 4.27 g Na_2HPO_4, 2.75 g NaH_2PO_4 x H_2O, 0.375 g KCl and 0.125 g $MgSO_4$ x $7H_2O$ in a final volume of 500 ml de-ionised water at pH 7.0. The always freshly prepared Z-buffer working solution is composed of 50 ml Z-buffer stock solution supplemented with 0.14 ml *ß*-mercaptoethanol. The reaction batches containing the Z-buffer and cell fractions were incubated at 30 °C for 2 minutes in the Thermomixer comfort (Eppendorf) and 20 µl of ONPG (4 mg ml^{-1}) were added to the mix. The tubes were incubated at 30 °C and 250 rpm until observing a change of the solution colour. The reaction was stopped by the addition of 50 µl 1 M Na_2CO_3 to the mix. The absorptions at 420 nm, 550 nm and 650 nm were measured with the µQuant

Microplate Spectrophotometer with the setting KC4 (Bio-Tek Instruments). The ß-galactosidase activity was calculated with the following formula:

$$Activity = \frac{(OD420 - (1.75 \times OD550))}{OD650 \times time \times vol} \times \frac{1nmol}{0.0045mlcm} \times 0.17ml$$

2.7 Staining methods of filamentous *E. coli* cells

2.7.1 Live/Dead stain

The L-7007 live/dead BacLight bacterial viability kit (Molecular Probes) was used in order to distinguish living and dead bacteria. The stain Syto 9 which is a green-fluorescent nucleic acid stain and the stain propidium iodide which is a red-fluorescent nucleic acid stain differ both in their spectral characteristic and their ability to penetrate healthy bacterial cells. Syto 9 generally labels all bacteria in a population – those with intact and those with damaged membranes. Propidium iodide penetrates only bacteria with damaged membranes and causes a reduction of the Syto 9 stain fluorescence when both dyes are present. Cells can be distinguished by microscopic analyses. Cells with intact membranes are stained with a green colour and damaged cells are red coloured.

The reaction started with the harvest of 100 µl S-layer expressing *E. coli* BL21(DE3) in the stationary phase at 10,000 x g for 5 minutes. The cells were washed in 1 ml of 0.9 % NaCl and suspended finally in 333 µl of 0.9 % NaCl. A mix of 0.5 µl Syto 9 and of 0.5 µl propidium iodide was added to the cells which were incubated on ice for 15 minutes in the dark. Afterwards the cells were washed in 0.9 % of NaCl. Microscopic analyses were done at extension/emission wavelengths of 480/500 nm for the SYTO 9 stain and at 490/635 nm for propidium iodide.

2.7.2 Staining of DNA by DAPI

In order to verify the disordered cell division with another method, S-layer expressing *E. coli* cells were stained with the DNA stain DAPI (Molecular Probes). DAPI (4',6-diamidino-2-phenylindole) is a AT specific minor groove-binding agent which penetrates intact cell membranes and exhibits an absorption maximum at a wavelength of 365 nm and an emission maximum at a wavelength of 450 nm (Russell et al., 1975; Tanious et al., 1992). S-layer expressing *E. coli* were harvested in the exponential and stationary growth phase, washed with TBS-Buffer [20 mM Tris-HCl, 150 mM NaCl, pH 7.5] and treated with 3 % DAPI-

solution [1 mg/ml DAPI in TBS] for 10 minutes. Subsequently these cells were washed again in TBS-Buffer and analysed with the Olympus microscope BX61 with incident fluorescent illumination (UV excitation and U-MNU-filter, 360-410 nm) (Tu et al., 1998).

2.7.3 Staining of membranes

In order to verify the membrane character of tubes which were expressed by S-layer expressing *E. coli* cells the membranes were stained with the membrane stain FM® 5-95 Lipophilic Styryl Dye (Molecular Probes). FM dyes are water soluble, virtually non-fluorescent in aqueous media and nontoxic to cells. These dyes are believed to insert into the outer leaflet of the surface membrane where they become intensely fluorescent. The method of membrane labelling has been used amongst others to selectively visualise plasma membrane in cultured bacteria. S-layer expressing *E. coli* BL21 were harvested in the exponential and in the stationary phase, washed in ice-cold HBSS buffer [137 mM NaCl, 1.26 mM $CaCl_2$ x 2 H_2O, 5.36 mM KCl, 4.16 mM $NaHCO_3$, 0.44 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 0.81 mM $MgSO_4$, 0.49 mM $MgCl_2$ x 6 H_2O, 5.55 mM glucose, pH 7.1] and stained for 1 min in membrane stain solution [5 µg/ml FM® 5-95 in ice-cold HBSS]. The cells were analysed with the Olympus microscope BX61 with incident fluorescent illumination (UV excitation and U-MSWG filter, 480-570 nm).

2.8 Microscopic and spectroscopic methods

2.8.1 Light and Fluorescence microscopy

Light microscope images of cells and purified tubes were taken with the Olympus BX61 microscope (Olympus Life Science) in phase-contrast mode. Cells expressing GFP fusion proteins were visualised by transmission through the GFP-Filter U-MNIBA2. Fluorescence microscope images of cells which were stained with the Live/Dead stain Kit (2.7.1) were taken with the filters U-MSWG (480-570 nm) and U-MSWB (420-460 nm). DAPI stained cells (2.7.2) were analysed with the filter U-MNU (360-410 nm) and fluorescence microscope images of membrane stained cells (2.7.3) were taken with the filter U-MSWG (480-570 nm). The fluorescence microscope filters were purchased from Olympus Life Science.

2.8.2 Atomic force microscopy

Atomic force microscopy (AFM) images of filaments and tubes were taken with the MFP-3D-Bio (Asylum Research) using AC-mode in air and in liquid. The in-air samples were applied on silicon wafers dried and scanned using OMCL AC240 cantilever (Olympus Life Science).

The in-liquid filaments and tube-like structures were incubated overnight at room temperature and fixed with 2 % glutaraldehyde in PBS, immobilised on silicon wafers and scanned in buffer using OMCL AC40 and OMCL TR400 cantilever in AC-mode (Olympus Life Science).

2.8.3 Transmission electron microscopy

For transmission electron microscopy (TEM) investigations with embedded cells and tubes, the samples were firstly fixed with 2 % of glutaraldehyde in PBS buffer consisting of 137 mM NaCl, 2.68 mM KCl, 8.1 mM Na_2HPO_4 and 1.47 mM KH_2PO_4 (pH 7.4) at room temperature. Afterwards the cells were fixed with 1 % of osmiumtetroxyd before being dehydrated in an increasing concentration of acetone (including a staining step with 1 % of uranylacetat) and embedded in Epoxy resin (Serva) according to the method described by Spurr (Spurr, 1969). Ultrathin sections (about 50-300 nm) of samples were prepared with a Leica EM UC6 ultramicrotome (Leica Microsystems) using a diamond knife (Diatome), and mounted on pioloform-coated copper grids (Plano). The air-dried samples were stored under dry conditions. The investigations were carried out with a Titan 80-300 transmission electron microscope (FEI) at 200 keV.

The polyelectrolyte capsules were air-dried for about 24 hours on carbon-coated copper grids (Plano). The morphology and chemical composition of the polyelectrolyte capsules and the Pd(0) nanoparticles were evaluated using a Titan 80-300 transmission electron microscope (FEI) at 300 keV.

2.8.4 Scanning electron microscopy and Energy dispersive X-ray spectroscopy

Samples for scanning electron microscopy and Energy dispersive X-ray spectroscopy investigations were prepared on RCA purified Si wafers (Kern and Puotinen, 1970). Samples were dried for about 24 hours at room temperature and analysed later with the scanning electron microscope. Scanning electron microscopy (SEM) images of polyelectrolyte capsules and Pd(0) nanoparticles were obtained using the crossbeam workstation NVision 40 (Carl Zeiss SMT) at 5 keV. Energy dispersive X-ray spectroscopy **(**EDX) analyses were obtained after activation scanning electron microscopy (SEM) with the EDX system Quantax 400 (Bruker AXS) with the Si-drift detector XFlash 123 eV.

2.8.5 IR-spectroscopy

The tubes of SllB_1 and SllB2-GFP were treated with chloroform and methanol for total lipid extraction (2.6.2.6). The resulting lipidic and lipid-free phases, as well as the precipitated

tube-associated proteins were analysed by IR-spectroscopy. Aliquots (10-20 µl) of the respective fractions were dried on a diamond ATR-cell (Resultec) and measured at room temperature. Spectra were calculated from averaging 256 interferograms recorded at 2 cm^{-1} resolution with a vector22 Fourier-transform infrared spectrometer (Bruker).

2.9 Development of applications for filamentous *E. coli*

Filamentous S-layer expressing *E. coli* were coated with polyelectrolytes (2.9.1), the cellular core was removed and the surfaces of the polyelectrolyte capsules were coated with S-layer polymer proteins (2.9.3). Pd(0) nanoparticles were synthesised on S-layer polymer coated polyelectrolyte capsules (2.9.4). These samples were analysed with light and fluorescence microscopy (2.8.1), transmission electron microscopy (2.8.3), scanning electron microscopy and Energy dispersive X-ray spectroscopy (2.8.4).

2.9.1 Preparation of polyelectrolyte capsules

Filamentous *Escherichia coli* cells were harvested in the stationary phase at OD_{600} = 2 and a pellet of at least 100 mg biomass was washed twice with 1 ml of 100 mM NaCl solution pH 7. The cells were fixed in the following step in 1 ml of 2 % glutaraldehyde (Serva) at room temperature for one hour as described elsewhere (Heard and Seaman, 1961; Moya et al., 2001). Afterwards the fixed cells were washed twice in 1 ml of 100 mM NaCl solution at pH 7 and six layers of freshly prepared PSS and PAH solutions were adsorbed onto the cells in the presence of 100 mM NaCl beginning with the polyanion. The polyelectrolytes (PE) sodium poly(styrene sulfonate) (PSS) (Sigma) of M_w ~70,000 Da and poly(allylamine hydrochloride) (PAH) (Sigma) of M_w ~56,000 Da were dissolved to a concentration of 1 mg ml^{-1} in 100 mM NaCl (Roth) solution pH 7. The final pH value of PSS solution was pH 6 and the pH value of PAH solution was pH 5. Each coating step lasted 10 minutes and was followed by four washing steps with 100 mM NaCl. After each step the cell pellet was concentrated by centrifugation at 12,000 x g at room temperature for 3-5 min. To avoid cell agglomeration the cell pellet was resuspended in 150 µl of 100 mM NaCl before addition of polyelectrolyte solution. In the following deproteinisation step with 1.2 % NaOCl the cells were destroyed, while the hollow polyelectrolyte capsules remained. The NaOCl solution was purchased from Sigma and diluted to a chlorine content of 1.2 % (Georgieva et al., 2004). Capsules were washed four times in 100 mM NaCl to remove residual NaOCl.

2.9.2 Linking of fluorescence dye to S-layer proteins

The fluorescence dye HiLyte Fluor™ 488 amine (MobiTec) was chosen for labelling of the S-layer proteins which were purified as described elsewhere (Raff et al., 2003). For coupling reactions the S-layer proteins were dissolved in 50 mM MES-buffer (Roth) at pH 5.6 and linked with the help of 200 µM cross-linker EDC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimid) (Sigma) to HiLyte Fluor™ 488 amine. The reaction took two hours. Afterwards uncoupled fluorescence dyes were removed by centrifugation and fluorescence labelled S-layer protein polymers were washed with buffer.

2.9.3 Coating of polyelectrolyte capsules with surface layer proteins

The natural S-layer proteins of *Lysinibacillus sphaericus* JG-A12 were purified as described elsewhere (Raff et al., 2003). The polyelectrolyte tubes were washed and resuspended in 1 ml of 10 mM $CaCl_2$ solution. Subsequently, 200 µg ml^{-1} of native or fluorescence labelled S-layer polymers were added to the polyelectrolyte capsule solution and bound to the surface of the polyelectrolyte tubes. The solution was stirred at room temperature for 20-24 hours. Afterwards the solution was concentrated by centrifugation at 12,000 x g at room temperature for 3-5 min and washed twice with de-ionised water. The supernatants were removed.

2.9.4 Synthesis of Pd(0) nanoparticles

Pd(0) nanoparticles were synthesised as described elsewhere (Fahmy et al., 2006). The S-layer polyelectrolyte tubes were concentrated by centrifugation. For this 2 mM Na_2PdCl_4 (Sigma) was dissolved in water and incubated overnight in the dark. The coating was started by addition of 10 ml Na_2PdCl_4 solution to the polyelectrolyte capsules. After 4 hours of incubation at room temperature under shaking in the darkness the tubes were washed twice in de-ionised water. Afterwards the bound Pd(II) was reduced by the addition of 30 µl of 100 mM dimethylamine-borane (Merck) (Fahmy et al., 2006). The sample was centrifuged and the pellet was washed twice and finally stored in de-ionised water. The Pd-solution was prepared 24 hours before usage.

2.10 Sources of supply

The sources of supply for instruments, materials and chemicals are listed in the tables M9 and M10.

Table M9. Sources of instruments.

Company	Instruments
Asylum Research, Santa Barbara, USA	Atomic force microscope MFP-3D-Bio
Biometra, Göttingen, Germany	T3 Thermocycler, Bio Doc Analyze System
Bio-Rad, Hercules, USA	Mini-PROTEAN II electrophoresis cell, Versa Doc 3000 Imaging System, Trans-Blot Semi-Dry Electrophoretic Transfer cell, Power Pac 300
Biotec Instruments Inc.	qQuant on Com plate reader, KC4 program
Branson, Schwäbisch Gmünd, Germany	Sonifier W250-D
Bruker, Karlsruhe, Germany	EDX system Quantax 400, vector22 Fourier-transform infrared spectrometer
Carl Zeiss SMT, Oberkochen, Germany	Crossbeam workstation NVision 40
CLC bio, Aarhus, Denmark	Genomics Workbench
Eppendorf AG, Hamburg, Germany	Thermomixer comfort, Microcentrifuge 5415R, Centrifuge 5804R, Concentrator 5301
FEI, Oregon, USA	Titan 80-300 transmission electron microscope
IKA Labortechnik, Stauffen, Germany	Rotating-blade bender IKA T8
Illumina, San Diego, USA	Hi Seq 2000
Leica Microsystems, Wetzlar, Germany	Leica EM UC6 ultramicrotome
Memmert, Schwabach, Germany	Incubator
Microfluidics Corporation, Newton, USA	M-110S Microfluidizer processor
Olympus Life Science, Hamburg, Germany	Olympus BX61 microscope
PE Applied Biosystems, Foster City, USA	ABI 494 Procise HT Sequencer, ABI PRISM 310 Genetic Analyzer, DNA Sequencing software
Peqlab Biotechnologie GmbH, Erlangen, Germany	PerfectBlue Gelsysteme Maxi M, Monochrome 8-Bit-CCD camera
Pharmacia Biotech, Cambridge, Great Britain	Ultrospec UV/Vis spectrometer
ThermoFisher Scientific, Waltham, USA	NanoDrop 2000/2000c UV/Vis Spectrophotometer
Tuttnauer, Breda, The Netherlands	autoclave 2540 EL

Table M10. Sources of materials and chemicals.

Company	Chemicals and Materials
Analytic Jena, Jena, Germany	InnuScript Reverse Transcriptase, InnuPrep RNA Mini Kit
Becton Dickinson, Heidelberg, Germany	Yeast extract, meat extract
Bio-Rad, Hercules, USA	40 % of Acrylamid/Bis (37.5:1); Nitrocellulose blotting membrane, Immun-Blot Assay Kit, Bradford Protein Assay, SYPRO Ruby, PVDF blotting membrane
Biozym, Hessisch Oldendorf, Germany	Midori Green Direct, DNaseI
Boeringer, Ingelheim am Rhein, Germany	Shrimp alkaline Phosphatase
Clontech, Mountain View, USA	pGFP vector
Diatome, Biel, Switzerland	Diamond knife
Difco, Augsburg, Germany	Bacto Tryptone, Bacto Peptone
EdgeBio, Gaithersburg, USA	Quick Step™ 2 PCR Purification Kit
Epicentre Biotechnologies, Madison, USA	MasterPure Gram positive DNA Purification Kit

Fermentas, Ontario, Canada	Fast Digest enzymes, T4-DNA-Ligase, Pfu-DNA Polymerase, PageRuler™ Unstained Protein Ladder, dNTPs'
Fluka, Buchs, Switzerland	Ammonium peroxosulfate, sodium acetate waterfree, uranylacetate
Invitrogen, Carlsbad, Germany	Agarose, DNA Ladder
Merck Millipore, Darmstadt, Germany	LiChrosolv ultra pure water
Merck, Darmstadt, Germany	D-(+)-glucose-monohydrate, $MgSO_4$ x $7H_2O$, dimethylamine-borane, kanamycindisulfate, NaOH, acetone, n-butanol, $MgCl_2$ x $6H_2O$, $NaHCO_3$, Na_2CO_3, $CaCl_2$ x $2H_2O$, $(NH_4)_2SO_4$, NaH_2PO_4 x H_2O, KH_2PO_4
MoBiTec, Göttingen, Germany	HiLyte Fluor™ 488 amine
Molecular probes, Life technologies GmbH, Darmstadt, Germany	L-7007 live/dead BacLight bacterial viability kit, DAPI, FM® 5-95 Lipophilic Styryl Dye
New England Biolabs, Ipswich, USA	BSA
Novagen, Gibbstown, USA	pET-30 Ek/LIC cloning Kit, BugBuster Protein extraction reagent
Olympus Life Science, Hamburg, Germany	OMCL AC 240 cantilever, OMCL AC40 cantilever, OMCL TR400 cantilever
PE Applied Biosystems, Foster City, USA	BigDye® Terminator v1.1 Cycle Sequencing Kit, HiDi®-Formamid
Plano, Wetzlar, Germany	pioloform-coated copper grids, carbon-coated copper grids
Prolab Scientific, Laval, Canada	Ethanol (95 %)
Promega, Madison, USA	Wizard® Plus SV Minipreps DNA purification system
Resultec, Illerkirchberg, Germany	Diamond ATR cell
Riedel de Haen, Seelze, Germany	KCl
Roth, Karlsruhe, Germany	DEPC, BrilliantBlue G250, BrilliantBlue R250, Guanidine hydrochloride, glycerol, urea, EDTA, MES, NaCl, TEMED, RNase away solution, osmiumtetroxide, acetic acid, ethanol (pure), isopropanole (pure), methanol (ultra gradient grade), IPTG, Tris, Tris-HCl, *o*-H_3PO_4, DTT, chloroform, glycine, Na_2HPO_4 x $2H_2O$, dialysis tubings
Sarstedt, Nümbrecht, Germany	syringe filter Filtropur S
Sartorius, Göttingen, Germany	Vivaspin Concentrators
Serva, Heidelberg, Germany	glutaraldehyde (25 %), Epoxy resin
Sigma, Taufkirchen, Germany	boric acid (99 %), HEPES, Na_2-EDTA, PAH, PSS, sucrose, SDS, Triton-X-100, NaOCl, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimid, Na_2PdCl_4, Ampicillin, *ß*-mercaptoethanol, *o*-nitrophenyl-*ß*-D-galactopyranoside, DNase II, RNase A
VWR, Darmstadt, Germany	HCl

3 Experiments, Results and Discussion

3.1 Identification of multiple putative S-layer genes partly expressed by *Lysinibacillus sphaericus* JG-B53

Authors Lederer FL, Weinert U, Günther TJ, Raff J, Weiß J, Pollmann K
Submitted to Microbiology (2012)

Abstract *Lysinibacillus sphaericus* JG-B53 was isolated from the uranium mining waste pile Haberland near Johanngeorgenstadt, Germany. Previous studies have shown that many bacteria that have been isolated from these heavy metal contaminated environments possess surface layer proteins (S-layers) which enable the bacteria to survive by binding metals with high affinity. Conversely, essential trace elements are able to cross the filter layer and reach the interior of the cell. This is especially true of the S-layer of *Lysinibacillus sphaericus* JG-B53 which is therefore of high interest for both environmental studies and technical applications. Particularly the latter due to the high amounts isolatable from biomass and the outstanding recrystallisation and metal binding properties.

In this study, S-layer protein gene sequences encoded in the genome of *L. sphaericus* JG-B53 were identified using next generation sequencing (NGS) technology followed by bioinformatic analyses. The genome of *L. sphaericus* JG-B53 encodes at least 8 putative S-layer protein genes with distinct differences. Using mRNA analyses the expression of the putative S-layer protein genes was studied. The functional S-layer protein B53 Slp1 was identified as dominantly expressed S-layer protein in *Lysinibacillus sphaericus* JG-B53 by mRNA studies, SDS PAGE and N-terminal sequencing. B53 Slp1 is characterised by square lattice symmetry and a molecular weight of 116 kDa.

The S-layer protein B53 Slp1 shows a high similarity to the functional S-layer protein of *Lysinibacillus sphaericus* JG-A12, being isolated from the same uranium mining waste pile Haberland described by previous research. These similarities indicate horizontal gene transfer and DNA rearrangements between these bacteria. The presence of multiple S-layer gene copies may enable the bacterial strains to quickly adapt to changing environments.

3.2 Heterologous expression of the surface-layer-like protein SllB induces the formation of long filaments of *Escherichia coli* consisting of protein-stabilized outer membrane

Authors Lederer FL, Günther TJ, Flemming K, Raff J, Fahmy K, Springer A, Pollmann K.

Published in Microbiology 156(Pt 12):3584-3595 (2010).

Abstract

Escherichia coli is one of the best studied microorganisms and is the most used host in genetic engineering. The Gram-negative single cells are rod-shaped and filaments are usually not found. Here we describe the reproducible formation of elongated *E. coli* cells. During heterologous expression of the silent S-layer protein gene *sllB* from *Lysinibacillus sphaericus* JG-A12 in *E. coli* Bl21 (DE3), the cells were arranged as long chains which were surrounded by highly stable sheaths. These filaments had a length of >100 µm. In the stationary growth phase, microscopic analyses demonstrated the formation of unusually long transparent tube-like structures which were enclosing separate single cells. The tube-like structures were isolated and analyzed by SDS-PAGE, IR-spectroscopy and different microscopic methods in order to identify their unusual composition and structure. The tube-like structures were found to be like outer membranes, containing high levels of proteins and to which the recombinant S-layer proteins were attached. Despite the entire structure being indicative of a disordered cell division, the bacterial cells were highly viable and stable. To our knowledge, this is the first time that the induction of drastic morphological changes in *E. coli* by the expression of a foreign protein is reported.

3.3 *E. coli* filament formation induced by heterologous S-layer expression

Authors Lederer FL, Günther TJ, Raff J, Pollmann K.

Published in Bioengineered Bugs 2(3):178-181 (2011).

Abstract *Escherichia coli* is a rod-shaped intestinal bacterium which has a size of 1.1-1.5 µm x 2.0-6.0 µm. The fast cell division process and the uncomplicated living conditions have turned *E. coli* into a widely used host in genetic engineering and into one of the best studied microorganisms of all. We used *E. coli* BL21(DE3) as host for heterologous expression of S-layer proteins of *Lysinibacillus sphaericus* JG-A12 in order to enable a fast and high efficient protein production. The S-layer expression induced in *E. coli* an unusual elongation of the cells, thus producing filaments of >100 µm in length. In the stationary growth phase, *E. coli* filaments develop tube-like structures that contain *E. coli* single cells. Fluorescence microscopic analyses of S-layer expressing *E. coli* cells that were stained with membrane stain FM® 5-95 verify the membrane origin of the tubes. Analyses of DAPI stained GFP-S-layer expressing *E. coli* support the assumption of a disordered cell division that is induced by the huge amount of recombinant S-layer proteins. However, the underlying mechanism is still not characterized in detail. These results describe the occurrence of a novel stable cell form of *E. coli* as a result of a disordered cell division process.

3.4 Development of functionalised polyelectrolyte capsules using filamentous *Escherichia coli* cells

Authors Lederer FL, Günther TJ, Weinert U, Raff J, Pollmann K.

Published in Microbial Cell Factories 11:163 (2012).

Abstract

Background: *Escherichia coli* is one of the best studied microorganisms and finds multiple applications especially as tool in the heterologous production of interesting proteins of other organisms. The heterologous expression of special surface (S-) layer proteins caused the formation of extremely long *E. coli* cells which leave transparent tubes when they divide into single *E. coli* cells. Such natural structures are of high value as bio-templates for the development of bio-inorganic composites for many applications. In this study we used genetically modified filamentous *Escherichia coli* cells as template for the design of polyelectrolyte tubes that can be used as carrier for functional molecules or particles.

Diversity of structures of biogenic materials have the potential to be used to construct inorganic or polymeric superior hybrid materials that reflect the form of the bio-template. Such bio-inspired materials are of great interest in diverse scientific fields like Biology, Chemistry and Material Science and can find application for the construction of functional materials or the bio-inspired synthesis of inorganic nanoparticles.

Results: Genetically modified filamentous *E. coli* cells were fixed in 2 % glutaraldehyde and coated with alternating six layers of the polyanion polyelectrolyte poly(sodium-4styrenesulfonate) (PSS) and polycation polyelectrolyte poly(allylamine-hydrochloride) (PAH). Afterwards we dissolved the *E. coli* cells with 1.2 % sodium hypochlorite, thus obtaining hollow polyelectrolyte tubes of 0.7 µm in diameter and 5-50 µm in length. For functionalisation the polyelectrolyte tubes were coated with S-layer protein polymers followed by metallisation with Pd(0) particles. These assemblies were analysed with light microscopy, scanning electron microscopy, energy dispersive X-ray spectroscopy and transmission electron microscopy.

Conclusion: The thus constructed new material offers possibilities for diverse applications like novel catalysts or metal nanowires for electrical devices. The novelty of this work is the use of filamentous *E. coli* templates and the use of S-layer proteins in a new material construct.

4 Conclusion

Strategies to handle difficult and fast changing environmental conditions were developed most efficiently by prokaryotes. In the present work, the genomes of several bacteria, which were isolated from soil samples from a uranium mining waste pile Haberland that is located near the town of Johanngeorgenstadt, were sequenced. The genome sequences were analysed in order to identify genes which are involved in the surviving strategies of these bacteria. In this study, the genome sequencing data of the strain *Bacillus* sp. JG-B53 were analysed in detail particularly regarding putative S-layer proteins. Within the genome of *Bacillus* sp. JG-B53 15 putative S-layer protein genes were detected indicating that S-layer proteins play an essential role in the heavy metal defence and tolerance strategy of this strain. Furthermore, up to know three heavy metal specific transporter proteins were detected within the *Bacillus* sp. JG-B53 that could be additionally part of the heavy metal tolerance mechanism of these cells. The identified putative S-layer protein genes as well as several putative heavy metal transporter protein genes point to the well adaption of the uranium mining waste pile isolate *Bacillus* sp. JG-B53 to its heavy metal polluted environment.

Furthermore, the present study has demonstrated that drastic changes in the morphology of *E. coli* cells are induced by the expression of the S-layer-like protein SllB derived from the uranium mining waste pile isolate *Lysinibacillus sphaericus* JG-A12. The heterologous expression of SllB was induced by the addition of IPTG while growing at room temperature. Extraordinary amounts of recombinant proteins were expressed and localised in the culture medium, the periplasm and the cytoplasm of the cells pointing to the secretion of the proteins. Using SDS-PAGE and enzyme assays the secretion was verified by the detection of SllB accompanied by the absence of cytoplasmic enzymes in the supernatant and the periplasm of SllB expressing *E. coli* cells. However, the transport pathway was not identified yet. SllB expressing *E. coli* cells presented the formation of filamentous cells with extraordinary length that point to the inhibition of the cell division in the exponential phase. In the stationary phase the cells produced stable long tubes exhibiting an outer-membrane-like structure associated with the recombinant protein. *E. coli* single cells were observed to leave the tube-like structures. The stability of the outer-membrane like tubes was contributed with the affinity of S-layer proteins to lipid membranes. Additionally, the interactions of SllB with cellular components in the cytoplasm that possess essential properties in the cell division process were blamed to induce the formation of filamentous cells. This is probably the first description of a massive alteration in cell morphology in response to the expression of a recombinant protein. These microtubes are highly interesting for technical applications such as the generation of

microcontainers or microwires by metallisation. The unexpected extracellular secretion process of recombinant SllB needs further investigation.

The newly designed bio-functionalised polyelectrolyte tubes that are described here are unique due to its starting material. Specific regulations of template organism, temperature and amount of activator induce the formation of *Escherichia coli* filaments with defined diameter and cell wall stability. The template bacteria provide up to several 100 µm long structures with defined 0.8-1 µm in diameter which were encapsulated by layer-by-layer method with polyelectrolytes. After removing the bacterial core these polyelectrolyte hollow capsules can be bio-functionalised with S-layer polymer proteins which support the synthesis of metal nanoparticles in the protein pores. These filamentous polyelectrolyte tubes may provide an interesting matrix for the development of microcontainers and metal microwires with possibly novel physical and chemical properties. In combination with S-layer coupled palladium nanoparticles these materials could find application as novel catalysts or metal microwires in electrical devices.

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6 Attachments

Vector card of the used pET vector (Novagen)

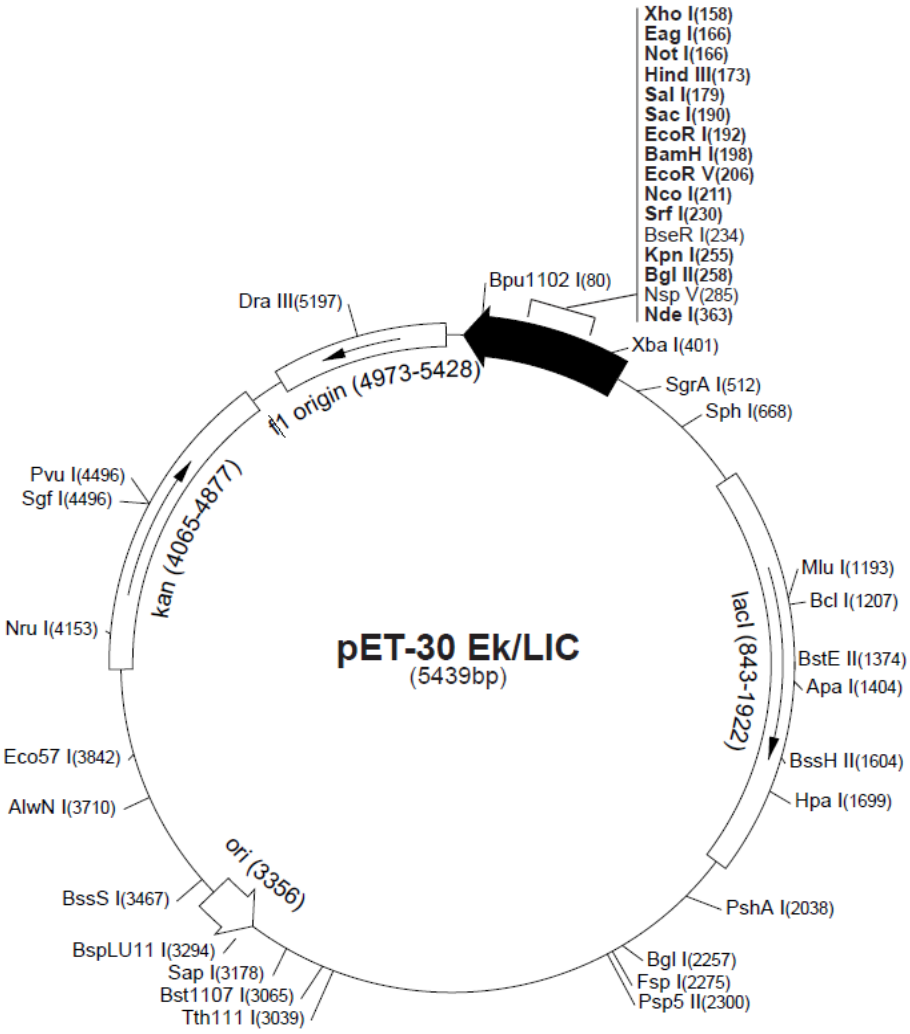

Figure A1. pET-30 Ek/LIC vector card (Novagen)

Gene and protein sequences of putative S-layer proteins of *Bacillus* sp. JG-B53

The italic blocks in front and behind of the putative B53 S-layer gene sequences show the upstream and downstream regions of these genes, respectively. The red underlined letters mark the -35 box, the -10 box and the ribosome binding site in each upstream region and in the downstream region the inverted repeat is marked with red underlined letters, respectively.

B53 *slp1*

TTTTACTTGAAAAATAACCTTAGATAGCCGACAATGGTAAAAGAGAGTTAGACGTCCTATTTTTTCCGGAAA
TGAATTAGAAACTTTCCCAAAATATGATTGACAACTTTTAGGGATACAACTATACTTTTATGTGTAGTTGTTTA
TTTACTGCATATCTATGTGGTATAAACATAATTTTTGAACTTAGAGGAGGAAATTTTTCT

ATGGCTAACCAACCAAAGAAATACAAAAAATTCGTAGCTACGGCTGCAACAGCTACTTTAGTAGCT
TCTGCTATCGTACCAGTGGCTTCTGCAGCAGGATTCTCAGATGTAGCAGGTAACGACCACGAGGTA
GCAATCAACGCACTAGTTGAAGCAGGTATCATCAATGGATATGCTGATGGCACATTCAAACCAAAC
CAATCTATTAACCGCGGTCAAGTGGTAAAATTATTAGGTCGTTGGTTAGAAGCTCAAGGTCAAGAA
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AGTACACTGTATCTCTTGGCGAAGACAAAATCGGTACTTTCAAAGGTATCGCAGCTGTAAATCCAA
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AGTTTTTGAAGCAGAAGCGATTGTTACTTCTGGATCGACTACTGTTACAGGATATAGCCAAAAATA
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AAAAGTTGTTGTGAAAATTGCACAAGCTAGCGCAATTCCTACTACTACTGCAATCACTGCGTTCAC
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AAAATAA

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ATACCACATAGCCTTTTTTCATATTTTCATGTTGTATACATAATACTTTCCAACAGAAATCTAAGAATCGATTA
ATC

B53 Slp1

MANQPKKYKKFVATAATATLVASAIVPVASAAGFSDVAGNDHEVAINALVEAGIINGYADGTFKPNQS
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ALQAEYTIDVTREGGEVAARGVNNGREYKVTVKDKDGKLAKNEIINVAFNEDLDRVISTETKAYFIDV
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DANHYNSLVDLAGKISADSKTGGVKAVVNAGSTGLDLTGNAKGENFTYKIGALTAVSTINGVVGKDA
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NSDVTIGSITLK

B53 *slp2*

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GTAGTATACAAGAAAGACATATATGGGAATAAACAACAGAATGCAGAACAAGAAAGAGTGTCCGT
AAATGAAGGAGATTATATTGAGTTGACACATCTTGAAGGCGGAAAACGGGCTATGATCACAAATG
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AAGAAGGTAGAAAAAGTACCAGAAGCAACAATTTTGGATGGTAACCAATTTACATGGTCGTTGAA
AGGTATTGGAGATTTTGAGTTTGCGAATGTGAACTTGAATAAATTGACTGAAGAAATACAAATCAA
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ATTATGAAAATAGACAATGGTGAACAAGAAAGTTTTGGGGAAAAAGTAGTTTATGAAGTCACTTCT
ACAGGTTTGTTAATAAAGTTAG

CAACGTTATTAAAAGGTTTAAACATTACAATCCATAATGAAGCGCATTTATATAAGGTTTCTATCCGTTTCTA
GCTTCATCTGAATTAGGTCCTTCCAAAAAGTAGCTTTA<u>ACTTCTGATAAATATGGGTTTTATGAAAAGT</u>GTAT
AAAAACGTAGTTTATTATTATTATGGCGTATGGTGGATGAATGAAAAGGAAGCGAATTTATCTCATGACGAC
GCTTTCTATGCCTT

B53 Slp2 [S-layer domain protein]

MMKRRQKQKTKKLVMATALFSMFATGITPSLEVFAEEQTQQQKVSNVLKNEKSVEVENRKFAVPGKG
DIAKFQNSERRDRQFSPYEPTGIYARPNEQIIIQVAGNQNIEAYIGTFSYDASWREDSKIKSFTLKPGSNTI
QSPNGGMIYFYNKQQGGTIQTTITTGGTATPLYELGKHTKQDLIDMLNQYPNAHTVELKGERVLITASP
ERVKKYLIGSNTDPTQLLKKLDEATRIQDKISGLSEEQVDKHYLHYVEDNNSLDYFMYAYPYRTAYVG

DAIQHVLDINKFINDGWGPWHEAGHMRQQNPWAFYNMTEVQNNIYSLAVEKAFGQPTRLEKEGVYPK
AFHYLEQQNKNYDEISDLFVKLAMLWQLHLAYGEEFYPKLHQSYRDMPDSMLPKNNEDKKQLFMIEA
SKAAQQNLMPFFEKWGLRPTNDTIRKVAALGYPNLTAEIWKSTDSNPIKPNTSESGNILDGNEFTWSLK
GIGDVEFAKVDLNKSTGEMQVELKAGVPHHYFDKMYASIKVQNLAGVVVYKKDIYGNKQQNAEQER
VSVNEGDYIELTHLEGGKRAMITNVDNGKQESFEEKGIYKVTEEGLKKVEKVPEATILDGNQFTWSLK
GIGDFEFANVNLNKLTEEIQINLNAGVPHNYFDKTYASIEVQNSSGQVVYNKNIYGNKQQNAELQKVPV
KIGDKIELTHLEGGHRATIMKIDNGEQESFGEKVVYEVTSTGLLIK

B53 *slp3*

ATCGTAAAGGCCAAGAGAGCACCGATGCTTCTTGGCTTTTTATAATACATAAAAGTAATTTCAAGACAGCTC
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CAGTATTCTAGCCATTATGAAAATATTTTAAAATTATACTCTCAGGGTGCCATTAGTGGGTTTGCAG
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TGCACTTCCCTTTAGGTATAGCCGATTATAGTATATAAAGATTTAACTAAAAAATCGAAAAGGCTATAGGTTG
CGAACAATAAACTAAACTCTATAAAGCAGTATAACAAGACATTCATCTCATTATGAACTGAGTCCGAATAGT
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B53 Slp3

MDKTKIQKVNKALIATVFATSGIAVVIPPPQKVAAATSPFIDINQYSSHYENILKLYSQGAISGFADKTFR
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RGQLAKILVLGFKFEVASNYNHSFQDVNSQTSNAAYIQTLVDLQVTEGTTPVTFSPFNAVTRGQIASFIV
RSQEKKSNATSYKVTGVEDDIIYINAEPYTVPESLSHIFNEYNADVLKGALIEGDLSGKTLYSVSKLTLN
ASGTSSRFLELDGENGSFGATIVVNGNYIEFSNVTLTGTMLVNETVRPPLHLDVSSYKPLSIGRVASNNV
SFINWSNPEKPEEDNSTNSKPSTDLQNWTEQNPDKDKPFVNWSKDKVTMKNVEKHIEFYNSSVSRLVV
SQSGTKIETNTKLPRVDIIGNVREFEIQGNIGTLNLDTETKLTIYGDSNIDWINYNSFTDLELYIDGRVGTL
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SISGVKVLNGSEIQADFHSNEVGTYYYYIVREKGAETPTKREMVNRHSVENVASGTGAAVNGTNSIKVSN
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FKTGALDNIHPFVEKTKLEPAGKENQFYVYFNEALDPESARNVNNYDLSGTVIVNTTGQSKIKPSAVEY
KEGDKKVLLTIPSETGFVYGDTLRVTVLPSVKDLAGNDFENANTVNAGENVRNYAEYVHEKLDMPVIS
IENVVSKPDQSDNFKRVEVEFKPNKAGTYYYMVLPDTVVDNGETKTFKQYLADKGITERDFVNEFASD
SAIKSGYFQLGGQDIYIERGSGPADLEEKTKKFPISISKDKLNPFLSYSVYMVLKDRGGEISKITSKEILGD
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VSVDRSNKINATFDFTFADPSVQPGEEVNFYYKVYSKTYSQTDIDKVKPIEVIDPNTSGALTSLSEGKGK
TPLTNGRTVQPITNNIFPFIEGDYIVIVIVDKYGNKYLVQDKISK

B53 *slp4*

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TCGATTACTTTTTTTAAT

B53 Slp4

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LSKVLKLEANDGKVFTDVPDTNMYLTDIQLAATAGIITGYADGSFKPDAAISRQHMAIMLERAIDYLKIP
KGTSSITFKDNASIIKDYRPAVAVGAHLGIINGSNGYFMPEKNATIGQAATFIQRLMLLSGDSAPDTSTY
AIKEIANGTLVGNQGFPSFDAADKALTKNTQVIVQKDKIVKMTSGYVVTNKYVALNSETIKDQIAVAG
NTEMEYISSDATQVKVRLAGQVGYLKQADVTLIPFSLSKGRSYYSNENGEIKHTLFDYNTNKYSSSYVY
GKAPAFMKQGEQYFSWNGINFTNGNGSSKGEAYNYYQFLPARATTQYTAEELDAYIMNKLAEMESTG
ITLYKDATTKSKLIGLGQTLKEVEANSKINAMLILALAQHESAYGMSEHAQKLNNLFGLYVYDTNPLN
KEFESVAVNINELVEKFLQPNYITPGGSPGRNYANGAVVGSKALGFNVKYASDPYWGAKIAGHYYRAE
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YISKEYIQVINTVK

B53 *slp5*

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TCTCTGATTTAAATATGACTTTATAAACTATTTAGGCAGTGGAAAGTTCGATGTAATCGAACTCTCCACTGTT
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B53 Slp5 [Glycoprotein]

MKQKYSKWVVGAASAALVASAIVPVASAASFSDIEDNDHKDAILALADAKIVGGYPDGTFKPNAVVT
RGNVTKFLGKWLVSENYEIPTDFATEARFTDLPTTAPDKELLQYAALVKDAGVFKGSNNKLMHTNNM
SREQMAVVLVRAIKTVYNVDLVADYKESDFKSTITDLDNATAEENREAIIALEYAGLTNVTAFNPKNSL
TRGQFASFLNRTITNLAEETLSVKAVKVVDATTLEVTLSDDSKHTVTLETALIENEETKVDFVIDGKSYS
AVVTYEVTELKVKSVDAVNAKTLSVTFNKAVETEKAKFELKKDGFKSNFSTITWNEDKTVATIELTSKI
TKGEFTVSVTGLSDQAVTGSVKTEDEKVAGIEILGEVAPSTGTTSATVGYQVTNQYGEDITKLNSSSLTL
SAAGADSAVANADGSITITKAAGLKEGDKVVLTVIHGSTATTTTKTVTVSAKTVVSEATIGTLYNKDGK
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VASIVASGDTNVIVIAKATGKNAQAAVKVAEGVRADSVTLGAPTKVVTAGADILFPLSVLDKQGNAIK
ETVALNGSKGITITGGTLFEKDGELFAKVAAGSVVENTPVTVVVTSSTGKVATQTVIPKAATTPKVITGL
DSKISTSIRELADAKVDITAKDIVVEDQFGQVISADELLAKLGTAGYTIQPFTDADAPFTVTGEIKDAVTN
KITVTYKAGATKTAANVTFKLVKTADNTAVEASSYSKQFSVVKDSSFASYKVEDIKPIYVTGDNTAGY
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ITINATGEEIVKDVTFSNVAPAVEKVAVVENNKAAAYIAGETVNFVTTNSYNVATDFNLDAFFQLADV
VVTDQYGVMATVAEADAAGVVKGQAKFNDVATATTTLTLSKVSGDVVFSANGTTAASAKGKANDVF
NARVNIGGQSATPVKVTAKVDF

B53 *slp6*

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ATTACACGTGAGCAGTTGGCAAAT

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TTGAGGCAATGCAGGCTAGTCGTGTCTTCCAAGAGTTTAAAACACAAGACTTTGGAGCCGATTATC
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AATCGATGACGAAGGAAACTATGAAGTAGTCGAATCTCAAAAATATAAGATTGTGATTACAAAAG
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TTGAAGCAGATTTAGGCTTTAATCCAAAGCATATTAAGTTAACAACTGCGGCAGGTCACGCAGTAA
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GGGCAAGTTATGCGCTGAAGTTAATGCAGGCGGGTGATTATATTGCTACATTCTCTGATGATGCTG
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CTGTAAACAGAGCAGGCAAAGGAGATGCCATTTTTGCTATTCGTCTAATTGGAGCAAAAGGGGAA
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TTAATAGGAAAATGCTGATAGAATCCGATAACAATAGAAGTATCTATATGAACCCGTTGATTTCCACTCCGG
CGGACGCTTTTCGCGGGCATGGCTTCAGTCCATATCTGTCAACTTTAGATTTCCAA<u>CCATTCATTTGTTTAT</u>
<u>TTTTTCAAGAAGAATGG</u>

B53 Slp6

MLTKAYQLNNYAYETTLPFTDVIKYSEAYYAVGPLYDNHITKGVTETTFGLKETVKRSQLALFINRIEA
MQASRVFQEFKTQDFGADYLEAFSYNNWTEDQEQEFFRIYSMNDGVKFEALSEGSGYFVLTGYTIDDE
GNYEVVESQKYKIVITKVDGQLQMTCQQTDEIAPSTSLFFEADLGFNPKHIKLTTAAGHAVSDKVYAYQ
PFEFDGWDEGSIPKGASYALKLMQAGDYIATFSDDAGKSVRVGIHAETDGYDLYTSHAVEKSSVFIPTS
EVGFAVTDYKIEQYTGAVHDHKIIDVTSSPEGVTVNRAGKGDAIFAIRLIGAKGEKLYMHGMIYELSGV
TSMYYELSTEKEMNGSFR

B53 *slp7*

GACGCTAGTTGGTTCGTCCGCGGAAAGCGAGTGGCTTTTTCCGCAACAAATTATTCATACTTTCTACAGTC
AGAAGCCTTCATTAAAATGAAGGCTTCTTTTTTGTATA<u>TTCACA</u>GGGTACGTCACTTATTTTA<u>GGGTAAATT</u>A
ATAATAAATTAACATAATTTGTATTGAGCGGCATAAACTATTTAGTAAA<u>GGAGACA</u>GACTCAT

ATGAGAAAATATATATATAATGTCATCATTATATTATTTGTTCTACAAATGAGTCATGTGCAAGTAT
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ACAATTAGCGCAAGATCATACATGGGATTATGCAAGTTACCCGGCAGCAACAAGTGAAGACTATTC
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TAATAAAGGAGGTGTCCCATTTTGCTTTCGGGGCACCTCCTTTCTCATGTGCAAGTTAA

TGCGCGTTTAACGCAGTATTTAAAATTTCCATATTGTTTTGCATTAACGTGAAGTATGTCTCATTATTTTTTAC
ATCATCCGTTGTTAAAACGCTTAAGTTATGCAGGACAAGTGATTCCGCACCAACTTCTTTTTGAATGACTTC
TGCTA

B53 Slp7

MRKYIYNVIIILFVLQMSHVQVFASYEQKTIATATTIETLQYQIQKEVMQLTTEFDIRYTGDTSALKDELT
ELIKHAIKDPYFYANISSFKWKYDGYANNIVIEFQFTYHISQKEADFVERTLTDIIAPMHGLSELEKLQAA
HDFIVLTSEYSKETKGSQYSPYTLLTENKGVCQAYALVLFRMLEMLGFEVQYVTGEVGDQLHAWVLV
KLDKDWYHIDVTWDDPLPDRQGEVRYNYFLLSDRQLAQDHTWDYASYPAATSEDYSALQQDSKVEV
MTKPLVYSNLNNDRGLSILGQNKLYTMQLQEQATHLEKRYTKSDEPLKIASYDMNKHMVFTRSYAYL
SVMPTIEEVEVAHLLLPSIHRTEKRMPQEKLVIIKEVSHFAFGAPPFSCAS

B53 *slp8*

ACATTTCATTTTAGTTGATTGATAAAGTAGAAGAGTTGTTTTAATAGGAATCGTAAAAAGTAAAAGAATCTGC
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CAAAAAGAAACAAAAGGTAAATTAACAGAAACGTTTAGCCTAAAACTAACAGCAAGCAATGGAGC
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CGTGCATTGGGGTTAGAAAACAAAGCACCAGACCCTGGTTATATTACGAAATTTGTTGATGATGCG
CAAATACCAAACTATTCCCGAGATGGTATATACATCGTCAATGAGTTAGGTTTAATGACAGGTGAT

CCTGTAACAGGTCGATTTAATCCAAATCAACCGTTAACACGGGCACAAGCATCTGTTATGCTTGAT
CGCTTCCTAAATTATTTAGAAAATGACTTAAAACAAAACTATCGAGACGATGTATTGTTCTTTAACT
AA

AGAAAGGACTGAAACGAATGACATATGCTAAAAAAGTAGTGCTAATACTTGCTTGTTTTGTACTCGTCTTAA
TGACAGCTACGCCAGTTACTCTAGCTGCCACGACGATTCAGGATGTTCCGACGAATAATAGTAAGTACAAA
GCGATTTCTTGGGCTGTCGATAATGATTTACTATCATTAAATGGTGCAAATAATTTCTTACCGAACGAACAG
GTA

B53 Slp8

MRKHISKVMLAFLVVFVIVANIPVDASAASAETTGAVKNEYTYEEAVFITGTPIVFKGTSKDIKITQKET
KGKLTETFSLKLTASNGATLTRNMAYESDVVDYATIGQKTSNGVVKKYSEKIVAGNITYTLVDFQFSQ
GTVTDNRAASDYFSGNVISRKTYTYQTGTGKNAKKNTVTVDTDSRHVGYENFWGATETQITESIYSYS
NGTTSHVKNRLSTSKSRVINYEENPASLASFDGGYAVVSEKDVISEYTYDLASGAKGTVDLDTEYMPTI
ERLIIPKFRDLTNHYAKDAVDKLYSLGIYTDASNFFSPNTPMKRLDFTIAIGKAVDLRVMEEKKTKKTST
TSVFKDVKRTVKDYPYIESAVNKGIIRGVTSEYFKPDNAITRAQAAAIFVRALGLENKAPDPGYITKFVD
DAQIPNYSRDGIYIVNELGLMTGDPVTGRFNPNQPLTRAQASVMLDRFLNYLENDLKQNYRDDVLFFN

B53 *slp9*

AGAGATTTAGAAAACGGACAGCTTGAAGCATTAGGCGCCAATATTTCTATGACGCATGAGGATTTTATGATT
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CTGGGCAATTTAACAACAATTAATATGCCTAAGTTGAATAAATAGGAGATGAGCAG

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CAAGGCTTTAACGGGTATGTATCCTTTCTCGAAAAAGCAAGAGAGCTTCAGTATAGCTAA

TGGGTTCACATTAAGCTAGAATATCGAAGGAAAAGGAGTTGTCTCAATGGTGAGACAACTCCTTTCTGCGT
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AGAA

B53 Slp9

MKRLLGLISVTLLTITLGVSSAFAQAENYVAIGDSLAAGQTPYQEIDTGYSDLIAMRLGLIGQIGHYTKE
LAFPGFTTMDVLQRVKSAEASELLANATLITISAGANDLLRLVQVNPTAGTLTFSQLQTDYALNTARKN
MEEILAELKTRAPHAKVYVMGYYFAYPTVHASQKEGTNEQLLKLNTILQQQAQQAGAVYINVYDAFG
LQATNYLPNSSDVHPNFEGYRQMVNAFLKTYSSSDMLAISSEELPKPNPMTFEEIVKKQAKVKQRPDES
TQESVATVRRIQGFNGYVSFLEKARELQYS

B53 *slp10*

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AATTTATT

ATGAAAAAATGGTTACTAAGTGGTGTAGCATTATTGATGTTAAGCCCAACTGCTGCATACGCACAA
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CAAGTGAGTTCTTTGTGCTTTTTTTGTATATAATGTAAGTAGGAAACGGTTGGAGGTGAGGATTTTGTGGAA
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B53 Slp10

MKKWLLSGVALLMLSPTAAYAQNAENHTVETSLKSAANVMSSSKVTTWDKFINEVVQQMNEFSTEIK
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VLKTIIKPSMTQFQKVKAVNDYIVSNAAYGNKTKASPHSAYALLMEGQAVCQGYALLAYKMLTQAGI
ETEYVVGFVNSNQGHAWNMVKIDGKWYHLDTTWNDPLPNRVGASSYDYFLVTDAQLKKDHSWIASD

YPAATSTTYNYMQNVHYSYQINNTLYFSNTADNDKLYKLDLTNGKKTKVIDTRALYITGVGDNLYYS
DYSNGGYLTKLNLKTLKAGVLVKQSISDLEIRDNNLVYKVNAKEQKLKIN

B53 *slp11*

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TATTGGTATATCCTTTGGAATAAATCTATCTTTCTTTTATAATAGAGATGAACAGAATTAGTAGGAGGTTATTA
AT

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B53 Slp11

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GNVTKFLGKWLVSEHYQVPVDYNKKVRFTDLQPASTKDKELLQYAALIYDTGISKGTNNKLSPSENITR
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FASFLHRTMTNVTDMLKEHEVPLLIQTVKVVDANTLQVTLTDGTSHKVTLATPLTENVETKVDFKIND
KSYSAVVTYEVNELKVQSIEGINASQIKVVFNLAIDPLSVLKTDGKLQDNIAAFSNVDTLRALSVVKTEI
SADRKSIIFTVNEPLKGTHRYVLNNIKSEKGLLLKRVDANFVIAGDTQPPTILGTTQGNNSSIVKVQFSKP
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NPATVTVKKGDKDGIPPTLSSVTQTGPNTFQLLFSEEIQPLYAYDLLIKNGQTSISVNKVEKDPKNGRLIN
VTVDPKSILQGITTIGTASSRVITDLSGETATFSTVYNFIKDDRAPVLMNSEIVYEDNVEYLQLSFDKPVQI
GAYAKASFTGSYMNNHILYEVSRGSQSDIHTVKDQPTKLRVKIAGLLGPYDTKGALYDGKLTLFNVTN
LYGVPINEVQNVKFTRNGDLSVNGNKLTLTQYNAIQTSATDYSIKDNNIVYLNFNYPVDPALAQNVQN
YIIDNAQVESAVVEASNLNRIKLTVKKDSNYFNGIRNFTIKGLRAANSMESFDEVRTTVNLKENIAPKVV
NVNVSNSQTLELVFSEPVINVNSMDFDISVNGSSVAASTYAQGNDRVLITISQSGYLFENGRNVTIQASP
RNAITDNNGNKLDFTSQTITVQR

B53 *slp12*

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GTAAATAGAAAGGGAGGAATGTAAA

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ACAATA

B53 Slp12

MKKIYSLLIALFAFFLVLPNTSLAATFNDVPTSHQNYSDIMYLLEKNVINKSSNYGVNDIVTREEVAVM
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GNKTFSDVPVNHTAYTAVKQLAAAGITTGYNDGTFKPEANLSRAHISAFLARAINYQEEQLVEPELIPEK
PEATKGTTRNNAVKINETVLIEVDDEFDGVQKYELTLTEVISGQTAWSMIKEANMFNDAPDPGMQYVL
AKFKVKILYLEKEPFDINHAQFSAVSKNGTKYNFISIVAPSPRLHIDLYSGAEYEGWVRFGVAENDSPKV
VYKSERDAERWFDLGL

B53 *slp13*

TGACTATCTCACTTAAGTTAGGGATTTTTATTTTATGTGAAGTTGATAGAAAGTGAAGCAACACAACAATCG
GTCTTATTTATTATTTTTTTAATGTTAATAGTCAAACCCTTAACATTCCTCAGTATCGCAACAACTATTTGTTA
TTTTACAGTGTAAAAGAGACGTTTTTCGAGGAGGAATAAATAG

ATGAAAAAAAATAAACGGGTGGGATTGCTGGTGGTTATTACTTTATTTTTATCCGTAATAGTACCA
GACTTTACGCCACTTGCAGCAGGAGGTACAGTAACAAAATCCTATACAGCTACTGCAGATACGATG
GATTTACAAGGCCATATTCAGTATGACGGCATTCATGATAATGATGGGGATGGTTTTCTTAGTATCG
GTAGATCTAATGACGAATACGGAGATGTCCGGATGAGAGCTGCGGCAATGTTCGATCTGGGTGTCC
CAGAAGGAAATATTGTGTCAGCAGAGCTGGTTTTGACTGTTGCTACTGTCATTAGAAACCCAAATC
ATACTTTGTATATGGAGGCAAGAGGTTCTGATGCGAATGATTTGAATTATGTAAATTTCCCCTCAAT
AGATACGAATAGCCCCTATGCAGATAAGTTCACCGCTAAAAGTACTGCTGAGGTGCCGATGGGTAC
TTATCTTGAGAATCAAACGATTATGCTCAATGTCAAGAGCGCTATTGACGCTTTCACAGATAGTTCT
GATCGTAAAATAACTCTTATACTCAACGGCAATGAAGCTGATGCAGATAGCGGCAGATTTCTACTT
TATTCTTTGGAAAATTCGAATGCTGCTTTTCGACCGAAGCTGATTGTTACTTATGAGACAGGGACAC
TTGCTAATAACCCTCCAGTGGGTTCCTTCACGATTCTGGAAGGCGCAATGACAAGTACTAGTACTG
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TGGCGGAGATGGGGCGAAGACCATCTATATGCAATTGAGGGATTCCAACAACGGGATCTCAGCCA
ATTCTTCTCAGTCCATTCTATTGGATCAGACAGCACCAACGGGTACACTGATCATTAATGATGGTGC
TACATGGACGAAATCGGACACGGTTACATTGAAAGGAACCTATACGGATGGAAGCGGTTCAGGAG
TTGAGCAAGCACGTCTCTCAAATATCATTGGCAGTTGGCAGACTAGCTGGTTTAACATCGCTGACTT
GAACGGGAAGTCGTGGGCCCTTCCGGCTGGCGGGGGAGCGAAAACCGTTTATGTGCAATTTAAAG
ATAAAGTTGGTAATACGAGTACTGGTACAATTAGCAGTATGATTACAGTTGATACGATAGCCCCGA
TTATTTCGAATGTAGAGCATGGCAAGGTATATAACAACAAGGTGAATGCGGTATTTAATGAGGGTA
GCGGGCTACTCAATGGCAACCCCTACACAAGTGGTACTGATATCACCCAGGATGGAACGTATACGC
TAATCGTAACGGATACAGCAGGCAATAGTACGACGGTGACCTTCAAGATTGATACGACTGCGCCTA
TTGTGACGGGAGTAACGAACGGTGGGATATATAAATCGAATGTTACCGTTACATTCAACGAAGGA
ACGGCGACATTGAACGGTACAGCTCTTATTAGCGGTATGCTGGTTACAATGGATGGTATGTATACA
CTTGTAGTAACAGATTCAGCCGGTAATGTCACGACGGTAAATTTCATGATTGATACGGAAGCACCG
CTCGTAACAGGTGTAACAAATGGTGGTATTTACAAAGACAAGGTGACAATCACATTCAATGAGGG
AACGGCAACGTTGAATGGAGCGGCTTTTGCCAGCGGTACGGAAATTGGTCACGATGGAAGCTATA
CACTAATCGTAACGGATGCGGCAGGCAATGTTACGACGGTAAATTTCATGATTGACAGGACAGCAC
CTACTGTAACGGGCATAACCAATGGTGGTGCTTACAAAGACAAGGTAACAATCGGATTTAATAAG
GGAACCGCGACGTTAAATGGAGTAGCCTTCACGAGTGGAACGGAAGTGGACCAGGAAGGAATGTA
CACGTTCGTCTTAACAGATGCATCAGGTCATGTTACAACGATACACTTCACGATTGACAAGACAGC
ACCAACTGTCACAGGAGTAACAAATGGAGGTAATTACAAAGATAAAGTAACAGTCGGATTCAATG
AAGGAACTGCAACGTTGAATGGAGTAATTTTTGCCAGTGGTCACGAAATTAATCAGGATGGAAGCT
ATACACTGGTCGTAGCGGATACAGCAGGCAATGTCACAACAATAAGCTTCACTATTGACAGCACAG
CACCAACTGTCACAGGAGTAACAAATGGAGGTGTTTATAAAGACAAGGTAACAATCGTATTTAATA
AGGGAACGGCGACATTGAATGGAGTAGCCTTCACGAGTGAAACAAAGGTAGACAAGGAAGGAAC
GTACACGTTGGTTGTCACAAATCCAGCAGGCCGTGTCACAACGATAAATTTTAAGATTGATAGAAC
AGCGCCCACAGGAACAATTGTTATTAATGGAGGTTCTGCTACGACAAATAGTAATTCGGTTACACT
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AAAGTGGTCAGCTTGGGAAAAAGTATCACTCTCAAAAAAATGGAGCTTTACTGAAGAAGTGGGAC
AGAAGAAGCTTTACGTTCAATTCCGAGATGCTGCTGGTAATAGTAGCATAACAAGCATGGCAACAA
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CACAGATCGTTACAACAAATGGCATAATAACTGTTCCAGTAGGAAGCTCTGGTGAAACTAGTGTTG
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TGTCGACAAGTCTCAGCCAATCTCTTTTTCAGATATTAATGAACATTGGGCACAAGACATGATTGA
AGATATTGCGGCTCGTGGGATTATCACAGGCTATCCTGATGGCTCTTTCCGACCGAATGAACCGAT
TCAACGCCAGCATGTTGCGGTGATGGTTGCACGTGGGTTCCAATTAACAGCAAAACGGGATGCTGT
AGCGTTTAGTGATGTACCGACAAGTCATCCGTATGATGAAGCGATTACCTTACTTCAGCAAGCAGG
CATTGTGGATGGGTCAAATGGAGCTTTTTATCCAAATGCAAATATGACACGTGCACAACTGGCGAA

AATACTTGTACTCGCATTTGGTATCACACCAAGTGGAACGAGTACCTTCCAAGATGTTCCAGTTAC
GCATTGGAGTTATGATTACATTGCTGCACTTGCAGATAACGGCATTGCCTTAGGGGATAAAGGCAA
CTTCAGACCAGATGAGTTTGTGACACGTGCGCAATTCGTGGCCTTTTTGTATAGAGCGCTGAATCA
ATAA

CTATTGAAAAGGGCTGGGACAAAACAACTCACCTCAAAAATGAAAAACCTCGAAAATTTTACGATTAGTAAA
ATCTTCGAGGCTTTGTTTTTTTAGTCTACTTCGTAGTCATTTTTCTCCGCTACGGCGGACGTTTTCCGCGGG
CACA

B53 Slp13

MKKNKRVGLLVVITLFLSVIVPDFTPLAAGGTVTKSYTATADTMDLQGHIQYDGIHDNDGDGFLSIGRS
NDEYGDVRMRAAAMFDLGVPEGNIVSAELVLTVATVIRNPNHTLYMEARGSDANDLNYVNFPSIDTNS
PYADKFTAKSTAEVPMGTYLENQTIMLNVKSAIDAFTDSSDRKITLILNGNEADADSGRFLLYSLENSNA
AFRPKLIVTYETGTLANNPPVGSFTILEGAMTSTSTVNLSITGSDPDIGDSVTHMRFANSAANLSAASWL
PFSNTATFSLTGGDGAKTIYMQLRDSNNGISANSSQSILLDQTAPTGTLIINDGATWTKSDTVTLKGTYT
DGSGSGVEQARLSNIIGSWQTSWFNIADLNGKSWALPAGGGAKTVYVQFKDKVGNTSTGTISSMITVD
TIAPIISNVEHGKVYNNKVNAVFNEGSGLLNGNPYTSGTDITQDGTYTLIVTDTAGNSTTVTFKIDTTAPI
VTGVTNGGIYKSNVTVTFNEGTATLNGTALISGMLVTMDGMYTLVVTDSAGNVTTVNFMIDTEAPLVT
GVTNGGIYKDKVTITFNEGTATLNGAAFASGTEIGHDGSYTLIVTDAAGNVTTVNFMIDRTAPTVTGITN
GGAYKDKVTIGFNKGTATLNGVAFTSGTEVDQEGMYTFVLTDASGHVTTIHFTIDKTAPTVTGVTNGG
NYKDKVTVGFNEGTATLNGVIFASGHEINQDGSYTLVVADTAGNVTTISFTIDSTAPTVTGVTNGGVYK
DKVTIVFNKGTATLNGVAFTSETKVDKEGTYTLVVTNPAGRVTTINFKIDRTAPTGTIVINGGSATTNSN
SVTLSLTSSDGSGSGVAEMRFSTDELKWSAWEKVSLSKKWSFTEEVGQKKLYVQFRDAAGNSSITSMA
TIDYRPSGGSDSGSSNNGGSGSNNSTANNSNSGNSNSNVGNESPPTQIVTTNGIITVPVGSSGETSVGEDI
NVSIPVGVAQQELTITVEKLRDITNLLSNGQRLISPIFELHKNSSGNIKKPLTLTMKFDSSKVGKDQYASIF
SYDPLKKEWLEMKGTTKGDFITISTDEFTKFAVFIVDKSQPISFSDINEHWAQDMIEDIAARGIITGYPDG
SFRPNEPIQRQHVAVMVARGFQLTAKRDAVAFSDVPTSHPYDEAITLLQQAGIVDGSNGAFYPNANMT
RAQLAKILVLAFGITPSGTSTFQDVPVTHWSYDYIAALADNGIALGDKGNFRPDEFVTRAQFVAFLYRA
LNQ

B53 *slp14*

GTAAAGATGGTAAGACTTATATTTATGCAAACAAACCATTAACGCGTGGACAAATGGCGAAAATACTAAATA
ATTCTTTAGATTTCTTCTATGAGTTTGAAGAATAACAATAAGCATTAAAATAGCAACTTCCTTTCTCACCCAG
TGAGGAAGGAAGTTACTATTGCTTCTAAAGAAAAATGGGGGAATTTCC

ATGATGAAAATAAATAGAAAAAACTTTCTTTTGTTAGCGTTCAGTGCTGTTTTTTTAGCCTTTCTCC
CTCAATTTGTTTGGATGCATGTACAAGCCGAAGAAAATGTCATAGAAAATGGATCTTATCAAGTTG
AACTAAGTTTTCCATCCATTGATGGAGTGGAACAAAGTCGTTTTTTCAGTAAAGAAGCAACACTTA
TTGTCGAGAATGGACACTATACTTTAGCTTTGTCGATAGAAAATAACAATAGCCTGACGAATTTAC
ATATTGAACAAAGCGAAAGAACGCTTCCTTTCAAATTAGAGAGTACTGAAAATTTAGTTCAGTTTG
ATGGCATAGATTTAACGCAACCGATTTTTGTAAAGGGTTTAATGGCCCTTCCATTTGAAGAAGATA
ACCGTCCTTTTACCCAAGAGCTTCTAGTAAAAGTAGCGTCTATTAAACCAATAGAGATACAAAGTG

AAGAGTCCTTATTAGTTAATGAGGTAGAACAAAACTTACCAAATGACATAGCTGAAAAAGAATGG
ACAATGAACTATGTCCTGCTTGTGGATGGCAAAAAAGAGAGCTCAATTATGAATACATATGTAAAG
CCAGTGGCAAAGATGATAGAAAAAGAAGGGAAAATCTTTGCGCAGATGTCGATAGAAAAATCGGC
TTGGATAACAGGTTTAACGGTAGAGCGACAAGGGGAGCAAGTGGCACCGAAGCTTATCTCTCTTAT
AGACAATATGCGAATTATTGAATTTGAAATTGAGGATTTAGAGCAGCTACTAAGAATGTGGGTAAA
GGTTGATATTGCGGAGCTTGACTATCATCATCAATATTTCGTAAATCTAAAATTTGACCAACTACAG
GTCGATAAATTTTTAAACAAACCACAAGCGGAATCTTCAGAACAGATAGACATAGTGAAGCCCCC
AGTCATTGTTAAAGAGAAAGTTAAGAGTACACCGGGTAAATTGACAGATGAGTCTGTGACAAAAC
CACCCCTACTAGCGCCATCATCAGTTCAACCTACCCCTACTGTACCAAAGGAAGAACTGTTAGCTTT
TGACCGGACACTTGATGCAAATACTGCAGGAGATGCTGAAGATGAACCAAATGAAGCTGAAGTCA
AAAAGGAATCAGCTACTAAAACGGTAGTGATGGACAGAAATACAACCCAGCAACTTGCTCAATTA
GATAAGGTGAAAATGGCCTTGCTCATCATTATTTGTATTTTATCTGGGTGGTTACTTGTTCGCCGCA
TCAAATATTCTAAAAAAGAAGCAACTGAACAGAAATAA

GTAGGAGATGACAATATGCAAAAAAAGTGGAAAGCGCTCATGCTCTTCATGGCACTCATGACATTATTCTTA
ATTACTGGTTGTAGTGGTGGAGATGACCAAGACAAAACAAAGGAAACTGCTGAAAAAATAAAAACAACTAC
ACAAAGCGAAAATCGTATTATTGCAGGTACAGTTGTCGTTGCGGACATTTTAGATAAGCTAGAGTTGGACG
CGATTGCTGTGCC

B53 Slp14 [cell surface protein; iron transport-associated domain protein]

MMKINRKNFLLLAFSAVFLAFLPQFVWMHVQAEENVIENGSYQVELSFPSIDGVEQSRFFSKEATLIVEN
GHYTLALSIENNNSLTNLHIEQSERTLPFKLESTENLVQFDGIDLTQPIFVKGLMALPFEEDNRPFTQELL
VKVASIKPIEIQSEESLLVNEVEQNLPNDIAEKEWTMNYVLLVDGKKESSIMNTYVKPVAKMIEKEGKIF
AQMSIEKSAWITGLTVERQGEQVAPKLISLIDNMRIIEFEIEDLEQLLRMWVKVDIAELDYHHQYFVNLK
FDQLQVDKFLNKPQAESSEQIDIVKPPVIVKEKVKSTPGKLTDESVTKPPLLAPSSVQPTPTVPKEELLAF
DRTLDANTAGDAEDEPNEAEVKKESATKTVVMDRNTTQQLAQLDKVKMALLIIICILSGWLLVRRIKY
SKKEATEQK

B53 *slp15*

GCGCGTTGCCTGTTTGATTATACAAAATAGGACATAGTTGAACAAAAAAAGAGGGGTAGTCCCAAGGTGAT
TTATGCATCACATTTGGGATTGCCCTTTTTTGTCTACCTATCTAAATCTTAAGAGTTTATGAATATTCATGAA
GATATCATAAGAAATGGAACAAA

ATGCAATTTAAATCGTATAATTATGTAATCATCTATATGTTAAGTAAAAAAGGGGTTGAGCTTTTGA
AAAAAACGAAGATCACGGCACTTATTCTACTATTAATGTTTGTTATGAGCTTTATGCTTCCTGTTTC
ACAGGCGAGCGCCGCACCGACTTTAGAAGTGCAGGCGAAAGCTGGAATGGTAGGAAAAGCAAAAT
ATCAATCTGTAGTGCCATTACAAGTAACAATAAAAAATAATGGTGCAGATTTTTCGGGGGACATGG
CAATTAATGCCTCAAGTTCCTATGAGGCTGCATCGGCACTTGTACTGCCTCTAGATATAGCAGCAG
GTGAAGAAAAAACTGTTGAGTTATATTTGGATGGACTAGCAGACTATAGTTATTCAGATGCGGATT
TGTTTGCCTTTTATGAGGGGAGCATTGAAAAAGGGAAAAAAGTTGCCTATAAAGGAACAAAACGT
TTACAAGCAAACTTTTTAGATCCAACAGCAATATTTGTCTATACGTTAACGGAGAAAAGCGATCGC
TTATCTGCTTATTTGCGCTTATCGCAATTTATTCCATCAAGTAATGTGGAAGTTTTTAATTTGAATCA

AATAAAAGATTTTACCTTGCCAGAGGATGCACAGGGCTACGCGATGGCAAATATTATTGCCGTCGA
TGAAATTTCCATTGTGGATTTATCTCAAAATCAACAAGAAGCTCTACTGAAATGGGTACAGGATGG
AGGTACTTTACTTCTTGGTGCTTCTGATCAAGTAAATACAACAGCGGGCATTTTTAAAGATTATTTA
CCGCTTACTTTATCACAACAAATGAAGACGATTTCAGCAAATAGTTTAACAAAATTATCTGGTGGA
GGTATTTTTACTAAGGATATTTCCGTCTATACAGCAACGGATAATGAAGGAAGCGTTCCTGTTTTAA
AAGATAATGATACAACGCTAGCTGCAAAGAAAAAGCTAGGTAGTGGGGAAATTGTACAAACAACG
TTTTCGTTAGGGGATGAGCCTTTAGCTTCAATGGATGGATACTTAGCATTAACTGCAAACATGTTAA
ATATTCAAAGTTTGTCTCAACAAGGTATGATGCAAGGTCAATCAACAATGGATCAGATTTCTTATG
AGCTACGTACCATAAATGAACTATTCCCATCATTCGAAGTGTCCGTCAGCTACATGTTAATCGTCAT
CGTTCTCTATATTTTAATTATCGGACCTATTTTGTATTTTGTTTTGAAAAAGATGGATAAGCGTGAA
CATGCATGGTGGTTAATACCGGCCATTTCAGTTGCATTATCTATTGCGCTCTTTATTTTCGGCGCTA
AAGATCGTATTGTTCAGCCACAAGCACAGCAATCAGCTTTTTATAAAGTAAATGAGGATAGTAGTG
TAAACGGCTACTATGTAGAATCGATTTTAACAAATCGTAGCGGTGATTTTGTTGTAGAGGCAGATA
AAGATACAACTGCACTGGCATTACGTAGAAATAATAATTTTACTGGAACAATGGGCGATTTACATG
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CTGTTCAATCATTTGCAGGAAAAACAGCTGCAAAAAACATCGGTAAGATGGATATCGATATTACGT
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ACGAAGAAAGAAGATGTAGACCCACTACGTATCGAGCGCATGAAAACGTTCGCACTACCGCTTGC
AGAAAGTGACAAGCAACCTGTAATTACGGCTTGGGCAGAGCAAGCAATTGTTGGCGTAGAGCTTG
AAACAAGTGCTAACATGTCACCTATCTCTTATTTCATCCAGCCATTTGAAGGAAAAGTAAATCTGTC
AGGTCCATTTACCATGAAACGTACCAACTTTACTTATACAGTGAGTCCGCAATCAGCGAATGCCTA
TTATGAAAAAATAGACGAGCAACTAAATAACTGGTATCTATCAGATGGTTTATTTGAAGTCACGAT
GGCTTTGCCAGATAATTTTATGGCATCTGTTCAATCATTAAATGAACTGGTTATTTCAAATAAGGAC
GTGAAGCGCATGCAGCTTTCCATTTGGAATAATGAAACGAGCATCTTTGAGCCACTAGTAGATACA
AAGCAAGCATTTACAGAAAATATTACCCAATACTTAAATGAAAATGATGAAATACTTGTACAAATT
AAATTTGGACCAGATCAAACTGGCGAACAGACAAAATTACCAGATATAGAGCTGAAAGGAGTGGC
GAAGGAATGA

TTGAAATTCGTGATTTAACCAAAAGATATGGCTCCTTTACAGCGTTAGACCATTTAAACCTATCACTTGAGG
AGGGGGTTGTGTTCGGCTTTGTTGGAGCCAATGGTGCTGGTAAATCGACAACATTTTCGATTTTAGCAACA
TTACTATCTCCGACTTCTGGCGATGCCCTTATCAACGGCAAAAGCGTCATCAAGGAACCAAAGGAAGTACG
CAAGCAAATCGGCTATA

B53 Slp15 [hypothetical protein with S-layer similarities]

MQFKSYNYVIIYMLSKKGVELLKKTKITALILLLMFVMSFMLPVSQASAAPTLEVQAKAGMVGKAKYQ
SVVPLQVTIKNNGADFSGDMAINASSSYEAASALVLPLDIAAGEEKTVELYLDGLADYSYSDADLFAFY
EGSIEKGKKVAYKGTKRLQANFLDPTAIFVYTLTEKSDRLSAYLRLSQFIPSSNVEVFNLNQIKDFTLPED
AQGYAMANIIAVDEISIVDLSQNQQEALLKWVQDGGTLLLGASDQVNTTAGIFKDYLPLTLSQQMKTIS
ANSLTKLSGGGIFTKDISVYTATDNEGSVPVLKDNDTTLAAKKKLGSGEIVQTTFSLGDEPLASMDGYL
ALTANMLNIQSLSQQGMMQGQSTMDQISYELRTINELFPSFEVSVSYMLIVIVLYILIIGPILYFVLKKMD

KREHAWWLIPAISVALSIALFIFGAKDRIVQPQAQQSAFYKVNEDSSVNGYYVESILTNRSGDFVVEADK
DTTALALRRNNNFTGTMGDLHETSYIKEHANGSTLTLRNLSYWSVQSFAGKTAAKNIGKMDIDITLKN
EKLSGSIKNNFPFKLKDVTLISGIKEVKLGDIEPNGTLKVDKELKSTVLQKPSSFNSYNYNYPTKKEDVD
PLRIERMKTFALPLAESDKQPVITAWAEQAIVGVELETSANMSPISYFIQPFEGKVNLSGPFTMKRTNFTY
TVSPQSANAYYEKIDEQLNNWYLSDGLFEVTMALPDNFMASVQSLNELVISNKDVKRMQLSIWNNETS
IFEPLVDTKQAFTENITQYLNENDEILVQIKFGPDQTGEQTKLPDIELKGVAKE

Putative heavy metal resistance supporting proteins

Putative magnesium and cobalt transporter CorA

MGISKDQQLLKGFPLEDIKDKYFEWFWVDFNSPTAEEELLLDTFFHFHPLAIEDCLMRLQRPKLDFYDD
YHFFVIHRLNEETLIAEELNIFVSDKFIVTYHKNETPEIDKVQKLLEEQPKNWERGTVFLTYQTIDKIVDS
YFPLVYKIEDHLNTLEDELTYQSSVNAMQIVFEFRSDLLHLRRTILPMRDLLNRVLSSYRFALKKSERAY
FGDIHDHLVKLTEIVESNRELTADMRDNYMAMSSSRMNGIMMTLTIVSTIFIPLTFIAGVYGMNFDIMPE
LHGRYSYFIVLGIMILIVIFMLSFFKYKGWFKLFKP

Putative arsenite resistance protein ArsB

MGNESLTKQLSFLDRYLTLWIFVAMGIGVLLSITMPTIGEALESMSVGTTSIPIAIGLIVMMYPPLAKVKY
EEMWRVFKDWKVLLLSLLQNWLLGPFLMFFLAILFLHDYPEYMAGLIMIGLARCIAMVIVWNDLARG
DREYVAGLVAFNSIFQIVTYSIFAYFFLNVLPGWFGLKNFNVSISMWEITKSVLIYLGIPFAAGFLTRWIGI
KTTGKQWYEEKLLPKISPLTLIALLFTIVMMFALKGEQLVELPLDVVRIAIPLFIYFVVMFAVSFFSSRKA
GASYPVTAALSFTAASNNFELAIAVTVGVFGLHSGVAFAAVIGPLVEVPVLIGLVWVALRWQKKYFKN

Putative cadmium ATPase A

MNMYTKLSRLLMNMRKRGLTMAATPTKQEYRLQNLSCASCAAKFEKNVKAIPEVEDAQVNFGASKIT
VFGEINVDQIEEAGAFDGIKVSQSPKSSIEKSTSFYKKTENILAGIALLFVILGYVLVTMRGETDPFAIGMF
IVAILVGGVGIFKTGFRNLARFEFDMKTLMTIAVIGAAIIGEWEEAAVVVFLFAVSEALEAYSMDKARQS
IRQLMDIAPATATIKRAHGEHFHEMEVPTEEIEIGDILIVKPGQKIAMDGIVIRGLSAVNQAAITGESIPVN
KSKDDEVFAGTLNEEGALEVRVTKRVEDTTIAKIIHLVEEAQAEKAPSQQFVDRFAKYYTPAIMMVAFL
VAVIPPLFIGDWQHWIYQGLAVLVVGCPCALVVSTPVAIVTAIGNAARQGVLIKGGVHLEQLGHIEAVA
FDKTGTLTKGKPAVTDIFTHRNMTEDSVLQLVAAVEKQSQHPLAKAILTALHEKGLTELEPTDFQSVTG
KGAFATIEGKKVSVGSLKWISTLTDVDEATKEQANQLQAQGKTVVAVVSDHHFIGIIGIADQLRGESNS
VLQNLTTLKVKHTVMLTGDAKPTAEAIATALGMSDVRAGLLPAEKLTAIKELRTKYGAVAMVGDGVN
DAPALASANVGIAMGGAGTDTALETADIALMGDDLTKLPYTIDLSRKTLRIIKENIIFALALKLIALLLVI
PGWLTLWIAIFADMGATLLVVFNSLRLIKTKKYK

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Selbständigkeitserklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbständig verfasst und keine anderen als die angegebenen Hilfsmittel verwendet habe.

Dresden, Franziska Lederer