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REVIEW S- LAYER PROTEIN : TAILOR - MADE NANOPARTICLES

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I. Table of Content

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Abstract: All bacteria and archea (with few exceptions) have an outermost covering of 'Surface' layer proteins which is having unique property of recrystallization or self assembly in suspension, in air-liquid or liquid-solid interphase and having uniform pore size (2-8nm). These proteins are having many functions on bacterial surfaces like adsorption of exoenzymes and protection of cell. The unique properties of S layer proteins viz. uniform pore size, weak non covalent interactions, large surface area and to recrystallise on various surfaces (eg.copper grids, silica plates) can be used in applications, viz. ultrafiltration, vaccines, biosorption, immobilization and synthesis of Nanoparticles. This review focuses on the general properties, methods of isolation, characterization and applications of S layer proteins specially in synthesis of inorganic nanoparticles, as they give uniformly shaped and sized tailor-made nanoparticles when fixed on a inert surface (as uniform pores are present on their surfaces).

Keywords: Bacteria, archea, S layer protein, tailor-made nanoparticles.

I.INTRODUCTION

While studying bacterial surfaces, surprisingly, it was found that, despite differences between Gram positive, Gram negative and archeal cell surfaces, in all three types of bacteria, an outer membrane which is proteinaceous, interacts with external environment and is commonly known as S (surface) layer protein.[12] This is a crystalline structure contributing to approximately 15% of total bacterial proteins. Generally S-layer proteins are weakly acidic (pI 4 - 6) and contain a high proportion of uncharged non polar amino acids(Hydrophobic) as they are present on outermost surface of a bacterial cell and have very low or no Sulphur containing amino acids. Naturally their functions are determined based on the adsorption sites for exo-enzymes. They increase virulence in pathogenic bacteria and protect the bacteria from some bacterial parasites. This structure of S layer was first observed in bacteria of the genus *Spirillum* in 1953 [58],[68].

Crystalline structure and pore size (2-8 nm) of these S-Layer proteins have lead to several applications in many fields like, ultrafiltration, immobilization, vaccines, biosorption, and nanotechnology. This review reveals the study on S-layer proteins and their applications particularly focusing on synthesis of tailor-made nanoparticles.[39],[68] Nanotechnology is a study of production of nanopaticles (bottom up and top down) and their uses in the field of medicines, chemical sciences and molecular electronics. Conventional methods for synthesis of nanoparticles involve physical and chemical methods.[28] These can be partially replaced by using S-layer proteins, as they provide weak covalent interactions with a large surface to weight ratio and uniform pore size. Thus S layer protein on an inert surface, can act as porous surface of required shape and size. (as S-layer are having a property of recrystallization and form self assembled structures of various shapes with uniform pore size of 2-8 nm).[53] S layer protein can form weak non covalent interactions which can act as adsorbent for a substrate (Salt form of inorganic material whose nanoparticles can be produced) and can be detached easily after nanopaticles formation.[11]

II.UNIQUENESS OF S LAYER PROTEIN

S-layer protein are the outer protenacious covering of all the bacteria with few exceptions. They are made up of single protein or glycoprotein specific for that particular bacteria. [50] .These proteins are arranged on a surface with a lattice symmetry which is of following types[53],[11] Fig. 1, 2 and 3 depicts the ultrastructure and properties of S layer proteins.

1) oblique (p1, p2)
2) square (p4),
3) hexagonal (p3, p6)

In archaea hexagonal symmetry is predominant [38]. S-layers are 5 to 25 nm thick, and they reveal a rather smoothouter surface and a more corrugated inner surface. In archaea pillar-like outgrowth were observed on the inner surface of S-layer lattices [33],

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[43],[45],[46]. Molecular weight of S-layer is in the range of 40 -200 kda [68]. Promoters of S-layer gene are very strong because if bacteria have generation time of 20min and S-layer is fully covering the surface of Bacteria then it should be synthesized in a very short time, its rate is 500 S-layer subunits per second continuously incorporating in to the existing S-layer. [68] At neutral pH, crystalline structures of S layer proteins are weakly acidic and content of hydrophobic amino acids is also high. Besides these general characters, there are some unique characters of S-layer proteins which make them indispensable for the synthesis of tailor made nanoparticles. They are as follows-

1) They have a property of recrystallizing as closed monolayers onto solid supports, at the air/water interface, on lipid films, and on liposomes even after detaching from bacterial cell surfaces

2) Pore size on S-layer are having uniform size (2-8 nm) and of same morphology

3) Different functional groups are arranged in very precise fashion which provide uniform weak binding forces on S-layer proteins [68]

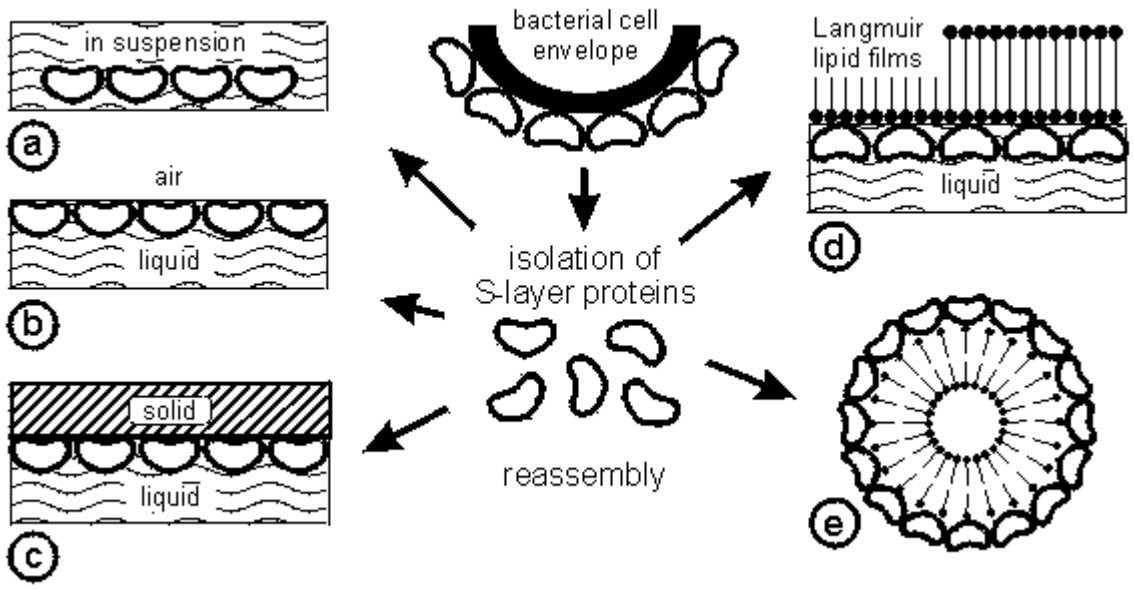

Fig. 1 Self assembly of S layer protein in different phases a) in suspension b) in air liquid c) in solid liquid[15]

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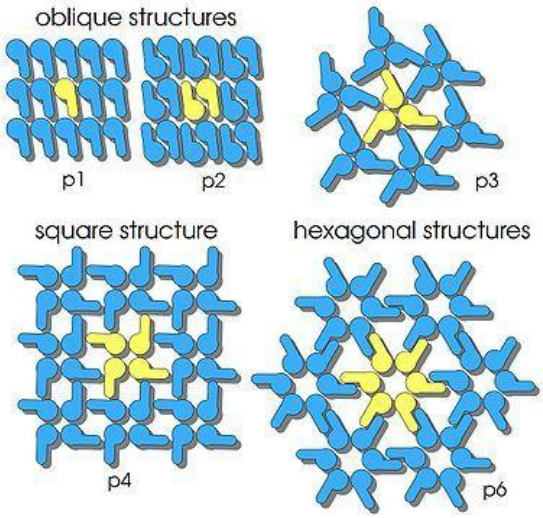

Fig. 2 Schematic drawing of different S layer lattice [10]

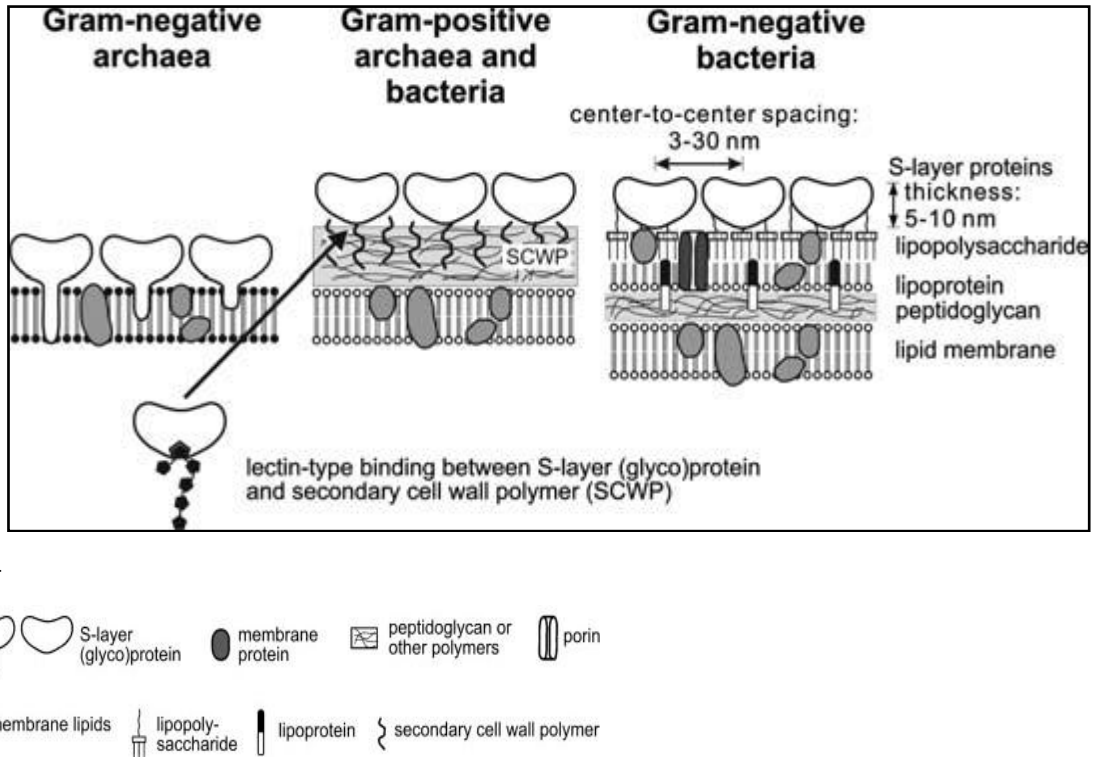

Fig. 3 Schematic illustration of major classes of prokaryotic cell envelopes containing crystalline cell surface layers i.e.S-layer (glyco)proteins.[41]

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III. ISOLATION AND CHARACTERIZATION OF S LAYER PROTEIN

a) Isolation of S layer proteins

For isolation of S layer proteins it is very important to detach it from the cell surface. It can be achieved by following methods

1) Treating the cells with high concentrations of Hydrogen bond breaking (chaotropic)agents like guanidium hydrochloride or Urea[30]
2) Using chelating agents like EDTA [12]
3) By altering the pH of the medium. [12]
4) Breakdown of Peptidoglycan layer by lysozyme [31]
5) Treatment with LiCl followed by centrifugation [2, 62]
6) By treatment with Glycin hydrochloride. [15]
7) By applying pressure(16,000 lb/in^2) [15]

- Extraction of S layer with guanidium hydrochloride leads to more loss of viability as compared to extraction with LiCl [62]
- Most commonly applied methods for isolation are given below in fig.4

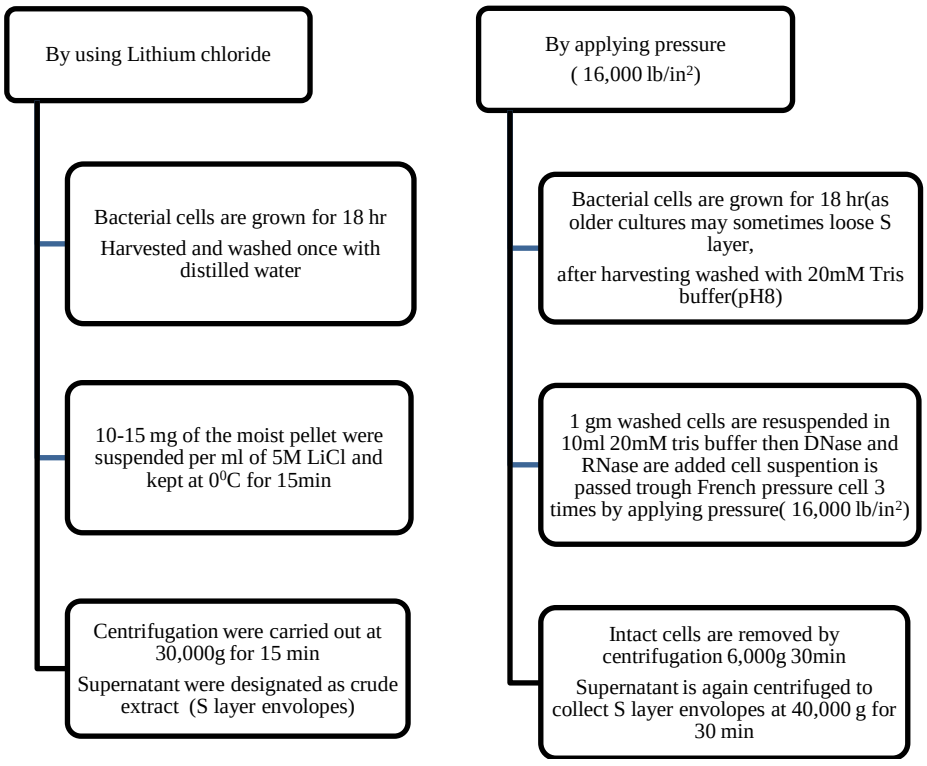

Fig. 4 Methods of isolation of S layer protein

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After isolation it is necessary to purify S-layer protein for further characterizations. Some methods followed for purification are [15]

1) Ultrafiltration
2) Dialysis
3) Column Chromatography(DEAE-Sepharose)

B. Characterization

S layer have to be characterized to understand their detailed structure of pores, their size and recrystallization pattern so that they can be used in different applications. Table 1 lists the bacterial strains with their strain no, S layer lattice symmetry and application. Because of very small size it is very difficult to characterize S layer proteins.Some techniques used for characterization are as follows:

1) Chemical characterization is done by SDS-PAGE gel electrophoresis to find out how many subunits are present in the protein and molecular weight of the protein[15],[62]
2) Structural characterization is done by [42], [62]
   - SEM/TEM imaging of freeze-etched cells given in fig.5 and 6.
   - Negative staining
   - atomic force microscopy (AFM)

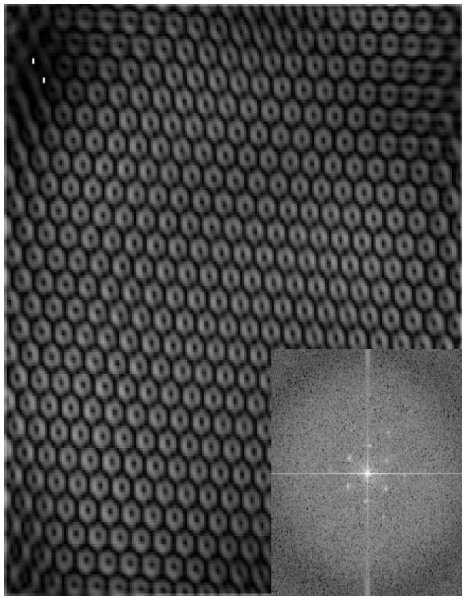

Fig. 5 Computer enhanced TEM image of isolated S-Layer from *D. radiodurans*, from image at left. (Inset) Fourier transformation of image at left.[71]

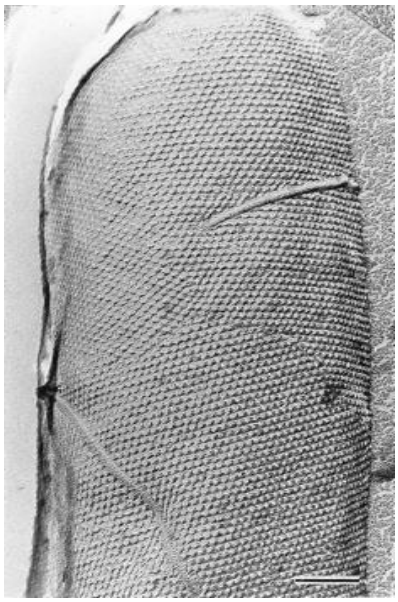

Fig. 6 Electron micrograph of a freeze-etched preparation showing a whole cell *Thermoanaerobacter thermohydrosulfuricus* with a hexagonally ordered S-layer lattice. [31]

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Table 1- List of bacterial strains with their strain no., S layer lattice symmetry and application[30], [32],[65]

Sr. No.	Species	Strain	Lattice symmetry	Applications	Reference
1.	*Aeromonas hydrophila*	TF7	L2		64
2.	*Aeromonas salmonicida*	A 450	L2		9
3.	*B. alvei*	CCM 2051	L2	➢ Uultrafiltration Membranes ➢ Vaccines ➢ Act as Matrices in functional molecule Immobilization, ➢ Biosorption of metals eg.lead, Uranium, copper, Aluminium. ➢ Synthesis of nanoparticles	3
4.	*B.aneurinolyticus*	-	L4		1
5.	*B.anthresis*	-	L6		65
6.	*B.brevis*	CCM 1089	L2		30
7.	*B.cereus*	ATCC 4342	L4		16
8.	*B.circulans*	CCM 1084	L2		50
9.	*B.circulans*	CCM 2048	L4		50
10.	*B.coagulans*	E36-68	L2		48
11.	*B.fastidiosus*	-	L4		20
12.	*B.licheniformis*	NM 105	L4		63
13.	*B.macroides*	Strain A	L4		20
14.	*B.polymixa*	CCM 1459	L4		50
15.	*B.polymixa*	NCIB 4747	L4		8
16.	*B.psychrophilus*	W16A	L4		20
17.	*B.schlegelii*	DSM 2000	L4		55
18.	*Bacillus sps.*	KL8	L6		25
19.	*B.sphaericus*	P-1S	L4		26
20.	*B.sphaericus*	CCM2120	L4		50
21.	*B.stearothermophilus*	39 strains	L6,L4,L2		34
22.	*B.stearothermophilus*	PV72	L6		50
23.	*B.stearothermophilus*	E465	L4		60
24.	*B.stearothermophilus*	NRS 2004/3a	L2		51
25.	*B.stearothermophilus*	3a/V1	L2		51
26.	*B.stearothermophilus*	3c/p2	L2		51
27.	*B.stearothermophilus*	PV72/p2	L2		51
28.	*B.thuringinsis*	4045	L2		28
29.	*Campylobacter fetus,ssp.fetus*		L4	➢ Vaccines	5
30.	*Campylobacter fetus,ssp.fetus*	23B	L6		66
31.	*Campylobacter fetus,ssp.fetus*	82-40LP3	L6		15
32.	*Campylobacter fetus,ssp.fetus*	84-91	-		15
33.	*Corynebacterium glutamicum*	ATCC 17965	L6		42
34.	*Lactobacillus acidophilus*	ATCC 4356	-	➢ Biosorption	7
35.	*Lactobacillus brevis*	ATCC 8287	L2	➢ Immobilization	70
36.	*Lactobacillus helveticus*	CNRZ 1269	L2		36
37.	*Sufolobus acidocalcarius*		L6	➢ Synthesis of titanium nanoparticles	68
38.	*Thermoanaerobacter kivui*	DSM 2030	L6		44
39.	*Thermus thermophilus*	HB-8	L6,L4		22

L2-oblique, L4-square, L6- hexagonal Note- Applications are generalised not strain specific

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IV. APPLICATIONS OF S LAYER PROTEIN

Uniform pore size and recrystallization property of S layer proteins leads to its wide applications in many fields. This applications can be broadly discussed as follows-

a) *Recombinant S layer proteins-*
Naive S layer proteins can directly be used or they can be genetically modified as recombinant proteins specially in Nanotechnology where they must be tollerent to exteme temperature and pressure conditions. Genetically modified S-layers have applications in various fields eg. SbsB-streptavidin fusion protein (fusion of S-layer protein from *Geobacillus stearothermophilus* and streptavidin expresed in E.Coli) have 80% biotin binding capacity. [11] Recombinant S-Layer protein can act as fluorescent Biomarkers, S layer proteins with controlled fluorescence properties can be constructed by Recombinant DNA technology. They can also be used for building nanopatterned biofunctional surfaces and in drug and delivery system [39]

b) *In Vaccines-*
S layer proteins function as virulence factor only in gram negative bacteria thus in them it can be used as vaccines as it has all the cell surface display molecules (epitopes). But for gram positive bacteria and some strains of gram negative bacteria (in which direct s layer is not much effective vaccine) modified S layers or recombinant S layer proteins can be used as vaccines. Examples of some S layer used as vaccines are *B.antrasis* ,*Campylobacter fetus* for chickens, *Bacillus subtilis, clostridium tetani*(recombinant). [23],[40],[68]

c) *In Biosorption-*
In this process many methodologies are applied for Biosorption of heavy metals acting as xenobiotics. S layer present on bacteria are having adsorbing capacity for heavy metals due to presence of surface motifs. It was observed that s layer of *B.sphaericus* strain JG-A12 has been found to show a high capacity of uranium biosorption. [35] Along with uranium, biosorption of copper, gold, and cadmium by S layer is observed [47]

d) *In immobilization-*
For immobilization matrices of 1-2 µ microparticles are required, thus S layer microparticlea (SMP) can be used. [49] Amperometric, and optical biosensors matrices can be constructed by using S layer proteins.[37],[39],[52]

e) In Ultra filtration
Fragments of S layer, layered on microfiltration membrane can be used in ultra filtration as they have uniform pore size. [70], [40],[25] This application can reduce the cost of ultrafiltration but is still in developing phase.

f) *Synthesis of Nanoparticles –*
Nanotechnology, is the study of production and use of nanoparticles. Nanoparticles are the inoraganic nanostructures (size < 100nm) of metals. Because of small size they are having greater surface area as compared to larger molecules hence are more reactive species. They are having vast applications in medicines, chemistry and molecular electronics.

V.USE OF S LAYER PROTEIN IN PRODUCTION OF TAILOR MADE NANOPARTICLES

Conventional physical and chemical methods used for the synthesis of nanoparticles are of two types top down and bottom up approach which involves very high temperature thermal cycles, reactions involving application of high pressures, requirement of vacuum chambers, which are not ecofriendly, and are having disadvantages like getting nanoparticles of broad size distribution(10-1000nm), varied particle shape, and impurities. Table 2 gives the general procedure followed for the chemical and physical synthesis of nanoparticles listing their advantages and disadvantages.

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Table 2-General procedure followed for the chemical and physical synthesis of nanoparticles their advantages and dusadvantagesis as follows [27],[4]

Sr. No.	Method	Advantages	Disadvantages	Nanoparticles Synthesized
a)	*Chemical Methods*			
1)	Chemical reduction	Simplicity and availability	large amount of admixtures remain in a colloid system along with nanoparticles, High temperature	Au (2-5nm), Ag(3.3-4.8)
2)	Reactions in micelles, emulsions, and dendrimers	synthesizing particles of definite sizes	Temperature dependant stability and application of high pressure	Bi(3.2nm),rhodium(2-3nm), Cu(7nm)
3)	Photochemical and radiation-chemical reduction	No impurities, can be done in low temperature	Radiations are used	Ag(2-3nm),
4)	Cryochemical synthesis	-	Requirement of Very low temperature (4–12 K) reactor , difficult to determine reagent ratio, Varied particle size in micro and macro level	-
5)	Sonochemical method	Bimetallic nanoparticles can be synthesized	particles of average diameter less than 50 nm can be synthesized	Au-Pd, Fe, Cu
6)	Microwave method	simplicity, ease of operation, high yield, short time requirement	Particle size is large (90nm to 260nm)	Cu
7)	Electrochemical method	No vacuum systems like other physical methods, low costs, simple operation, high flexibility, pure product and ecofriendly	Particle size is large (29nm to 200nm)	Cu
8)	Solvothermal method	Pure nanocrystals are synthesized	High temperature and pressure requirement	Cu
b)	*Physical methods*			
1)	Pulse laser ablation/deposition	Small particle size (2-50nm)	Vaccume chamber and high-power pulsed laser beam required	Cu
3)	Pulsed wire discharge method	high production rate and high energy eficiency	Very expensive, Vaccume chamber requirement	Cu

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b) Biological Synthesis of Nanopartcles-

These physical and chemical methods can be replaced by ecofriendly biological methods.

The biological synthesis of nanoparticles which is still in developmental stages for large scale production of nanoparticles mainly involve biotransformation by live microorganisms or plant extract [54,30]. Which is requiring very large quantity of biomass of fungi or plant and nanoparticles formed are not uniform in size (12-25nm). A unique property of self assembly is depicated by S layer. Size of pores of self assembled S layer ranges from 2-8nm hence the S layer lattices represent the perfect arrangement for metal assembly thus providing uniform nanoparticles on S layer template.

Procedure for the synthesis of tailor-made nanoparticles from S layer proteins is given schematically in fig. 7 and 8. [56]

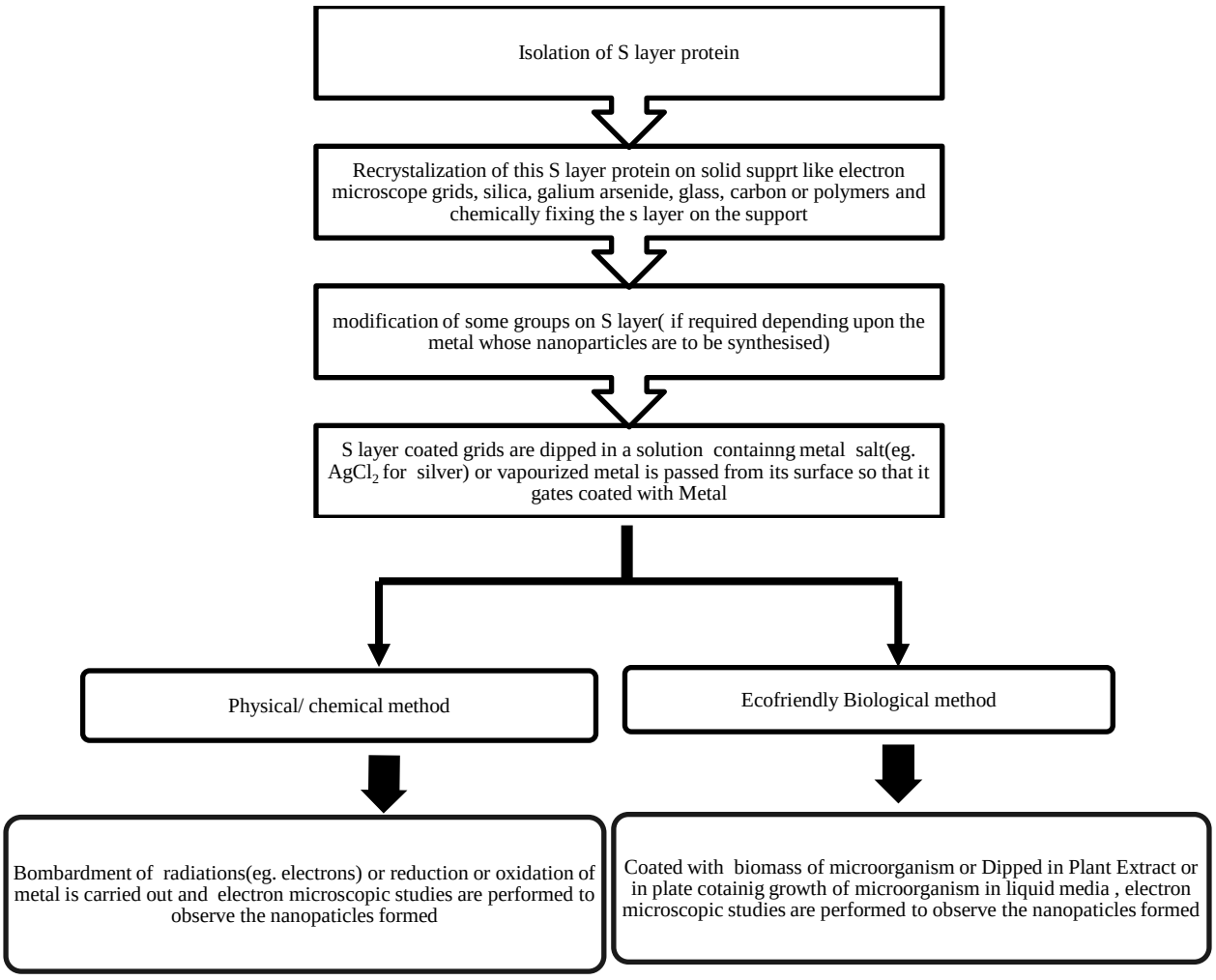

Fig.7 Flowsheet for production on tailor- made nanoparticles.

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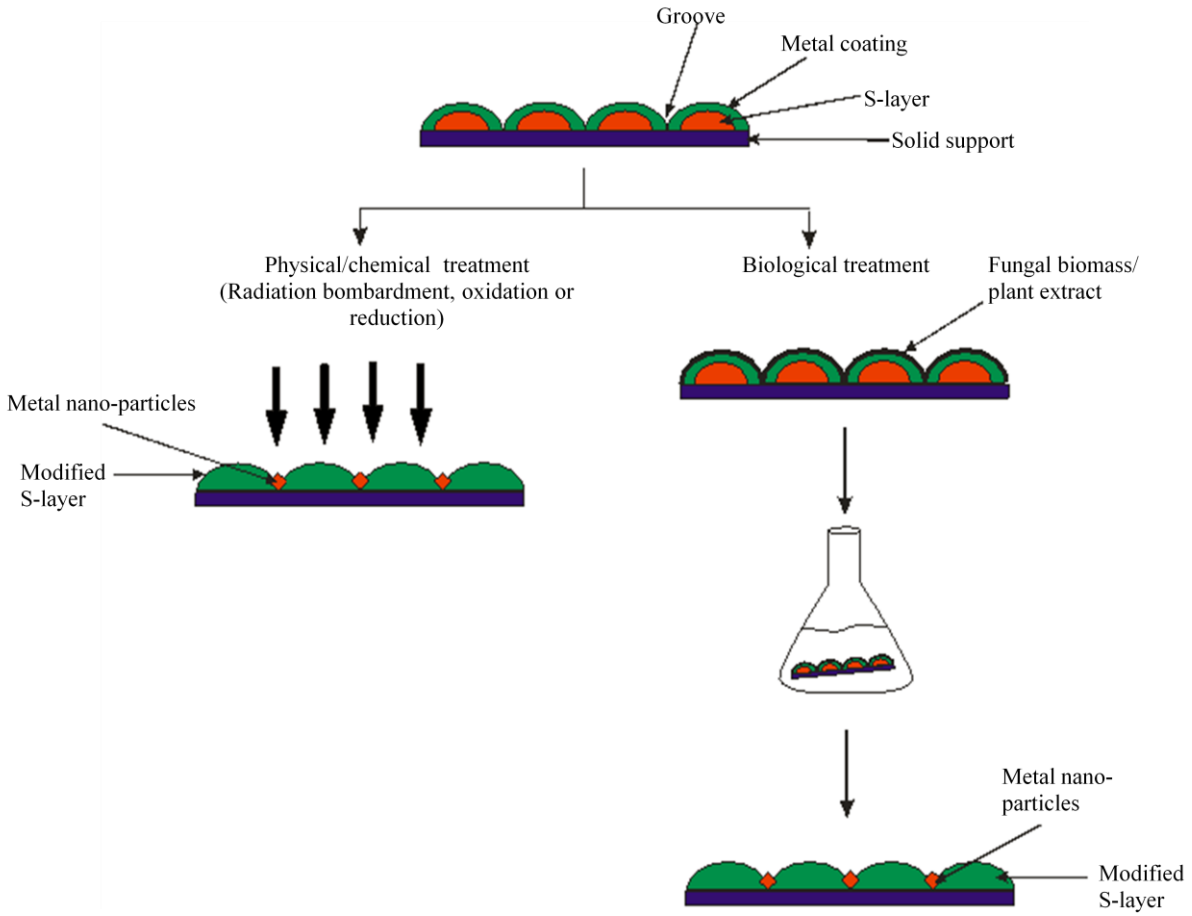

Fig.8 Schematic illustration of metal nano-particle formation using S-layers

VI. CONCLUSION

S-layer proteins are surface proteins present on all prokaryotic cell surfaces with few exceptions. They have unique properties like self assembly/ recrystallization in solid- liquid and air interphases after detachment from the cell. After recrystallization they give uniform pore size in between 2-8nm and can reform in to wide variety of shapes. These properties make them applicable in various fields like vaccine development, biosorption, recombinant DNA technology, ultrafiltration and Nanotechnology.Synthesis of tailor made nanoparticles by using S-layer proteins is successfully done in case of Au [55] and many other metals.

VII. Future Prospect

In future we can hope for the development of ecofriendly Biological methods for nanoparticle synthesis by S-Layer proteins.

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REFERENCES

[1]Abe, M. & Kimoto, N. Distribution of two types of regular- array particles in the cell wall of *Bacillus aneurinolyticus*(Kimura et Aoyama), Microbiol lmmuno.l28, 841-846, 1984.
[2]Allievi, Mariana C., F.Sabbionne Metal Biosorption by Surface layer proteins from *Bacillus* sps
[3]Altman, E., Brisson, J.-R., Messner, P. &Sleytr, U. B. Structure of the glycan chain from the surface layer glycoprotein of *Bacillus alvei* CCM 2051.Biochem Cell Biol69, 72-78, 1991.
[4]Asimumer, Shahid Naveed, Naveed Ramzan , and Muhammad Shahid Rafique, Selection of a suitable method for the synthesis of copper nanoparticles. nano: brief reports and reviews vol. 7, no. 5 1230005 (18 pages), 2012.
[5]Blaser, M., and E. C. Gotschlich.. Surface array protein of *Campylobacter fetus* cloning and gene structure. J. Biol. Chem. 265:14529–14535, 1990.
[6]Brockel, G., M. Behr, S. Fabry, R. Hensel, H. Kaudewitz, E. Biendl, and H.Konig. Analysis and nucleotide sequence of the genes encoding the surface-layer glycoprotein of the hyperthermophilic methanogens *Methanothermus fervidus and Methanothermus sociabilis*. Eur. J. Biochem. 199:147–152. 1991.
[7]Boot, H.J., C. P. A. M. Kolen, and P. H. Pouwels. Identification, cloning, and nucleotide sequence of a silent S-layer protein gene of *Lactobacillus acidophilus* ATCC 4356 which has extensive similarity with the S-layer protein gene of this species. J. Bacteriol. 177:7222–7230, 1995.
[8]Burley, 5. K. & Murray, R. G. E.. Structure of the regular surface layer of *Bacillus polymyxa*. Can J Microbiol29,775-780, 1983.
[9]Chu, S. S. Cavaignac, J. Feutrier, B. M. Phipps, M. Kostrzynska, W. W.Kay, and T. J. Trust. Structure of the tetragonal surface virulence array protein and gene of *Aeromonas salmonicida*. J. Biol. Chem. 266:15258–15265, 1991.
[10]Designed by: Timo Wolf Group: iGEM11_Bielefeld-Germany S-Layer SbpA from Lysini *Bacillus sphaericus*. 2011-09-10.
[11]Dietmar Pum, Jose Luis Toca Herrera and Uwe B. Sleytr. Review S-layer Protein Self- Assembly.International Journal of Molecular Sciences, 14, 2484-2501, 2013.
[12]Dietmar Pum*, Angela Neubauer, Erika Gyoervary, Margit Sára, Uwe B. Sleytr S-layer proteins as basic building blocks in a biomolecular construction kit. A draft paper for the seventh foresight conference on Molecular Nanotechnology.
[13]Dieter Moll, Carina Huber, Birgit Schlegel, DietmarPum, Uwe B. Sleytr, and Margit Sara. S- layer- streptavidin fusion proteins as template for nanopatterned molecular arrays. PNAS Vol.99 No. 23, 14646-14651, 2002.
[14]Dooley et al. Isolation and biochemical characterization of S layer protein, 1988.
[15]Dworkin, J., M. K. Tummuru, and M. J. Blaser. Segmental conservation of sapA sequences in type B *Campylobacter fetus* cells. J. Biol. Chem.270:15093–15101, 1995.
[16]Ellar, D. & Lundgren, D. G. Ordered substructure in the cell wall of *Bacillus cereus*. J Bacteriol94, 1778-1780, 1967.
[17]Fonet A., Mesange S., Tosi-Couture E., Gounon P., Mock M.. *Bacillus anthracis* S-layer.*FEMS Microbiol. Rev*. 20, 55–59, 1997.
[18]Grogono-Thomas R., Dworkin J., Blaser M.J. The role of S-layer proteins in ovine *Campylobacter* abortion. *FEMS Microbiol. Rev*. 20, 133–135, 1997.
[19]Helmut Schmidt Nanoparticles by chemical synthesis, processing to materials and innovative applications Applied Organometallic Chemistry Appl. Organometal. Chem; 15: 331–343 DOI: 10.1002/aoc.169, 2001.
[20]Holt, 5. C. &Leadbetter, E. R.. Comparative ultrastructure of selected aerobic spore-forming bacteria : a freeze-etching study. Bacteriol Rev 33, 346-378. 1969
[21]Howatson, A. F. & Russell, W. C. Observations on cell wall structure in Bacillus subtilis. J Appl Physiol35, 3086-3087, 1964.
[22]Faraldo, M. M., M. A. de Pedro, and J. Berenguer. Sequence of the S-layer gene ofThermusthermophilus HB8 and functionality of its promoter in *Escherichia coli*. J. Bacteriol. 174:7458–7462, 1992.
[23]Fonet A., Mesange S., Tosi-Couture E., Gounon P., Mock M. *Bacillus anthracis* S-layer.*FEMSMicrobiol. Rev*. **20**, 55–59, 1997.
[24]Ilk N., Kupcu S., Moncayo G., Klimt S., Ecker R.C., Hofer-Warbinek R., Egelseer E.M., Sleytr U.B., Sára M. A functional chimaeric S-layer-enhanced green fluorescent protein to follow the uptake of S-layer-coated liposomes into eukaryotic cells. Biochem J. 379:441–448. [PMCID: PMC1224085] [PubMed: 14725506] , 2004.
[25]Kari, M., Rt)nkkB, R., Valtonen, A., Piparinen, H., Kukkonen, M., PByry, T., Nohynek, L., Nurmiaho-Lassila, E.-L. &Lounatmaa, K.. Ultrastructure of a Bacillus sp. strain KL8 isolated from indoor dust. J Basic Microbiol 30, 99-104, 1990.
[26]Lepault, J., Martin, N. & Leonard, K. Three-dimensional structure of the T-layers of *Bacillus sphaericus*P-1.J Bacteriol 168,303-308, 1986.
[27]Lezione 2_cap 7-synthesis and stabilization of Nanoparticles
[28]Luckevich, M. D. & Beveridge, T. 1. Characterization of a dynamic S-layer on *Bacillus thuringiensis*. J Bacteriol 171, 6656-6667, 1989.
[29]Lupas, A., Engelhardt, H., Peters, J., Santarius, U., Volker & Baumeister, W..Domain structure of the Acetogenium 153-163.168,303-308. kivui surface layer revealed by electron crystallography and sequence analysis. Bacteriol 176, 1224-1233, 1994.
[30]Maan Singh Sidhu and Ingar Olsen. Review article S-layers of *Bacillus* species, Microbiology, 143, 1039-1052, 1997.
[31]Majumder D. R., Bioremediation: Copper Nanoparticles from Electronic-waste International Journal of Engineering Science and Technology (IJEST) Vol. 4 No. 10, 4380-4389, 2012.
[32]Margit Sara and Uwe B. Sleytr. Minireview S-layers proteins. Journal Of Bacteriaology, Vol. 182, No. 4. p. 859-868, 2000.
[33]Mayr, J., A. Lupas, J. Kellermann, C. Eckerskorn, W. Baumeister, and J. Peters. A hyperthermostable protease of the subtilisin family bound to the surface layer of the archaeon *Staphylothermus marinus*. Curr. Biol.6:739–749, 1996.
[34]Messner, P., Hollaus, F. &Sleytr, U. B. Paracrystalline cell wall surface layers of different *Bacillus stearothermophilus* strains. lnt J Syst Bacteriol 34, 202-210, 1984.
[35]Mohamed L. Merroun, Johannes R. Comlexation of Uranium by cells and S layer sheets of *B. sphericus*
[36]Morelli, L., and M.-L.Callegari. Surface layer of *Lactobacillus helveticus* CNRZ 892. FEMS Microbiol.Lett. 20:118–121, 1997.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 9, September 2013

[37]Neubauer A., Pum D., Sleytr U.B. An amperometric glucose sensor based on isoporous crystalline protein membranes as immobilization matrix. *Anal.Lett*.26, 1347–1360, 1993.
[38]Neubauer A., Hodl C., Pum D., Sleytr U.B. Amultistep enzyme sensor for sucrose based on S-layers microparticles as immobilization matrix.*Anal.Lett*.27,849–865, 1994.
[39]Nicola IIK, Eva M Egelseer, and Uwe B. Sleytr.S-Layer Fusion Proteins – Construction Principles ans applications.CurrOpinBiotechnol. 22(6): 824 – 831, 2011.
[40]Pablo Mobili, Maria de los Angeles Serradell, Ciaudine Mayer, Veronique Arluisol and Andrea Gomez – Zavaglia. Biophysical Methods for the Elucidation of the S –Layer Proteins/ metal interaction.International Journal of Biochemistry Research and Review. 3(1): 39-62, 2013.
[41]Paul Messner, Christina Scha¨ffer, Eva-Maria Egelseer, and Uwe B. Sleytr, Occurrence, Structure, Chemistry, Genetics, Morphogenesis, and Functions of S-Layers, Chapter 2.
[42]Peyret, J. L., N. Bayan, G. Joliff, T. Gulik-Krzywicki, L. Mathieu, E.Shechter, and G. Leblon. Characterization of the cspB gene encoding PS2, an ordered surface layer protein in *Corynebacterium glutamicum*. Mol. Microbiol. 9:97–109, 1993.
[43]Peters, J., M. Nitsch, B. Kuhlmorgen, R. Golbik, A. Lupas, J. Kellermann,H. Engelhardt, J. P. Pfander, S. Muller, K. Goldie, A. Engel, K. O. Stetter, and W. Baumeister. Tetrabrachion: a filamentous archaebacterial surface protein assembly of unusual structure and extreme stability. J. Mol.Biol. 245:385–401, 1995.
[44]Peters, J., M. Peters, F. Lottspeich, and W. Baumeister. S-layerprotein gene of *Acetogenium kivui*: cloning and expression in *Escherichia coli* and determination of the nucleotide sequence. J. Bacteriol. 171:6307–6315, 1989.
[45]Peters, J., W. Baumeister, and A. Lupas. Hyperthermostable surface layer protein tetrabrachion from the arachaebacterium *Staphlyothermus marinus*: evidence for the presence of a right-handed coiled coil derived from primary structure. J. Mol. Biol. 257:1031–1041, 1996.
[46]Phipps, B., R. Huber, and W. Baumeister. The cell envelope of the hyperthermophilic archaebacterium *Pyrobaculum organothrophum* consists of two regularly arrayed protein layers: three-dimensional structure of the outer layer. Mol. Microbiol. 5:523–528, 1991.
[47]Pollmann K., RaffJ.,Merroun M., Fahmy K., Selenska- pobell S. Metal binding by Bacteria from Uranium mining waste piles and uts technological applications. BiotecnolAdv . 24;58-68.[Pubmed :16005595, 2006.
[48]Pum, D., Weinhandl, M., Htrdl, C. &Sleytr, U. B. Large- scale recrystallization of the S-layer of *Bacillus coagulans* E38-66 at the air/water interface and on lipid films.] Bacteriol 175,2762-2766, 1993.
[49]Sara M., Sleytr U.B. Use of regularly structured bacterial cell surface layers as matrix for immobilizing macromolecules.*Appl. Microbiol. Biotechnol*.30, 184–189, 1989.
[50]Sara, M., Moser-Thier, K., Kainz, U. &Sleytr, U. B. Characterization of S-layers from mesophilic *Bacillaceae* and studies on their protective role towards muramidases.Arch Microbiol 153, 209-214, 1990.
[51]Sara, M., Pum, D., KUpcU, S., Messner, P. &Sleytr, U. B. Isolation of two physiological induced variant strains of *Bacillus stearothermophilus* NRS 2004/3a and characterization of their S- layer lattices. J Bacteriol 176, 848-860, 1994.
[52]Sara M., Sleytr U.B. Biotechnology and biomimetic with crystalline bacterial cell surface layers (Slayers), 1996.
[53]Sara M., Sleytr U.B. S-layer proteins.*J. Bacteriol*. 182, 859–868, 2000.
[54]Sarang S. Puranik, Hrushikesh M. Joshi, S. B. Ogale and K. M. Paknikar. Hydrazine Based Facile synthesis and Ordered Assembly of Metal Nanoparticles(Au, Ag) on a Bacterial Surface Layer Protein Template. Journal of Nanoscience and Nanotechnology, Vol. 8, 3565-3569, 2008.
[55]Schenk, A. &Aragno, N. *Bacillus schlegelii*, a new species of thermophilic, facultatively chemolithoautotrophic bacterium oxidizing molecular hydrogen. J Gen Microbiol 115, 333-341, 1979.
[56]S. Dieluweit, D. Pum, and Uwe B. Sleytr. Formation of gold superlattice on an S-layer with square lattice symmetry.Supramolecular Science Vol. 5, No. 1-2, 1998.
[57]Sergey R. Konstantinov, HaukeSmidt, Willem M. de Vos, sven C. M. Bruijns, SatwinderKaur Singh, Florence Valence, Daniel Molle, Sylvie Lortal, Eric Altarmann, Todd R. Klaenhammer and Yvette Van Kooyk. S-layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T-cell function. PNAS, Vol.105, No.49 19474-19479, 2008.
[58]Sleytr U.B. Regular arrays of macromolecules on bacterial cell walls: Structure, chemistry, assembly and function. *Int. Rev.Cytol*. **53**, 1–64, 1978.
[59]Sleytr, U. B., P. Messner, D. Pum, and M. Sara. Crystalline bacterial cell surface layers. Springer-Verlag, Berlin, Germany, 1988.
[60]Sleytr, U. B., Sara, M., KUpcii, Z. &Messner, P.. Structural and chemical characterization of S-layers of selected strains of *Bacillus stearothermophilus* and *Desulfotomaculum nigrificans*. Arch Microbiol 146, 19-24, 1986.
[61]Sleytr, U. B. P. Messner, D. Pum, and M. Sa´ra.. Crystalline bacterial cell surface layers. Mol. Microbiol. 10:911–916, 1993.
[62]Sylvie Lortal, Jean Van Heijenoort, Karin Gruber and Uwe B. Sleytr. S layer of *Lactobacillus helveticus* ATCC 12046: isolation chemical characterization and reformation after extraction with LiCl, Journal of general Microbiology, 138, 611-618, 1992.
[63]Tang, M., Owens, K., Pietri, R., Zhu, X., McVeigh, R. &Ghosh, B.K. Cloning of the crystalline cell wall protein gene of*Bacillus licheniformis*NM105.*J Bacteriol 171*, 6637-6648, 1989.
[64] Thomas, S. R., and T. J. Trust. Tyrosine phosphorylation of the tetragonal paracrystalline array of *Aeromonas hydrophila*: molecular cloning and high-level expression of the S-layer protein gene. J. Mol. Biol. 245:568–581, 1995.
[65]Toumelin, 1. E., Sirard, 1. C., Dulflot, E., Mock, M. &Fouet, A. Characterization of the *Bacillus anthracis* S-layer : cloning and sequencing of the structural gene. J Bacteriol 177, 614-620, 1995.
[66]Tummuru, M., and M. J. Blaser. Rearrangement of *sapA* homologs with conserved and variable regions in *Campylobacter fetus*. Proc. Natl. Acad. Sci. USA **90:**7265–7269 1993.
[67]Uwe B. Sleytr, Paul Messner, DietmarPum, and Margit Sara. Review Crystalline Bacterial Cell Surface Layers (S Layers): From Supramolecular Cell Structure to Biomimetics and Nanotechnology. Angew. Chem. Int. Ed. 38, 1034 – 1054, 1999.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 2, Issue 9, September 2013

[68]V.G.Debabov,. Bacterial and Archaeal S- layer as subject of Nanobiotechnology. Molecular Biology, Vol 38. No.4 pp.482-493.translated from Molekulyarnaya Biologiya, vol.38, No.4, 2004, pp 578 – 591, 2004.
[69]Vidgren, G., I. Palva, R. Pakkanen, K. Lounatmaa, and A. Palva.S-layer protein gene of *Lactobacillus brevis*: cloning by polymerase chain reaction and determination of nucleotide sequence. J. Bacteriol. 174:7419–7427, 1992.
[70]Weigert, S., and M. Sara. Surface modification of an ultrafiltration membrane with crystalline structure and studies on interactions with selected protein molecules. J. Membr. Sci. 106:147–159, 1995.
[71] http://www.agavebio.com/Catalog/contentframe.html

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