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BACTERIAL SURFACE LAYER PROTEINS: A KEY TO METAL BIOSORPTION

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***Abstract*—According to various studies it has been shown that *Bacillus* species play a key role as biosorbent for metals. However a clear mechanism for this characteristic has not yet been deduced. It is believed that Bacillaceae family shows the property of sorption due to the presence of numerous envelopes and spores. Our studies include use of bacteria showing the capacity of producing surface layer proteins for biosorption. Copper was taken as the metal of interest and checked for its absorption. Biosorption analysis by following bacteria was carried out viz *B. subtilis* 2097, *B. licheniformis*2324, *B. sphaericus*2478, *L. brevis* 2090, *L. acidophilus 2*285. These cultures were obtained from NCIM. Bacillus species were grown in LB medium and Lactobacillus were grown in MRS medium. Extraction of surface layer proteins was carried out using 5M LiCl. Various parameters like culture age, pH, temperature, contact time were optimized. Comparison of biosorption by surface layer protein with that of whole cell was also studied. Maximum S layer production by *B. sphaerices* 2478 was found to be 38.9% in 24hrs old culture. Maximum sorption was found at pH of 7.0, temperature 37^{0}C. 500ppm of Copper Sulphate solution with 1mg of both S layer and whole cell were utilized. SDS PAGE analysis of S-layer protein was performed and 40kD molecular weight for *Bacillus sphaericus* was visualized.**

Keywords: Biosorption, S layer proteins, *Bacillus sps.* Lactobacillus *sps*., Copper.

I. INTRODUCTION

Due to the excesses of toxic metals being released into water bodies, it has become necessary to find new methodologies for the removal of heavy metals from these water bodies.Copper is one of the most common metals of industrial waste [13].Similar conditions have been noticed in heavy metal mining sites. Organisms isolated from these sites have been used for bioremediation purposes [1].Various metals like**,** Cu, Pd (II), Au (III) and U act as xenobiotics. Though these metals are important entities in various metabolic processes but if present in higher concentration they prove harmful to human health and the environment.

Biosorption can be termed as the capability of materials of biological origin to amass heavy metals from wastewater through a metabolically mediated or physico-chemical channel of uptake. This biosorption technique has been carried out using various biological entities including dead or alive fungal biomass, immobilized cells, weeds etc. [2,3and 19]. It has become a necessity to come up with cheaper technologies and conduct further exploration of available biosorbants.

Surface Layer proteinsarefound commonly in cell envelope of archae and bacteria. These are mono molecular assemblies of similar sub units of proteins or glycoprotein. Itranges from 2 to 8 nanometers [4]. S Layer proteins have metal binding capacity due to the presence of surface motifs. Recent studies involving S layer arewith reference to its biosorption capacity of heavy metals.Presence of spores shows an added advantage in metal accumulation [7]. Use of spores for biosorption of heavy metal has been compared with surface layer protein metal binding capacity [5]. The S Layer of *Bacillus spericus* strain JG-A12 has been found to show a high capacity of Uranium bisorption [6]. A direct relationship between S Layer protein and metal binding capacity has been represented with connection to the retention of S Layer protein by *Bacillus* cells [8]. We have studied Surface Layer producing *Bacillus* and *Lactobacillus* speciesfor their metal binding capacity by optimizing various parameters in order to gain maximum biosorption.

II. MATERIALS AND METHOD

Microorganisms and Media

The cultures used were *Bacillus sphaericus*NCIM2478, *Bacillus subtilis*NCIM2097, *Bacillus licheniformis*NCIM2324, *Lactobacillus acidophilus* NCIM2285, *Lactobacillus brevis* NCIM2090.

Bacillus cultures were maintained on nutrient agar slants and Lactobacillus cultures were maintained on MRS agar slants.

Whole cell preparation

Exponential cultures of all Bacillus *sps.*were grown in Luria-Bertani medium and all *Lactobacillus sps*. were grown in de Man, Rogosa and Sharpe(MRS) medium. They were subjected to centrifugation at 3000xg, the wet pellet was harvested and kept for drying in an oven at 55^{0}C for 24 hr and used for dry weight determination.

Extraction of S-layer protein

100ml exponential culture was centrifuged at 3000xg, harvested cells were washed with PBS (pH7.4). 15mg of wet pellet was treated with 1 ml of5M LiCl and kept at 0^{o}C for 15 min. Centrifugation at 15,000xgfor 20 min. Supernatant was collected andpellets treated with 10ml 5M LiClfor 30 min followed by centrifugation 15,000xg for 20 min.Supernatants collected as crude extract[9].

Purification of S-layer protein

Dialysis membrane of 10 cm length was pre-treated in boiling water bath for 30 min, the tubing was filled with crude extract. The crude extract was dialysed against Milli Q water at 4^{o}C overnight. This was followed by centrifugation at10,000xg for 20 min the pellet (S layer) was dispensed in Milli Q water and stored at -20^o C

SDS PAGE Analysis

12% polyacrylamide gel was used for SDS PAGE analysis. Slayer proteins were treated with Laemmli's gel loading buffer. Running buffer constituted of Tris 3.16g, Glycine 2.0 g, SDS 0.5g, and Distilled water 500 ml. Silver staining was carried out to observe protein bands.

Gel Doc EZ Imager, BioRad; Software: Image Lab 3.0 was used for documentation of gel image

Biosorption profile

$CuSO_4.5H_2O$ (analytical grade) was used to prepare 1000 ppm stock solution for Cu^{2+}

For biosorption, whole cell was used. This dry biomass (1mg dry weight) was added to a range of 100 to 1000 ppm Cu^{2+} solution. Cu^{2+}biosorption was analyzed till it reached equilibrium. Biosorbant was removed by centrifugation (15000xg). The unabsorbed metal was estimated using Ultra Violet Visible Spectrophotometer (Systronics 118 double beam model). Biosorption using S Layer proteins was done with 1mg of isolated protein in every experiment.

Determination of metal uptake

Metal uptake was calculated by $Q=V(C_f-C_i)/W$, where Q is metal uptake (mg Cu^{2+}/gm cell), V is Volume of metal ion solution, C_i is initial concentration of Cu^{2+} in solution, C_f is final concentration of Cu^{2+} in solution [13].

Optimization of pH was carried out using $CuSO_4.5H_2O$ solution of different pH ranging from 5 to 9.

III. RESULTS

A. Use of whole cell for metal biosorption

*Bacillus sphaericus*2478, *Bacillus subtilis*2097, *Bacillus licheni form is* 2324, *Lactobacillus acidophilus* 2285, *Lactobacillus brevis*2090 were all used for preparation of whole cell to analyze Cu^{2+} metal ion accumulation by specific whole cells. A considerable biosorption capacity of approximately 23% was shown by the cells of *Bacillus sphaericus.* (Fig.1).Equilibrium was established after 23% of Cu was retained by these whole cells.

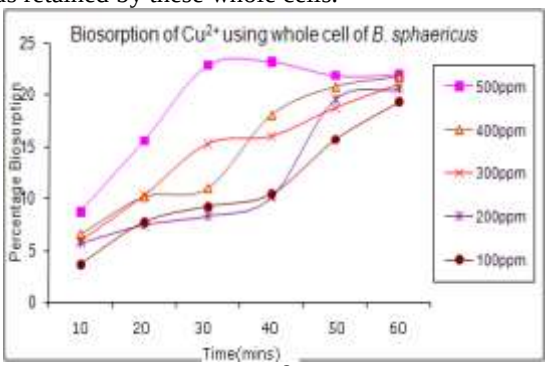


Fig. 1: Biosorption of Cu^{2+} using whole cell of *B. sphaericus*with different concentrations of Cu^{2+} solution (100-500 ppm)

The biosorption shown by whole cell of other organisms was remarkably low. This analysis was done in order to have a comparison between the capability of biosorption by S Layer and whole cell.

B. Use of S Layer for metal biosorption

It has been reported that the presence of metal binding peptides increases biosorption of metal ions [10]. The experimental profile carried out for Cu^{2+}biosorption by S layer protein isolated from *Bacillus sphaericus, Lactobacillus acidophilus, Lactobacillus brevis* displays a high retention capacity of copper ions. 1 mg of S Layer protein of *Bacillus sphaericus* in a 500 ppm of Cu^{2+} solution displays about 40% biosorption. After retention of Cu^{2+} to this extent biosorption stabilizes. (Fig. 2)

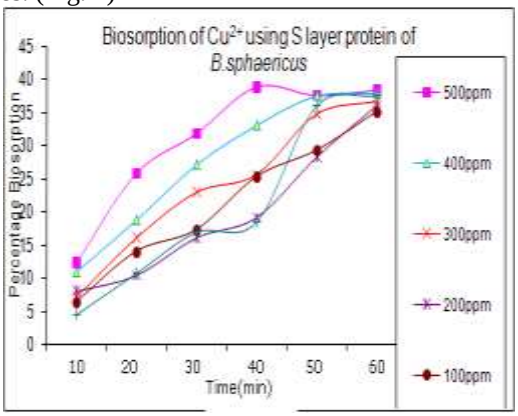


Fig 2: Biosorption of Cu^{2+} using S layer protein of *B.sphaericus*shows highest metal uptake of 40%

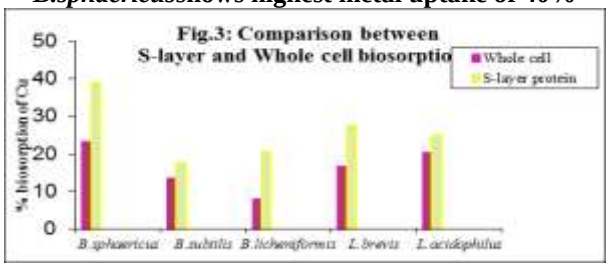


Fig 3: Comparision between the metal binding capacity of isolated S Layer protein and whole cell shows that with each organism S Layer definitely plays a more crucial rolein Copper biosorption. (Fig.4)

C. Effect of pH on Cu^{2+}biosorption

In any biosorption experiment pH of the solution affects the metal binding property of a particular biosorbant as well as may interfere with chemical aspect of metal solution. The other parameters were standardized and only a change in pH ramging from 5.5 to 8.5 was analysed using S Layer. An optimized pH for the following bacteria *Lactobacillus brevis,Bacillus sphaericus, Bacillus licheniformis*is shown in Fig 4.

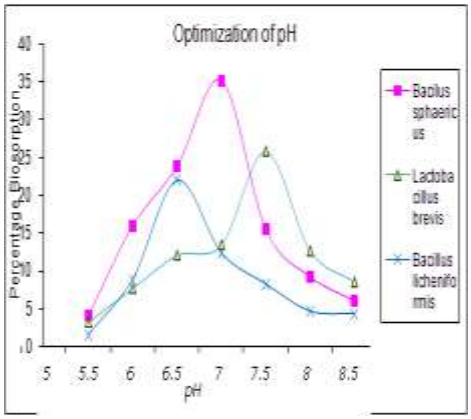


Fig. 4: S Layer of *Lactobacillus brevis* shows its maximum biosoeprion at a pH of 7.5 whereas that of *Bacillus sphaericus* and *Bacillus licheniformis* is found to be 7 and 6.6 respectively.

Effect of initial Copper metal concentration and biosorption capacity of S Layer.

The metal uptake capacity Q for Cu^{2+} was graphically represented with final metal concentration $C_{f.}$. 1 mg of S Layer retains maximum Copper metal till equilibrium was established

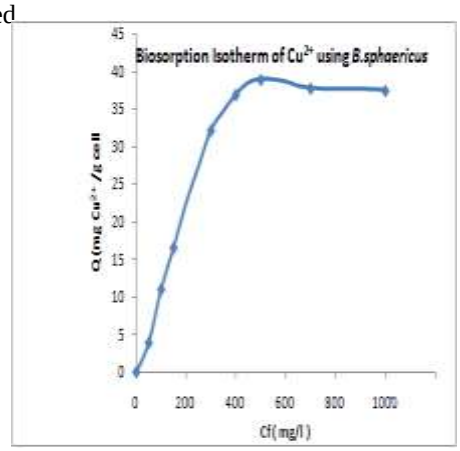
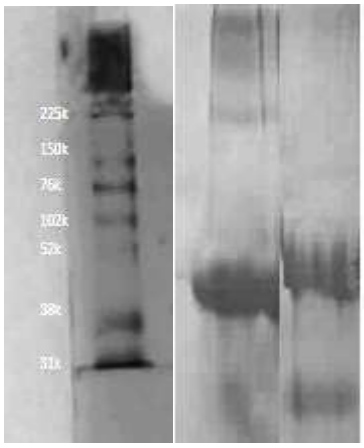


Fig 5: The biosorption isotherms of Cu^{2+} on S Layer of *B.sphaericus:*.1 mg S Layer of *B. sphaericus* .has shown maximum removal of Copper metal ions with various concentrations of Cu^{2+} till equilibrium. The equilibrium is reached at a maximum value of 38.9mg Cu^{2+}/gm cell.

D. SDS PAGE analysis



M CE PP

Fig 6: SDS PAGE analysis of S Layer protein of *B. sphaericu, M*- Molecular weight marker, CE- crude extract, PP- Purified protein after dialysis

SDS PAGE analysis for molecular mass of S Layer protein of *B. sphaericus* revealed molecular weight of S layer protein of *B. sphaericus* is 40-45k

IV. DISCUSSION

S-layers are crystalline protein structures arranged in particular shape with definite size in nanometers. This property of S layer gives them potential to be used in various applications fields like vaccines, ultrafilteration, immobilization, synthesis of nanoparticles and in Biosorption [21].Present study focuses on application of S-layer proteins in Biosorption of heavy metals. Use of bacterial and fungal biomass for heavy metal removal from liquid effluent has potential advantage like low cost and recycling[8]. *Bacillaceae* family shows high capacity for biosorption due to presence of number of surface structures and envelopes on its cell surface whichcan interact with heavy metals. S-layer proteins covering 15% of total protein in bacteria, when isolated, purified and used for biosorption of heavy metals showed comparatively high percentage of biosorption, than whole cells of bacteria.

Columbian *B. spharicus* strains were used for biosorption of Hg, Co, Fe and Cr. Biosorption relation between dead and live organism was analyzed. Living cell showed 6 to 47% of Co, Hg, and Fe and As accumulation. 25 and 44.5% of Cr accumulation was shown by dead and living *B. sphaericus* OT4b31. These results conclusively show that lack of active metabolisms affects biosorption of dead cells. However the presence of S Layer can still bring about metabolisms in living as well as dead cells as compared to dead fungal or bacterial cell mass they are active proteins which can reassemble by themselves and provide more surface area for biosorption [22].

In our studies the*B. sphaericus* culture which has maximum capacity of producing S Layer proteins brings about considrably high retention of Cu^{2+}. The comparative analysis of this with that of whole cell clearly depicts that S Layer protein is a more potent biosorbant. Previous studies of S Layerbiosorption have not shown significant result with S Layer of *L.acidophilus* and *L. brevis*which inthis study displays a substantial 22% and25% biosorption respectively. The difference between the metal chelating properties of the biosorbant S Layer is almost twice than that of whole cell. The use of various other biosorbants like dead or live fungal biomass, cyanobacteria, weeds has been rampant in bioremediation. [3, 11, 12 and 14]. When these are compared with S Layer, capacity of S Layer of bacteria used is found to be similar to the capacity of dead or live fungal biomass, cyanobacteria, and weeds.

V. FUTURE PROSPECTIVE

- The most unique characteristic of the S Layer biosorbant is its property of self assembly. The presence of lattice symmetry can be exceptionally useful in studying recovery of metals using these surface layer proteins.
- The recovery of metal ion in the form of nanoparticles of uniform shape and size using S Layer proteins can be done due to its symmetrical lattice [21].

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