Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0403.26**

PROBIOTIC MEDIATED SYNTHESIS OF SELENIUM NANOPARTICLES: CHARACTERIZATION AND BIOFILM SCAVENGING ANALYSIS

R. Kaur, T. Kaudal, A. Sharma*

Department of Biotechnology and Bioinformatics, DAV College, Sector 10, Chandigarh, India.

ABSTRACT: Bacteria in biofilms are innately more resistant to existing antimicrobial agents owing to protective covering of exopolysaccharides around microbial colonies. The eradication of biofilm is difficult thereby accentuating the need to develop alternative interventions. The present study is concerned with the development and characterization of selenium nanoparticles (SeNPs) prepared by probiotics against biofilms. The nanoparticles were characterized by TEM. The antimicrobial study showed that the generated nanoparticles were more effective against biofilm forming micro-organisms. As biofilms are generally composed of extracellular proteins, and polysaccharides;SeNPs also reduced the protein as well as carbohydrates content crucial to biofilm formation and antimicrobial resistance.

KEYWORDS: Selenium nanoparticles, biofilm, lactobacillus, antimicrobial activity.

Corresponding Author: Dr. A. SharmaPh.D.

Department of Biotechnology and Bioinformatics, DAV College, Sector 10, Chandigarh, India.Email Address: anjs108@yahoo.co.in

1.INTRODUCTION

A biofilm, sometimes referred to as slime, is a polymeric mixture generally composed of extracellular DNA, proteins, and polysaccharides [1]. Bacterial polysaccharides are a major component of the extracellular polymeric substance or matrix of biofilms, and mediate most of the cell-to-cell and cell-to-surface interactions required for biofilm formation and stabilization [2]. Cell communication in bacteria occur through the process called quorum sensing.Biofilm formation protects and enables single-cell organisms to assume a multicellular lifestyle, in which “group behavior” facilitates survival in adverse environments [3]. Biofilms usually consist of a mixed

bacterial population, but they may also consist of a single bacterial species [4].Traditional antibiotic resistance of free-living bacteria usually involves inactivation of the antibiotic, modification of targets, and exclusion of the antibiotic [5]. Resistance to antibiotics in both stationary phase cells and biofilms may be due to the presence of persister cells which are mechanistically distinct from multidrug resistance [6].Biofilm forming organisms have an inherent resistance to antibiotics, disinfectants and germicides. Unlike planktonic populations, bacterial cells embedded in biofilms exhibit intrinsic resistance to antibiotics due to several specific defense mechanisms conferred by the biofilm environment, including the inactivation of anti-microbial agents by exopolysachharide (EPS), over expression of stress-responsive genes, oxygen gradients within the biofilm matrix and differentiation of a subpopulation of biofilm cells into resistant dormant cells. It is now well documented that biofilms are notoriously difficult to eradicate [7,8,9]. The intrinsic resistance of bacterial cells within biofilms to conventional anti-microbials has motivated new approaches for elimination of biofilms.The use of nanoparticles can be considered as a new approach among the various methods for the prevention and elimination of biofilms [10]. Nanotechnology may provide the answer to penetrate such biofilms and reduce biofilm formation. Zinc, Silver, copper nanoparticles etc. are effective against the biofilm as they were able to penetrate inside the cell .There are various chemical and physical methods to synthesize nanoparticles but these routes for synthesis of particles are tedious and may be associated with environmental toxicity or biological hazards. The use of natural materials like plant extracts, bacteria, fungi and enzymes for the synthesis of nanoparticles offer numerous benefits as refraining use of toxic chemicals for the synthesis .The growing need to develop clean, non-toxic and ecofriendly procedures for synthesis of nanoparticles has resulted in researchers looking at biological systems for inspiration.To meet the increasing demands for commercial nanoparticles new eco-friendly “green”methods of synthesis, were discovered [11]. Numerous endeavours have been made to synthesize nanoparticles employing natural resources[12-16].In this study selenium nanoparticles werepreparedbybioreduction of selenium through Probiotics and were tested for their antibiofilm activity against biofilm forming microorganisms. Elemental selenium prepared employing Probiotics are least toxic of all selenium form [17].

2. MATERIALS AND METHODS

Commercially availableSporolac manufactured by Uni Sankyo Ltd. containing around 150 million spores of*Lactobacillus sporogenes*in 1g sachet was used in the study, Sodium selenite, Nutrient Broth, Nutrient agar, MRS broth, YEPD agar, YEPD broth, Trypticase soy broth manufactured by HiMedia. All the reagents of standard grade were used in the study.

Micro organisms

For evaluating the antimicrobial activity of selenium nanoparticles different microbial strains

employed were *Escherichia coli* (MTCC 118), *Staphylococcus aureus* (MTCC 96), 2488), *Klebsiella* (MTCC109), *Bacillus subtilis* (MTCC 121) and *Candida albicans* (MTCC 183).

Methods for detection of biofilm

Tube method:

10 ml of Tryptic soy broth with 1% glucose was inoculated with a loop full of test organism from overnight culture on nutrient agar individually. Broths were incubated at 37 ˚c for 24 hours. The cultures were decanted and tubes were washed with phosphate buffer saline pH7.3.The tubes were dried and stained with 0.1% crystal violet. Excess stain was washed with deionized water. Tubes were dried in inverted position and observed for biofilm formation. Biofilm Production was considered positive when a visible film lined the wall and bottom of the tube. Ring formation at the liquid interface was not indicative of biofilm formation. Tubes were examined for biofilm formation[18].

Congo Red Agar Method (CRA):

Prepared 250 gm nutrient agar medium andCongo red stain as concentrated aqueous solution separately, mixed and autoclaved at 121˚ C for 15 minutes. Plates were inoculated with test organism and incubated at 37 ° C for 24 to 48 hours aerobically. Black colonies with a dry crystalline consistency indicated biofilm production; weak producers usually remained pink, though occasional darkening at the center of colonies was observed [19].

Tissue culture plate method (TCP):

Isolates from fresh agar plates were inoculated on trypticase soy broth with 1% glucose (TSB media and incubated for 18 hours at 37°C and then diluted 1 in 100 with fresh medium. Individual wells of sterile polystyrene, 96 wells- flat bottom tissue culture plates were filled with 0.2 ml aliquots of the diluted cultures and only broth served as control to check the sterility and non-specific binding of the media. The tissue culture plates were incubated for 24 hours at 37°C.After incubation, the content from each well was gently removed by tapping the plates. The plates were gently submerged into PBS solution (pH 7.2). Shook out the PBS and repeated the process two to three times. Added 125µl of 0.1% solution of crystal violet in water to each well of the microtiter plate and incubated the plate at room temperature for 10-15 minutes the plate was rinsed 3-4 times with water to rid the plate of all excess cells and dye. Turned the micro titer plates upside down and dried for a few hours or overnight.125µl of 30% acetic acid was added in water to each well of the tissue culture plate to solubilize the crystal violet. The plates were incubated for 10-15 minutes at room temperature. Transferred 125µl of solubilized crystal violet to a flat bottomed microtiter dish and quantified the absorbance in a plate reader at 550 nm using 30% acetic acid in water as the blank [20].

Preparation of selenium nanoparticles

Strain

Lactobacillus (SPOROLAC)

Culture media:

The commercially available media MRS broth was suitable for the lactic acid bacteria (PROBIOTICS)

Production and Recovery of Purified Nano-Selenium [17]

Dissolved the MRS, 5.5grams in 100 ml distilled water andautoclaved it. After cooling down to 25˚C added 30 mg sodium selenite as selenium source and 1gm *Lactobacillus acidophillus* to 100 ml of selenite containing MRS solution in shaking incubator for 36-48 hours at 37 ˚ C (optimum temperature for lactic acid bacteria full reproduction cycle). At the end of the fermentation process the culture medium become red, because of the produced elemental selenium. The medium was centrifuged at 8000 rpm for 10-15 minutes, supernatant was discarded and pellet was washed thrice in distilled water. The formation mechanism of elemental selenium is mainly intracellular in lactic acid bacteria. To digest their very resistant cell wall, added hydrochloric acid (37% HCl) to the nanoselenium sample.This acidic hydrolysis took five days at room temperature. The acid was removed by centrifugation (6000 rpm for 10-15 minutes) and washing with distilled water until its pH returned to neutral. The samples were ultrasonicated for 10-15 minutes in order to disintegrate the cohesive selenium spheres. As a last step use vacuum filtration was employed to get rid of the rest of the bacterial cell wall.SeNPswere characterized by TEM.

Zone of inhibition assay by Disk diffusion Method [21]Media was prepared,autoclaved and poured into Petri dish aseptically. Each of the bacterial culture was diluted to match 0.5 McFarland turbidity.100µl of each bacterial strain was spread onto the plate containing nutrient agar. 30 µl of nanoparticle dispersed in distilled water dispensed onto the stack of discs(Whatman filter paper) was gently pressed to agar using a flame sterilized foreceps. Plates were incubated overnight in an incubator.Zone of inhibition was observed.

Biofilm inhibition:

Biofilm was grown in a microtiter plate by using the method of tissue culture plates where the culture and the nanoparticles samples were added as shown in table A. The cultures added were matched with 0.5 McFarland solution.

A1	A2	A3	A4	A5	A6
B1	B2	B3	B4	B5	B6
C1 100µl MHB 10 µl NPs 50 µl PA 90µl PBS	C2 100µl MHB 10 µl NPs 50 µl EC 90µl PBS	C3 100µl MHB 10 µl NPs 50 µl SA 90µl PBS	C4 100µl MHB 10 µl NPs 50 µl CA 90µl PBS	C5 100µl MHB 10 µl NPs 50 µl BS 90µl PBS	C6 100µl MHB 10 µl NPs 50 µl KP 90µl PBS
D1 100µl MHB 20 µl NPs 50 µl PA 80µl PBS	D2 100µl MHB 20 µl NPs 50 µl EC 80µl PBS	D3 100µl MHB 20 µl NPs 50 µl SA 80µl PBS	D4 100µl MHB 20 µl NPs 50 µl CA 80µl PBS	D5 100µl MHB 20 µl NPs 50 µl BS 80µl PBS	D5 100µl MHB 20 µl NPs 50 µl KP 80µl PBS
E1 100µl MHB 30 µl NPs 50 µl PA 70µl PBS	E1 100µl MHB 30 µl NPs 50 µl EC 70µl PBS	E1 100µl MHB 30 µl NPs 50 µl SA 70µl PBS	E1 100µl MHB 30 µl NPs 50 µl CA 70µl PBS	E1 100µl MHB 30 µl NPs 50 µl BS 70µl PBS	E1 100µl MHB 30 µl NPs 50 µl KP 70µl PBS

Table A- Depiction of different incubation mixtures in tissue culture plate for assessment of bilfilm scavenging activity [Row A1-A6 = blank containing MHB (100µl)+ PBS(150µl); Row B1-B6 = standard containing MHB(100µl)+culture(50µl)+PBS(100µl)] (MHB= Muller Hilton Broth; NPs= selenium nanoparticles with varying concentration in each column; PBS= Phosphate Buffer Solution; PA= *Psuedomonas aeruginosa*; EC= *Escherichia coli*; SA= *Staphylococcus aureus*; CA= *Candida albicans*; BS= *Bacillus subtilis;* KP=*Klebsiellapuenomina*).

The formula used for the determination of the biofilm inhibition was: $Biofilminhibition\ \% = [(O.D.ofcontrol - O.D.oftest) \div O.D.ofcontrol] \times 100$

3. RESULTS AND DISCUSSION

The Congo red method provides a reliable interpretation for biofilm depiction and its nature. As shown in fig 1(c),1(d) and1(f),*Klebsiella, Bacillus,Staphylococcus* produced black colonies which is the indicative of strong biofilm whereas Fig1(a),1(b)and 1(e) *C.albicans,P.aeruginosa* and *E.coli* produce dark red colony which indicate moderate biofilms.

Fig1(a)*Candida* albicans **Fig1 (*b*)** *Pseudomonas aeruginosa*

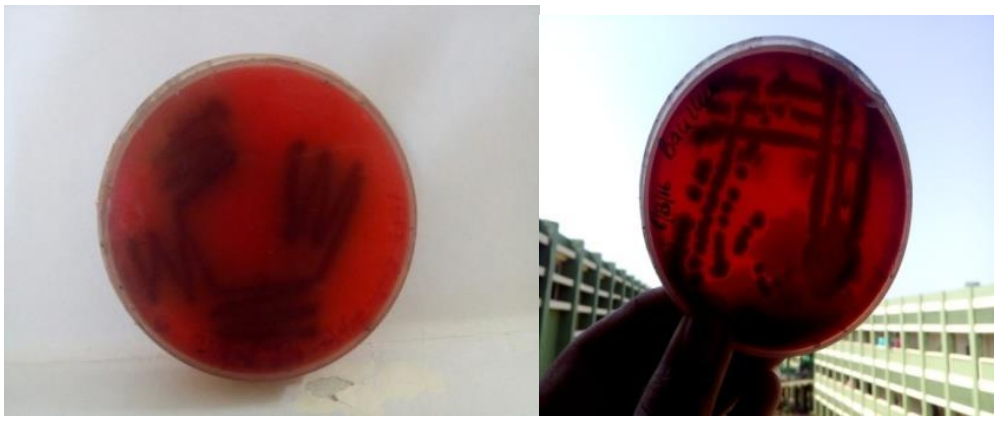

Fig1(c)*Klebsiella* **Fig1(d)***Bacillussubtilis*

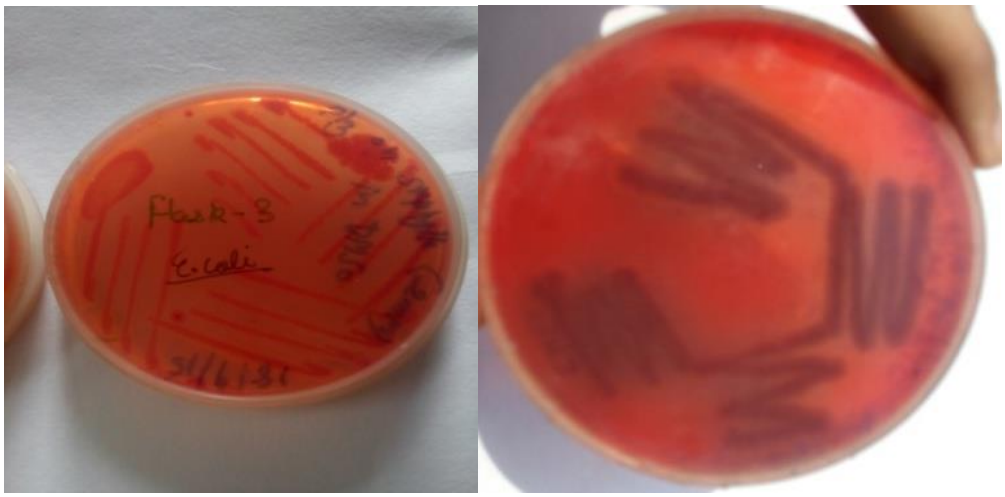

Fig1(e)*E.coli* **Fig1 (f)***Staphylococcus aureus*

Fig1 (a-f) Biofilm formation in different microbes.

Test tube method

In this method tubes were stained with crystal violet (0.1%).

Biofilm formation was considered positive when a visible thick film lined the wall and the bottom of the tube. As depicted in the figures all the organisms were forming blue ring on the walls and bottom of the respective tubes when cultured in TSB.

Dark blue ring depict –Strong biofilm

Light blue ring depict – moderate biofilm

No ring depict - biofilm not formed

As shown in fig. 2 the blue ring depicts biofilm formation

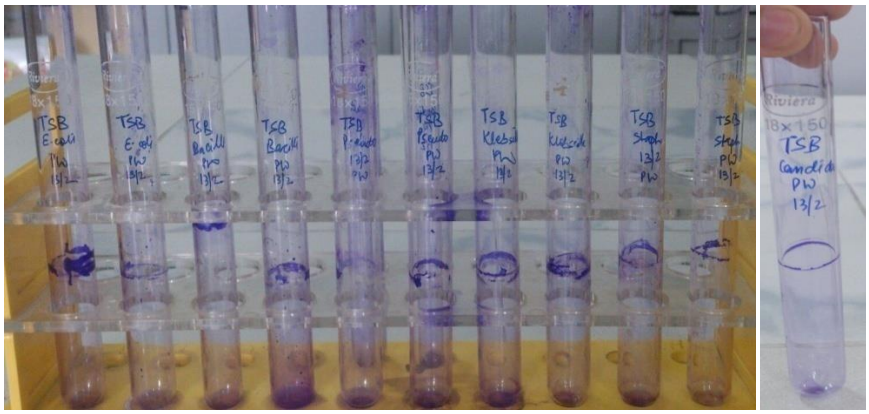

Fig 2 Depiction of blue color ring indicates biofilm formation.

Characterization of Selenium nanoparticles by Transmission Electron Microscopy (TEM)

Nanoparticulate selenium was synthesized employing *Lactobacillus*and characterized by Transmission Electron Microscopy. The nanoparticles ranged in sizes of 11-23 nm as depicted by electron micrograph (**Fig 3**)

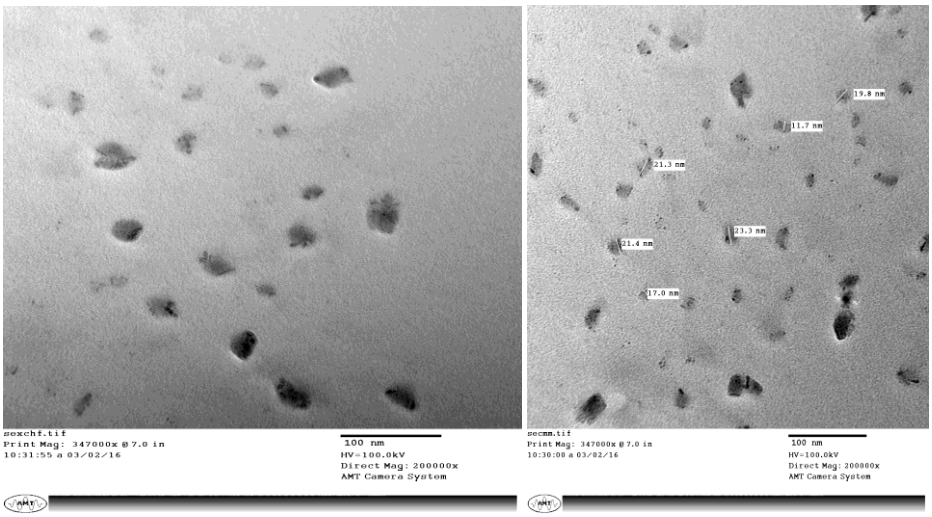

Fig 3 Electon micrograph of selenium nanoparticles

Antimicrobial Activity of Selenium Nanoparticles against planktonic microorganisms (Zone of Inhibition Assay)

Selenium nanoparticles at a concentration of 30 µg/ml (as determined as MIC for the same) were analyzed for their antimicrobial activity against test organisms in planktonic form (free living microbes). As depicted in **Fig 4(a-f)**nanoparticulate selenium exhibited a strong antimicrobial potential as shown by zone of clearance using disc diffusion method.

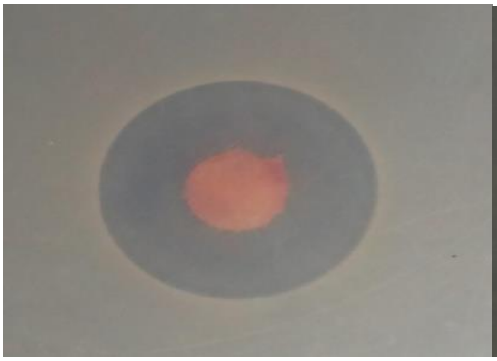

Fig 4 (a)*Staphylococcus*

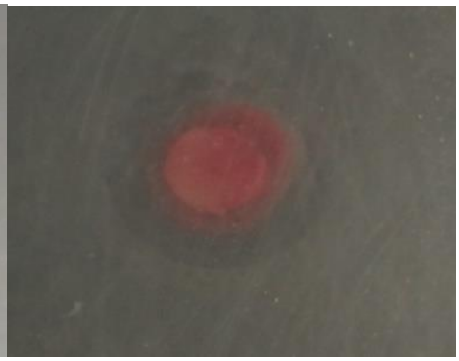

Fig 4(b)*Candida*

Fig 4(c)*Klebsiella*

Fig 4 (d)*E.coli*

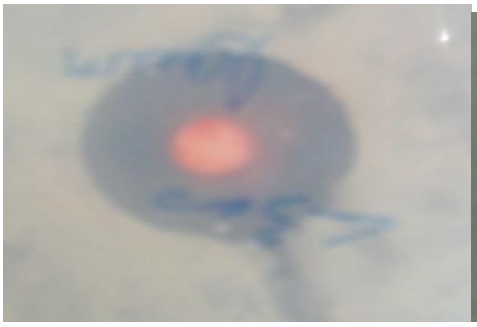

Fig 4(e)*Bacillus*

Fig 4(f)*Psuedomonas*

Activity of nanoparticles against biofilm:

Activity of selenium nanoparticles were determined by using ampicillin as control where the biofilm for test microbes was established in the 96-well ELISA plates for test organisms in the arrangement depicted in table A. The biofilm were incubated for 24 hours before their absorbance was measured at 540 nm. The variation of inhibition between different micro-organism is depicted in **Table 1**

where the concentration of selenium nanoparticles is at 30µg/ml (MIC). Ampicillin, a broad spectrum antibiotic was employed at its MIC.

Table 1: **Percentage Biofilm inhibition of different micro-organisms emloyingSeNps as compared to ampicillin.**

Microorganism	% inhibition for SeNPs	% inhibition for ampicillin
E.coli	48.19% ± 0.29	38.88%±0.61
Klebsiella	48.51% ± 0.25	46.55%±0.29
Pseudomonas	22.474% ± 0.61	38.88%±0.24
Staphylococcus	35.29% ± 0.24	45.54%±0.29
Bacillus	46.4% ± 0.15	62.65%±0.67
Candida	68.28% ± 0.66	27.09%±0.15

Evaluation of biochemical composition of biofilm matrix:

Biofilms are group of micro-organisms in which microbes produce an extracellular polymeric substances (EPS) such as proteins (<1-2%) including enzymes), DNA (<1%), polysaccharides (1-2%) and RNA (<1%), and in addition to these components, water (up to 97%) is the major part of biofilm which is responsible for the flow of nutrients inside biofilm matrix. Therefore in the present studies, the protein and carbohydrate content was evaluated upon incubation of sessile microbes with SeNps as a measure of biofilm disruption.

Carohydrate content estimation:

The carbohydrate content of biofilm matrix before and after incubation with nanoparticles in the biofilm is calculated by DNSA(3,5-Dinitrosalicyclic acid) and is depicted in the **Table 2**.

Table 2: Reduction of carbohydrate content in biofilm upon incubation with SeNPs nanoparticles

Micro organism	**Carbohydrate content before NP action (mg/ml)**	**Carbohydrate content after NP action (mg/ml)**
Escherichia coli	0.77 ± 0.01	0.64 ±0.02
Bacillus subtilis	0.78 ± 0.02	0.70 ±0.10
Staphylococcus aureus	1.27± 0.02	0.73 ±0.06
Pseudomonas aeruginosa	0.79 ± 0.03	0.56 ± 0.05
Klebsiellapuenominae	1.37±0.02	0.66 ± 0.04
Candida albicans	2.54±0.04	1.34± 0.03

Protein content estimation:

Due to the action of nanoparticles on the biofilms, protein content decreased accordingly in biofilm formed by the microorganism. The protein content of the biofilm was then determined by biuret method **(Table 3).**

Table 3: Reduction of protein content in biofilm upon incubation with SeNPs nanoparticles

Micro organism	Protein content before NP action (mg/ml)	Protein content after NP action (mg/ml)
Escherichia coli	0.66 +0.02	0.54 + 0.02
Bacillus subtilis	0.68 + 0.03	0.60 + 0.04
Staphylococcus aureus	0.87 + 0.04	0.63 + 0.05
Pseudomonas aeruginosa	0.79 + 0.03	0.56 0.03
Klebsiellapneumoniae	0.77 + 0.10	0.66 + 0,03
Candida albicans	1.54 + 0.20	1.04 + 0.05

Nanotechnology is an interesting emerging field having multidisciplinary approaches. The chemical methods of production are too costly to be implemented in industrial scale and the stability of the nanoparticles produced is also an unanswered question .The organic synthesis of nanoparticles open a window into the production of low cost and efficient nanoparticle production of stable encapsulated nanoparticles [22].In modern clinical microbiology, the establishment of bacterial biofilms is often considered a pathogenic trait due to quorum sensing. In order to cure these biofilms produced in the invasive devices, the biological method of nanoparticles formation [23] can be used as they provide a better alternative to chemical and physical methods.The study was designed to develop nanoselenium as a strategy to overcome resistance to antimicrobials in microbes, which are becoming a threat to a growing world. In the current study the focus on was on the green chemistry route used for selenium nanoparticles synthesis. It includes the bioreduction of selenium through probiotics and testing for their antibiofilm activity. The bacterial and candidal biofilms were characterized by various methods. Congo red method is a simple and reliable method for determining the potential for biofilm production. In this method the cultures were grown in the nutrient agar with concentrated Congo red with 1% glucose for increasing the growth of the microorganism. The biofilm produced was differentiated by the color formed by the colonies. Congo red (CR) in alkaline 80% ethyl alcohol solution saturated with NaCl stains amyloid selectively. CR is a linear and amphiphilic molecule. Its hydrophilic part includes two amino groups and a negatively charged sulphate groupand its hydrophobic part consists of a biphenyl group along with diazo groups. CR has striking spectrophotometric properties. Congo red is absorbed by hydrogen bonding between hydroxyl groups of the polysaccharide chains and the amino groups of the dye. Black coloration is due to the presence of curlie fibers which helps the formation of exo polysaccharides as curlie has the property to bind with congo red. The curlie fiber has the capability to produce slime. As shown in **Fig 1(c), 1(d)** and**1(f)** *Klebsiella, Bacillus, Staphylococcus* produced black colonies which is the indicative of strong biofilm whereas **Fig1(a),1(b)**and **1(e)** *C.albicans. P.aeruginosa* and *E.coli*

produce dark red colony which indicate moderate biofilm formation. Vasanthi *et al* [20] reported that *Staphyloccus aureus, Klebsiella species* and *Pseudomonas species* form moderate to high biofilm.The difference found in the color of colonies could be explained by the use of various types of media for culturing of microorganisms as composition varies from media to media. It could be also be due to the different Mcfarland standards used for the inoculation of the respective microorganisms. Another difference may also be observed due the varying concentration of glucose added to the culture medium for the growth of the microorganisms. Another method used for the detection of biofilm was the tube method in which formation of biofilm ring was detected by staining with 0.1% crystal violet. As depicted in the **Fig 2** all the organisms were depicting a blue ring formation on the walls and bottom of the respective tubes when cultured in TSB broth. Crystal violet is not as specific and binds indistinctly to bacteria, thus showing biofilm formation without taking into account the characteristics which the bacteria are using to form it. Tissue culture plate method or microtiter plate method was used to quantitatively detect the biofilm producing organisms. The tissue culture plates were incubated for 1 24 hours at 37°C containing isolates in trypticase soy broth with 1% glucose (TSBglu) media. After incubation, the content from each well was gently removed by tapping the plates and stained with crystal violet (0.1% w/v). Adherent cells usually form biofilm and Optical density (OD) of stained adherent bacteria was determined with a micro ELISA auto reader at wavelength of 540 nm. These OD values were considered as an index of bacteria adhering to surface and forming biofilms. These results are nearly identical as reported by Rewatkar and Wadher [24] for *Staphylococcus aureus* and *Pseudomonas aeroginosa*. Using the biological production method, selenium nanoparticles were produced using probiotics i.e. *Lactobacillus acidophilus* which was cultured with sodium selenium at the conc. of 20 mg/l in MRS broth. As the selenium got reduced in the elemental form, the color changed into red color [17]. ForTransmission electron microscopy samples were prepared by drop coating selenium nanoparticles solution on to carbon coated copper TEM grids. The films on the TEM grids were allowed to stand for 2 minutes. The extra solution was removed using blotting paper and the grids were dried prior to measurement. TEM micrographs were obtained on Hitachi (H-7500) with accelerating voltage of 120kV.TEM images of the prepared selenium nanoparticles showed a uniform distribution and spherical morphology. The depicted nanospheres in the TEM images were ranging in size from 11- 40 nm in diameter which characterizes its morphology. Figure shows selenium nanoparticles at the range of 11 nm to 35 nm on scale, respectively. The present study was similar to that of reported by Visha *et al* [23].The difference in the reported sizes of nanoparticles could be explained by the variation in the strain used as bacterial proteins play a major role in controlling the size and shape of nanoparticles .In the present study it was found that SeNPs was effective against biofilm forming microorganism like *E.coli*, *Staphylococcus aureus*, *Psuedomonas aeruginosa*, *Klebsiella*, *Bacillus*

subtilis and *Candida albicans* .The extracellular matrix is an intermediate environment for biofilm bacteria that stabilizes the three dimensional biofilm structures and mediates bacterial adhesion [2]. The composition of the matrix directly affects the biofilm architecture. The extracellular matrix is composed of cytoplasmic proteins that are recycled as components of the extracellular matrix during biofilm formation. This parameter focused on the investigation of potential of SeNPS to reduce the protein and carbohydrate content of the extracellular matrix. The estimation of protein content was performed by biuret method and carbohydrates by DNSA method. The protein and carbohydrate content reduction by SeNPs was found to be in all microorganisms respectively. The small size of nanoparticles alone [25]or combined with antibiotic activity of metals[26] has been employed by other researchers as well for the disruption of extracellular matrix in microbial biofilms. Therefore, SeNPs can act as an alternate for inhibiting the formation of biofilms. It was also found that SeNPs also reduces the protein as well as carbohydrates content present in the extracellular matrix of biofilms.

4. CONCLUSION

It has been concluded from the present study that selenium nanoparticles that are generated by the aid of Probiotics could effectively eradicate preformed biofilm and inhibit the biofilm formation, regardless of Gram-positive and Gram-negative pathogenic bacteria. SeNPs are also able to reduce protein as well as carbohydrates content of the biofilm matrix .SeNPs are of least toxic of all forms. In the present study, it was found out that even very small conc. Of SeNPs were effective against biofilm forming micro-organism.

5.ACKNOWLEDGEMENT

D.A.V. College, Sector-10, Chandigarh for providing the support and infrastructure

6. CONFLICT OF INTEREST

None

REFERENCES

1. Costerone JW, Stewart PS. Bacterial biofilms: acommon cause of persistent infections.Science. 1999; 284: 124-134.
2. Flemming H, Wingender J. The biofilm matrix. Natural Rev Microbiol. 2010: 8: 623–33.
3. Christensen GD, SimpsonW, Younger JA, Baddour LM, Barrett FF, Melton DM*et al*. Adherence of coagulase negative Staphylococci to plastic tissue cultures: a quantitative model for the adherence of Staphylococci to medical devices. J ClinMicrobiol. 1995; 22:996-1006.
4. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. ClinMicrobiol Rev. 2002; 15: 167–93.
5. Patel R. Biofilms and antimicrobial resistance. ClinOrthopRelat Res. 2005; 437: 41–47.
6. Lewis K. Riddle of biofilm resistance. Antimicrob Agents chemother. 2001;45:999-1007.

7. Kim W,Racimo F, Schluter J, Levy SB, and Foster KR. Importance of positioning for microbial evolution, PNAS 2014; 111 (16):E1639-E1647.
8. Keren I, Kaldalu N, Spoering A, Wang Y, Lewis K. Persister cells and tolerance to antimicrobials. FEMS MicrobioilLett. 2004;230:13-18.
9. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends Microbiol. 2005;13:34-40.
10. Gristina AG, Hobgood CD, Web LX, Myrvik QN. Adhesive colonization of biomaterials and antibiotics resistance. Biomaterials. 1987;8:423-6.
11. Vadlapudi V, Kaladhar DSVGK, Behara M, Naidu GK, Sujatha B. Green synthesis of silver and gold nanoparticles. Int J Chem Sci.2013 ;1:22-31.
12. Acharyulu NPS, Dubey RS, Swaminadham V, Kalyani RL, Kollu P, Pammi SVN Green synthesis of CuO nanoparticles using *Phyllanthus amarus* leaf extract and their antibacterial activity against multidrug resistance bacteria. Int J Eng Res Technol.2014; 3:639–641.
13. Aenishanslins NAO, Saona LA, Durán-Toro VM, Monrás JP, Bravo DM, Donoso JMP Use of titanium dioxide nanoparticles biosynthesized by *Bacillus mycoides* in quantum dot sensitized solar cells. Microb Cell Factories.2014; 13:90.
14. Ahmad A, Mukherjee P, Senapati S, Mandal D, Khan MI, Kumar R *et al*. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. Colloids Surf.2003; 28:313–318.
15. Anwar NS, Kassim A, Lim HN, Zakarya SA, Huang NM. Synthesis of titanium dioxide nanoparticles via sucrose ester micelle-mediated hydrothermal processing route. Sains Malays.2010; 39:261–265.
16. Aromal SA, Vidhu VK, Philip D, Green synthesis of well-dispersed gold nanoparticles using *Macrotyloma uniflorum*. Spectrochim Acta Part A 2012; 85:99–104.
17. Eszenyi P, Sztrik A, Babka B, Prokisch J.Elemental, Nanosized (100-500 nm) Selenium Production by Probiotic Lactic Acid Bacteria. International Journal of Bioscience, Biochemistry and Bioinformatics. 2011; 1(2): 148-152.
18. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. Infect Immun. 1982;37(1):318-26.
19. Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negativestaphylococci. J Clinical Path. 1989;42:872-74.
20. Vasanthi R., Kartihikeyan D, Jeya M. Study of Biofilm Production and Antimicrobial Resistance Patrern of the Bacterial Isolates from Indwelling Devices. International Journal of Research in Health Science. 2014; 2(1): 274- 281.

21. Bauer, A.W., W.M. Kirby, J.C. Sherris and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American.J Clin Pathol 45 (4):493-496.
22. SasidharanS. and Balakrishnaraja R. Comparison studies on the synthesis of selenium nanoparticles by various microorganisms. International Journal of Pure and Applied Biosciences. 2014;2(1): 112-117.
23. Visha P, Nanjappan K, Selvaraj P,Jayachandran S, Elango A, Kumaresan G. Biosynthesis and Structural Characteristics of Selenium Nanoaparticles using Lactobacillus Acidophilus Bacteria by Wet Sterilization Process. International Journal of Advanced Veterinary Science and Technology.2015; 4(1):178-183.
24. RewatkarAR and WadherBJ. *Staphylococcus* and *Pseudomonas aeruginosa* biofilm formation methods. Journal of Pharmacy and Biological Sciences. 2013;8:36-40.
25. Baelo A, Levato R, Julián E, Crespo A, Astola J, Gavaldà J, Engel E. Mateos-Timoneda MA, Torrents E. Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. J Control Release. 2015; 209:150–8.
26. Flores CY, Miñán AG, Grillo CA, Salvarezza RC, Vericat C, Schilardi PL. Citrate-Capped Silver Nanoparticles Showing Good Bactericidal Effect against Both Planktonic and Sessile Bacteria and a Low Cytotoxicity to Osteoblastic Cells. ACS Appl Mater Interfaces. 2013; 5:3149–3159.