Annexure – IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE FINAL REPORT OF THE WORK DONE ON THE PROJECT

1. TITLE OF THE PROJECT	: **Ecofriendly synthesis of $AgNo_3$ & AgI nanoparticles to the management and destabilization of biofilm forming bacteria**
2. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR	: **Dr.M.KANNAN** **Department of Microbiology** **V. H. N. Senthikumara Nadar College, (Autonomous)** **Virudhunagar – 626001** **Tamilnadu, India** **Tel. No.: 04562-280154,** **Cell : 09486140302** **Email: microkannan@gmail.com**
3. NAME AND ADDRESS OF THE INSTITUTION	: **V. H. N. Senthikumara Nadar College, (Autonomous)** **Virudhunagar – 626001** **Tamilnadu, India** **Tel. No.: 04562-280154**
4. UGC APPROVAL LETTER NO. AND DATE	: **42 – 485/2013 (SR) Dated 22/03/2013**
5. DATE OF IMPLEMENTATION	: **01/04/2013**
6. TENURE OF THE PROJECT	: **3 + 1 Years (Additional 1 Year extended by UGC)**
7. TOTAL GRANT ALLOCATED	: **Rs. 10,99,542/-**
8. TOTAL GRANT RECEIVED	: **Rs 10,28,768/-**
9. FINAL EXPENDITURE	: **Rs. 10,99,542/-**

10. TITLE OF THE PROJECT : **Ecofriendly synthesis of $AgNo_3$ & AgI nanoparticles to the management and destabilization of biofilm forming bacteria**

11. OBJECTIVES OF THE PROJECT :

- Isolation and Characterization of Thermophilic Actinomycetes Thermomonospora sp., and fungi such as Fusarium sp, and Verticillium sp. for the Biosynthesis of nanoparticles.
- Biosynthesis of nanoparticles from $AgNo_3$ and AgI using isolated Actinomycetes and Fungi.
- Characterization of the biosynthesized nanoparticles by an Ultra Violet spectrophotometer (UV-spec.), FT-IR, XRD and Scanning Electron Microscopy (SEM). Optimization of Nanoparticles biosynthesis by microbes.
- Investigation of biosynthetic enzymes and electron shuttling compounds by Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Thin Layer Chromatography (TLC).
- Investigating the antimicrobial and the wound healing activity of the biosynthesized nanoparticles.
- Screening of the Biofilm forming bacteria from the Intra Utrine Devises (IUD) and Identification of biofilm forming bacteria.
- The effect of biosynthesized nanoparticles will be tested against both gram positive and gram-negative bacterial biofilms. The present studies also aims to understand the ability of nanoparticles to kill microbial cells within established biofilms by XRD and SEM analysis.

12. WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS) : **YES**

13. ACHIEVEMENTS FROM THE PROJECT :

Eight research papers were published in UGC listed Journals which includes one research paper article in the book Nanostructures in therapeutic medicine, published by Elsevier, Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands. ISBN 978-0-323-46152-8. The title of chapter is "Silver iodide (AgI) nanoparticles as an anti-biofilm agent - A case study on gram-negative biofilm forming bacteria". One research scholar has almost completed his Ph.D., research work. Two M.Phil., and one M.Sc. student have done their project work and their project thesis published. Nine papers are presented in seminars that include four International seminars.

14. SUMMARY OF THE FINDINGS :

The three different microbial cultures were isolated from the infected tomato plant leaf and compost cow dung sample were isolated. Totally 32 samples were collected from six different locations. For the characterization of isolates, morphological analysis by both microscopic and macroscopic, slide culture and biochemical tests were done. Two MTCC cultures of *Thermomonospora* sp. and *Thermomonospora chromogena* were also chosen for the microbial synthesis of nanoparticles. All the isolates were taken to carry out the microbial synthesis of nanoparticles. The silver nitrate ($AgNo_3$) and silver iodide (AgI) was selected for the microbial synthesis of nanoparticles.

The silver nitrate resistant cultures were isolated by plating the sample in MGYP agar containing silver nitrate. Then the nanoparticles synthesis was carried out from the five different cultures under different pH, temperature and the colour change was observed. The silver nanoparticles of $AgNo_3$ and AgI thus synthesized were confirmed through Ultra Violet Spectrophotometer (UV-spec.), Fourier Transform Infrared Spectroscopy **(**FTIR) and XRay diffraction (XRD) studies. The peak at 420nm for the five different cultures and the size of nanoparticle within 1-100nm revealed that the synthesized nanoparticles were silver nanoparticles. The isolated three different cultures were further confirmed through 16S rRNA sequencing and phylogenetic analysis. From the BLASTN analysis and GC content of the isolates were confirmed as *Bacillus licheniformis, Fusarium solani* and *Thermomonospora*sp. and these three cultures were best suited for the silver nanoparticles synthesis. The microbially synthesized $AgNo_3$ and AgI nanoparticles of *Bacillus licheniformis* and *Fusarium solani* were taken for testing the control of drug resistant bacteria isolated from diabetic foot ulcer. Four different bacterial cultures were isolated from the patients of diabetic foot ulcer and among them only two cultures showed sensitivity towards the synthesized nanoparticles.

In biofilm Inhibitory studies the pH and temperature did not significantly affect the biofilm formation. The results indicate that the maintenance of intracellular pH homeostasis is the basis of the enhanced physiological status and acid tolerance of biofilm cells. The results shows that the bacteria isolated from medical devices were sensitive to silver nanoparticles but resistant to standard antibiotics. So infections in patient with individually catheter and IUDs are difficult to treat with high dose of antibiotics. In summary, the present approaches could prevent biofilm formation which is more desirable than treating biofilm related infection. In spite of the shortcomings of many of the approaches, improving biomaterial anti-biofilm properties remains the most effective and promising strategy to prevent the morbidity and mortality associated with biofilm infections.

Bacterial biofilms are a serious medical problem. Due to the increasing ineffectiveness of conventional antibiotics, numerous alternative methods to combat bacterial biofilms are being considered. Silver nanoparticles have recently received an increased attention for their antimicrobial effects and possible clinical applications. Despite numerous studies conducted over the last

decade there are still considerable gaps in our knowledge about the antimicrobial properties of AgNPs. Furthermore, the precise basis of their antibacterial activity has yet to be defined. This is mainly due to the pleiotropic effects of nano-silver on bacterial cells, which suggests multiple mechanisms of action on several cellular targets. Nonetheless, the strong anti-biofilm effect of AgINPs is indisputable. Several studies have demonstrated the inhibition of in vitro biofilm formation by a variety of bacterial species at specific nanoparticle concentrations. This raises the intriguing possibility of treating infections caused by biofilm-forming bacteria with AgINPs. However, the toxicity of nanoparticles to eukaryotic cells is a legitimate concern and still remains uncharacterized. One way of avoiding this potential drawback might be to target AgNPs to the specific site of an infection so that toxic silver concentrations are localized.

15. CONTRIBUTION TO THE SOCIETY :

Based on this research work, incorporation of the biosynthesized AgINPs into medical devices could increase their efficacy and diminish any side-effects, but considerable research effort is still required to perfect this technology.

16. WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT : One research scholar **(Mr.A.Maniraj (Research Fellow) Reg. No.: F9513 at Madurai Kamaraj University, Madurai.**) has almost completed his Ph.D., research work.

17. NO. OF PUBLICATIONS OUT OF THE PROJECT (PLEASE ATTACH) : **Eight research papers published in UGC listed Journals. One research paper published as book chapter the title of book Nanostructures in therapeutic medicine, published by Elsevier.**

(PRINCIPAL INVESTIGATOR) **(REGISTRAR/PRINCIPAL)**
(Seal)

(CO-INVESTIGATORS)

Enclosures –
Publication and Paper presentation Details

Research Publications in International and National Journals

1. Silver iodide (AgI) nanoparticles as an anti-biofilm agent. A case study on gram-negative biofilm forming bacteria. **M. Kannan**, K. Rajarathinam, S. Venkatesan, B. Dheeba, A. Maniraj. Nanostructures in therapeutic medicine, Published by **Elsevier.** Radarweg 29, PO Box 211, 1000 AE Amsterdam, Netherlands. **ISBN 978-0-323-46152-8**.

2. Biosynthesis, process optimization and characterization of Cadmium sulfide nanoparticles against wood degrading termite. Maniraj. A, Muthuram kumar.S, **Kannan.M** and Ganesan.R. Book Name: A treatise on recent advances in bioorganic and medical chemistry. Publisher: VHNSN College (Autonomous) Virudhunagar. February 2017. **ISBN: 978-93-8173-63-0**

3. Optimization and Characterization of Green Synthesized Silver Nanoparticles and Its Inhibitory Activity Against Biofilm Forming Bacterial Pathogens. A.Maniraj, S.Muthuram Kumar, **M. Kannan**, K. Rajarathinam and A. Pushparaj. Journal of Advanced Applied Scientific Research, Vol-1-9-March-2017: 97- 106 -**ISSN: 2454-3225.**

4. Biobased approach for the synthesis, characterization, optimization and application of silica nanoparticles by fungus *Fusarium oxysporum*. **M. Kannan**, K. Uma Sangareswari, P. Suganya, R. Ganesan, K. Rajarathinam. 11/12/2015. Pharmaceutical And Biological Evaluations. December 2015; vol. 2 (Issue 6): 223-233. **ISSN 2394-0859.**

5. Rajivgandhi G, Vijayan R, **Kannan M,** Santhanakrishnan M, Manoharan N. Molecular characterization and antibacterial effect of endophytic actinomycetes Nocardiopsis sp. GRG1 (KT235640) from brown algae against MDR strains of uropathogens. Bioactive Materials. 2016;1(2):140-150. doi:10.1016/j.bioactmat. 2016.

6. Biosynthesis and characterization of intracellular TiO_2 nanoparticles by Lactobacillus sp. and its potential application in decolourization of methyl orange dyes. **M. Kannan**, K. Rajarathinam, B. Dheeba, K. Nagheswari, K. Kannan. International journal of pharmacy and pharmaceutical sciences. Vol 7, issue 2, 3/12/2014, ISSN: 0975-1491. (Impact factor 0.55).

7. Isolation and identification of biofilm forming uropathogens from urinary tract infection and its antimicrobial susceptibility pattern. G. Rajivgandhi, J. Vijayarani,

M. Kannan, A. Murugan, R. Vijayan, N. Manoharan. International journal of advanced life sciences, ISSN: 2277-758X Vol7, Issue 2, 23/4/2014.

8. Extracellular Biosynthesis of Iron Oxide Nanoparticles by Bacillus subtilis Strains Isolated from Rhizosphere Soil. P. Alagu Sundaram, Robin Augustine, **M. Kannan.** Biotechnology and Bioprocess Engineering 17: 835-840 6/4/2012. ISSN: 1226-8372 (print) ISSN: 1976-3816 (electronic)/Impact Factor 1.277.

Paper Presented in Conference and Seminar

S. No.	Title of the paper	Conference/ Seminar	Organized by	Role
1.	Biosynthesis, process optimization and characterization of Cadmium sulfide nanoparticles against wood degrading termite	National seminar on Recent advances in bioorganic and medical chemistry (RABAMCHEM-2017)	VHNSN College (Autonomous) Virudhunagar on 15.02.2017	Presented
2.	Optimization and Characterization of Green Synthesized Silver Nanoparticles and Its Inhibitory Activity Against Biofilm Forming Bacterial Pathogens.	National seminar on "Recent advanced in chemical research (RACR-2017) "	VHNSN College (Autonomous) On 24.01.2017 &25.01.2017	Presented
3.	Drug resistance patterns analysis of biofilm forming bacteria isolated from clinical specimens	"National conference on Emerging infectious and curative measures"	Kamaraj College Thoothukodi 23.&24.12.2016	Presented
4.	Silver nanoparticles impede the biofilm formation by Proteus mirabilis and Klebsiella pneumoniae in indwelling medical devices	International conference on "Recent Trends In Biological Research"	Ayya Nadar Janaki Ammal College, Sivakasi on 22/12/2015	Presented
5.	Biosynthesis and fabrication of silver nanoparticles (Ag-NPs) and their applications against methicillin resistant Staphylococcus aureus (MRSA)	International satellite symposium on "Emerging Trends In Nanotechnology"	Thassim Beevi Abdul Kader College for women, Kilakarai on 17 & 18/12/2015	Presented
6.	Biosynthesis, characterization and application of silver nanoparticles using Thermomonospora sp. and	National seminar on "Current Research In Microbiology – 2015"	Ayya Nadar Janaki Ammal College, Sivakasi on 19	Presented

	Fusarium sp.		& 20/03/2015	
7.	Effect of AgI nanoparticles against biofilms forming Proteus mirabilis isolated from UTI samples	International conference on "Physiology and Medicine - 2014)	Periyar university, Salem on 15 - 17/10/2014	Presented
8.	Bio-Synthesis, Characterization, Optimization and Application of Tio2 Nanoparticles by Fusarium oxysporum	11thNational Conference of Indian Association of Applied Microbiologists (IAAM)	PSG College of Arts & Science, Coimbatore, 29 – 30 November 2013.	Presented
9.	Anti-quorum sensing & biofilm inhibitory potential of soil associated bacteria against human bacterial pathogens	International symposium on "Changing Etiology of Emerging Infectious Diseases"	University of Madras, Chennai on 6/02/2013	Presented