# EXECUTIVE SUMMARY
# UNIVERSITY GRANT COMMISSION RESEACH PROJECT
**(F. NO. 42-862/2013 (SR) dated 23 March 2013)**

1. Title of the project: Development of Antibiofilm Compounds from Marine Bacteria
2. Name and address of the Principal Investigator:

Dr. Joseph Selvin
Professor
Department of Microbiology
Pondicherry University
Puducherry 605 014
Email: jselvin.mib@pondiuni.edu.in
Mob: 91-9944263367

3. Name and address of the Institution:

Pondicherry University, R. Venkataramn Nagar kalapet, Puducherry 605014.

4. UGC approval letter no and date: UGC-MRP No. 43-459/2014(SR) dt. 30.09.2015

5. Date of implementation: 01.07.2015 (Project works started since 30.09.2015)

6. Tenure of the project: 3 years

7. Total grant allocated: Total Allocation (Rs.): 6,71,000/-

8. Total grant Received: 1st instalment (Rs.): 5,11,000/-

9. Final Expenditure: Rs.: 6,71,000/-

10: Objectives of project:

- To evaluate sponge and seaweed associated marine bacteria for antagonistic activity against pathogens
- To screen marine bacteria (extra-cellular products) for antibiofilm activity and disruption of pathogenic biofilms
- To perform characterization and production of antibiofilm compounds

11. Whether objectives were achieved: Yes

12. Achievements from the project:

Purification and characterization of antivirulent Methyl benzoate

The crude extract CBE12 (bacterial product) was fractionated by column chromatography. The activity of purified fractions matched with the initial activity of crude extract,

confirming the presence of active molecules responsible for antivirulence activity. The column purified active fraction (FA2, 10:90 (v/v) EtOAc/$CHCl_3$) was further characterized using TLC, HPLC, FT-IR and GC-MS analyses. In TLC using silica as a stationary support, a single discrete spot was observed with an Rf value of 0.57. The FT-IR and GC-MS analyses revealed that the compounds responsible for antibiofilm and antivirulence activity were fatty acid methyl esters such as methyl benzoate and methyl phenyl acetate produced by the coral associated bacterium CBMGL12. The $C_{13}$ and proton NMR studies of the purified active fraction suggest the presence of compounds having aromatic ring linked to methyl ester groups and responsible for antivirulence activity. In addition, synthetic methyl benzoate and methyl phenyl acetate when dissolved in ethyl acetate at varying concentrations such as 20µL and 30µL exhibited a marked antibacterial activity against MTCC96, *Vibrio* pathogens and CA-MRSA.

13. Summary of the findings:

Chemical characterization using analytical techniques such as FT-IR and GC-MS suggests the presence several fatty acid methyl esters. The NMR data suggest the presence of carbon atoms of benzene ringed structures in $C_{13}$ NMR and the proton types of ester groups. The two fatty acid esters such as methyl benzoate and methyl phenyl acetate produced by the coral associated bacterium CBMGL12 could be responsible for the antibiofilm and antivirulent activity. The pathway at which the active molecules were synthesized by CBMGL12 can also be a choice of study in future. In addition, the production of antivirulence metabolites by the bacterium CBMGL12 can be over enhanced using cheapest carbon and nitrogen sources so as to commercialize these bioactive molecules in a cost effective manner.

14. Contribution to the Society:

Though the earlier reports have suggested the anti-QS potential of marine bacterial communities, the actual chemistry behind the active molecules were meager and further

urges the researchers to explore novel biomolecules from marine bacteria. This study revealed an anti-biofilm strategy to combat antimicrobial resistance and biofilm pathogens.

15. Whether any Ph.D Enrolled/ Produced out of the project: No

16. No. of Publications out of the Project: 03

SIGNATURE OF THE PI

Dr. Joseph Selvin
Professor & Coordinator
Department of Microbiology
School of Life Sciences
Pondicherry University

SIGNATURE OF THE REGISTRAR

Registrar
Pondicherry University

Annexure IX

**UNIVERSITY GRANT COMMISSION**
**BahadurshahJafar Marg, Delhi**
**PONDICHERRY UNIVERSITY**
**PONDICHERRY**
**ASSESSMENT/EVALUATION REPORT UGC MRP**

**A.** DETAILS OF THE PROJECT

| | |
|---|---|
| 1. Title of the project | **Development of Antibiofilm Compounds from Marine Bacteria`** |
| 2. Total duration of the project | **3 years** |
| 3. Project status | **Completed** |
| 4. Subject | **Microbiology** |
| 5. File Number UGC reference number & date | **No. 43-459/2014(SR) dt. 30.09.2015**<br>**First installment received on 31.03.2016** |
| 6. Grant approval | **UGC MRP** |

**B.** Evaluation report of the Expert member

| | | |
|---|---|---|
| 1. | Name of the principal investigator | **Dr. Joseph Selvin** |
| 2. | Designation | Professor |
| 3. | Address , E-mail, phone | Department of Microbiology<br>Pondicherry University<br>Pondicherry 605014<br>E-mail: josephselvinss@gmail.com |
| 4. | Whether work is focused on the title of the project | Yes |
| 5. | Whether original work is done | Yes |
| 6. | Whether significant contribution made the principal investigator | Yes |
| 7. | Whether proposed work have relevance to the society/ scientific community | Yes |
| 8. | What type of contribution found in the final report theoretical/ practical. If there are theoretical contribution given by the principal investigator, whether real application are given | The contributions made in the project are useful for the development of anti-biofilm strategies. |
| 9. | Whether theoretical/ practical contribution and their results and findings are published | Yes, three manuscripts were published in international journals with impact factors >3. |
| 10. | Whether results and findings are significant | The frequent onset of multidrug resistant *S. aureus* infections among the worldwide humans demands the scientific community to search for the novel bioactive molecules that can replace the currently available treatment strategies in a cost effective way. However, expression of several virulence genes in *S. aureus* is controlled by a regulon called *agr* QS system. When mining the coral associated bacterial isolates for the presence of antivirulence molecules, the bacterium CBMGL12 was found to express several secondary metabolites with antibiofilm |

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| | | and antivirulent activities against the two test strains such as *S. aureus* MTCC96 and *S. aureus* CA-MRSA. Chemical characterization of the active fraction resulted in aromatic fatty acid ester compounds such as methyl benzoate and methyl phenyl acetate which were responsible for the initial antivirulence activity<br>Experimentation process in future shall reveal the actual cellular targets for QS which might be inhibited or interfered by active molecules produced from the marine bacterium CBMGL12 and also the extent at which these molecules can be exploited for future antivirulence therapy to combat multidrug resistant *S. aureus* infections. |
| 11. | Whether the significant publication are made by principal investigator in peer reviewed journal | Yes, the publications are in impact journals |
| 12. | The number of publication made by the principal investigator in standard reputed journal | 03 |
| 13. | Whether the contribution made by the principal investigator is sufficient | Yes |
| 14. | The findings and results of the sanction major research projects are justifiable | Yes |
| 15. | Whether completed project work meet the proposed objective | Yes |
| 16. | Give your brief comments on the overall work of the project | Excellent progress and the project outcomes can be utilized for anti-biofilm process development. |
| 17. | Any specific comments | The project outcomes need follow-up support and proceed development |
| 18. | Indicate your overall assessment of the project poor/ good / excellent | Excellent |

Date: 10.07.2019

Place: Trichy

Name and Address of Expert:

**(Prof. N. THAJUDDIN)**

Dr. N. THAJUDDIN
Professor
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Dr. Joseph Selvin
Professor & Coordinator
Department of Microbiology
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B. Chi Reddy
Registrar
Pondicherry University

Annexure IX

UNIVERSITY GRANT COMMISSION
Bahadur Shah Jafar Marg, Delhi
PONDICHERRY UNIVERSITY
PONDICHERRY
ASSESSMENT/EVALUATION REPORT UGC MRP

A. DETAILS OF THE PROJECT

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|---|---|
| 1. Title of the project | **Development of Antibiofilm Compounds from Marine Bacteria`** |
| 2. Total duration of the project | 3 years |
| 3. Project status | Completed |
| 4. Subject | Microbiology |
| 5. File Number UGC reference number & date | No. 43-459/2014(SR) dt. 30.09.2015<br>First installment received on 31.03.2016 |
| 6. Grant approval | UGC MRP |

B. Evaluation report of the Expert member

| | | |
|---|---|---|
| 1. | Name of the principal investigator | Dr. Joseph Selvin |
| 2. | Designation | Professor |
| 3. | Address , E-mail, phone | Department of Microbiology<br>Pondicherry University<br>Pondicherry 605014<br>E-mail: josephselvinss@gmail.com |
| 4. | Whether work is focused on the title of the project | Yes |
| 5. | Whether original work is done | Yes |
| 6. | Whether significant contribution made the principal investigator | Yes |
| 7. | Whether proposed work have relevance to the society/ scientific community | Yes |
| 8. | What type of contribution found in the final report theoretical/ practical. If there are theoretical contribution given by the principal investigator, whether real application are given | The marine sampling and the field investigations during sample correction and survey was well reported journals in addition to the laboratory experimental data collected and presented in the report. |
| 9. | Whether theoretical/ practical contribution and their results and findings are published | Three publications |
| 10. | Whether results and findings are significant | The main aim of the project was to determine whether these bacteria serve as a source of bioactive metabolites that interfere / inhibit agr QS system controlling the virulence gene expression in S. aureus. In this study, we report the alterations in the expression of virulence and biofilm production in *S. aureus* by the secondary metabolites of coral associated bacterium CBMGL12. Interestingly, the inhibition of |

11/7/19

Joydeep Mukherjee Ph. D.
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| | | biofilm was observed in the reference strain S. aureus MTCC 96 when treated with ethyl acetate extract of CBMGL12 whereas the same extract induced the biofilm formation in the test strain CA-MRSA. Bioassay directed fractionation of the crude extract resulted in the identification of two aromatic fatty acid esters such as methyl benzoate and methyl phenyl acetate as the potent inhibitors of virulence phenotypes in multidrug resistant *S. aureus* and pathogenic *Vibrio* strains. |
| 11. | Whether the significant publication are made by principal investigator in peer reviewed journal | Published in impact journals |
| 12. | The number of publication made by the principal investigator in standard reputed journal | 03 |
| 13. | Whether the contribution made by the principal investigator is sufficient | Yes |
| 14. | The findings and results of the sanction major research projects are justifiable | Yes |
| 15. | Whether completed project work meet the proposed objective | Yes |
| 16. | Give your brief comments on the overall work of the project | Very Good progress achieved in the project. |
| 17. | Any specific comments | The antibiofilm strategies can be extended to other pathogens. |
| 18. | Indicate your overall assessment of the project poor/ good / excellent | Excellent |

Date: 11.07.2019

Place: Jadavpur

Name and Address of Expert:

Joydeep Mukherjee 11/7/19

Joydeep Mukherjee Ph. D.
UGC Associate Professor
Under Faculty Recharge Programme
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Kolkata - 700 032 INDIA

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