ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 9, Issue, 2(J), pp. 24521-24524, February, 2018

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

SELECTION OF SUITABLE BACTERIAL ISOLATES FOR THEIR USAGE AS BIOCONTROL AGENTS AGAINST MEDICALLY IMPORTANT PESTS

Sivakami V[1]., Kannan S[1] and Selvaraj Pandian R[2]

[1]Department of Environmental Studies, School of Energy, Environmental and Natural resources, Madurai Kamaraj University- 625021, TN
[2]Department of Zoology, The American college, Madurai, TN

DOI: http://dx.doi.org/10.24327/ijrsr.2018.0902.1676

ARTICLE INFO

Article History:
Received 17th November, 2017
Received in revised form 12th December, 2017
Accepted 04th January, 2018
Published online 28th February, 2018

Key Words:
rhizosphere, *Pseudomonas fluorescens*, toxigenicity, bioinsecticide

ABSTRACT

Microbial populations are highly diversified in nature due to their metabolic diversity and genetic adaptability which enable them to survive in different ecological niches. The rhizosphere region of a specific plant inhabits specific microflora which produces numerous compounds involved in complex mechanisms such as biofertilization, phytostimulation and biocontrol activity. Among this, *Pseudomonas* genera plays a major role in biocontrol mechanisms due to their diverse metabolic compounds. These compounds are exploited as biocontrol agents against medically important vectors such as mosquitoes which pose a major threat to public health. Hence it is essential for identifying a potential candidate as alternative to chemical insecticides. In this context, the present study was conducted to isolate *Pseudomonas fluorescens* strains from different sources. Their toxigenicity were assayed and evaluated for biocontrol efficacy against medically important mosquito vector, *Aedes aegypti* (Linn).These effective strains proved to be potential candidate for bioinsecticide formulation and field application trails.

Copyright © Sivakami V., Kannan S and Selvaraj Pandian R, 2018, this is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Microbial community in the plant rhizosphere soil is highly dynamic in nature which compete for water, nutrients, space and also help in the growth and ecological fitness of their host. The diversity and predominance of microbial population depend on a number of abiotic and biotic factors of a particular ecological niche. Plant species, plant development stage and soil type have been indicated as major factors determining the composition of rhizosphere microbial communities (Broeckling *et al*, 2008). The rhizosphere microflora include bacteria, fungi, nematodes, protozoa, algae and microarthops (Raaijmaker *et al*, 2009). Among these, *Pseudomonas* spp and *Bacillus* spp are well documented bacterial populations which involve in biostimulation, biofertilization and biocontrol activities. Thus, rhizosphere zone acts as bioresource for bioactive substances such as antibiotics, biosurfactants, enzymes and osmoprotective substances (Berg *et al*, 2005).

Species of the genus *Pseudomonas* show remarkable metabolic and physiological versality, enabling colonization of diverse terrestrial and aquatic habitats (Palleroni,1992) and produce a variety of metabolites which posses a major role in biocontrol of phytophathogens and mosquitoes (Haas and Defago, 2005). *P.fluorescens* Migula (VCRC-B426) culture filtrate was found to be effective against pupae of mosquitoes such as *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti* (Prabakaran *et al*, 2003, Padmanaban *et al*, 2005). This strain plays a significant role in the degradation of cuticular regions and peritrophic membrane and binds to the midgut epithelium of larvae and pupae of mosquito species of *C. quinquefasciatus* (Usharani & Kummankottil, 2012). The secondary metabolite or exotoxin produced by *Pseudomonas fluorescens* was found to be a Rhamnolipid which act as biosurfactant (Prabakaran *et al*, 2015). Hence, an attempt was made to isolate novel strains from native environment which can be effectively used for field applications. In this context, the present study was conducted to isolate *Pseudomonas fluorescens* strains from both rhizosphere regions and non rhizosphere regions and to evaluate the biocontrol efficacy against dengue vector, *Aedes aegypti*.

Corresponding author:* **Sivakami V
Department of Environmental Studies, School of Energy, Environmental and Natural resources, Madurai Kamaraj University- 625021, TN

MATERIALS AND METHODS

Study site description and soil sampling

In the present study, a total of six soil samples were collected from different habitats such as such as paddy field, bamboo tree soil and garden soil and sewage effluent discharging sites. About 10 cm rhizosphere soil particles loosely adhering to the roots were gently teased out and the soil was aspetically transferred to sterile polythene bags. In addition, sewage sediment samples were collected from mosquito breeding sites. All the soil samples were added to a conical flask and shaken with 100 ml sterile distilled water to obtain standard soil suspension. This will enrich the growth of soil microbes. From this, soil suspension was serially diluted and plated on selective medium Pseudomonas Agar (King *et al*, 1954) and it was obtained from Hi Media Pvt. Ltd.

Identification of Pseudomonas spp from samples

All the isolates were subjected to biochemical characterization according to Bergey's Manual of Determinative Bacteriology. Biochemical tests such as Nitrate test, HCN production, IAA test, starch hydrolysis, gelatin liquefaction were done to the identify all the cultured isolates to species level (Holt *et al*, 1994). The isolated and identified strains were given reference codes as PX01, PX02, PX03, PY04, PY05 and PY06 respectively to specify the soil type and the type of ecological niches.

Production of extracellular proteins

Bacterial isolates were inoculated into Glucose Peptone Broth (GPS), enrichment media for the isolation of extracellular proteins. For this, one loopful of slant culture was inoculated into 10ml of GPS medium in a test tube and kept on a rotary shaker at 180 rev min_1 for 7h.

The culture was then transferred to 50ml of GPS medium and the flask was incubated on a rotary shaker at 30°C and 180 rev min_1 for 48h. The cell mass was harvested by centrifugation at 10000 rev min_1 for 15min and the cell-free supernatant was transferred to fresh sterile tubes for further studies (Prabakaran *et al*,2003). The protein concentration of extracellular proteins were determined using Lowry's method.

Hemolytic activity

Hemolytic activity was determined by incubating suspensions of sheep red blood cells with serial dilutions of each sample. Sheep blood was collected from slaughter house with anticoagulant EDTA (2.7g/100ml) and 1% S-RBC was prepared by centrifuging anticoagulated blood with PBS for thrice. Microhemolytic assay was performed using V bottom 96 well microtitre plate. Serial dilution of samples was done with PBS in different rows of microtitre plate.50 µl or S-RBCwas added in all the wells and incubated at room temperature for 1 hr. PBS was used as negative control and 10% Triton-X was used as positive control (Rodriguez *et al*,2014). Uniform red color suspension in the wells was considered as positive hemolysis and a button formation in the bottom of wells was considered as negative hemolysis. The hemolysis was calculated as OD values in Spectrophotometer.

Biocontrol efficacy

The exoproteins in culture supernatant of *Pseudomonas fluorescens* isolates were determined. The test solution for bioassay was prepared by diluting exoproteins with sterile distilled water. *Aedes aegypti*, mosquito larvae procured from CRME (Centre for Research in Medical Entamology), Madurai were used in this study. Larval food containing yeast and dog biscuit (1:1/w:w) was added in fine powder form to the bioassay cups (WHO, 2005). For every 25 ml of test solution, 10 larvae were transferred and a controlset along with three experimental sets were maintained. The mortality rate of larvae was observed after 24hrs. Using Abbott's formula, mortality rate was calculated and probit regression analysis was carried out using SPSS (statistical software package).

RESULTS

All bacterial isolates produced yellowish green colored colonies which showed fluorescence under UV light (λ =356 nm). Microscopic examination revealed Gram negative rods and also positive for motility test, Catalase test, nitrate test, Oxidase test, gelatin hydrolysis.

The extracellular proteins of *Pseudomonas fluorescens* were characterized for protein estimation. These proteins were further partially purified using 50% ammonium sulfate precipitation. Hemolytic activity with 1% S-RBC showed different hemolytic pattern with respective to their sources (fig.1). Biocontrol efficacy of exoproteins against mosquito vector, *Aedes aegypti* showed that LC_{50} values of PX01, PX02, PX03, PY04, PY05, PY06 are 144.8, 29.9, 19.2, 25.3, 38.6, 106.9 µg protein per ml and LC_{90} values are 214, 35.5, 28.6, 43.3, 47, 155.9 µg protein per ml respectively, indicating a diversity in the biological activity.

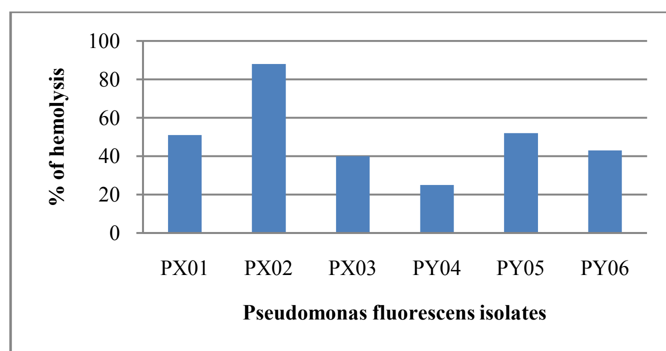

Fig 1 Hemolytic activity of *Pseudomonas fluorscens* isolates from different sources.

X- rhizosphere region, Y- non-rhizosphere region
(PX01-Garden soil, PX02-Bamboo rhizosphere soil, PX03-Paddy rhizosphere soil, PY04-Sewage sediment I,PY05-Sewage sediment II, PY06-Sewage sediment III)

DISCUSSION

Within the rhizosphere soil samples, fluorescent pseudomonads show different characteristics with respect to toxigenicity and biocidal activity. This is mainly due to ecological differences such as soil type, water availability, type of plant species etc. Earlier studies showed that there is a lack of diversity in sandy soils that are frequently tilled (Bronstad *et al*, 1996) and low microbial diversity could be associated with greater selective pressure than organic rich agricultural soils. Bamboo

rhizosphere soil remains as sandy soil with less moisture when compared to paddy rhizosphere and garden soils. Apart from physiochemical complexity of soil type, plant species also determines predominant microflora around their root region which in turn act against phytopathogens, stimulate plant growth and improve soil quality (Grayston *et al*, 1998).

In comparison with non-rhizosphere sampling sites such as sewage sediments, there is a chance of organic pollution and environmental stress which will reduce bacterial diversity. High organic substrates favours the growth of few fast growing bacterial population (Trosvik *et al*, 1996). Despite of these contributing factors, fluorescent pseudomonads with their high genetic adaptability and metabolic versality capable of growing in diverse ecological niches. The extent of hemolysis was quantified using microhemolytic assay and the variation in hemolytic ability of exoproteins of *Pseudomonas fluorescens* strains ranges from 25% to 88%. Similar results were also documented in earlier reports showed that they are highly diversified in nature with reference to their sources (Pushpanathan and Pandian RS, 2008; Vijaypreethi and Pandian RS, 2009). Some strains of non-spore forming bacteria are also toxic to larval stages of mosquitoes as well as lepidopteran insects. *Pseudomonas aeuroginosa* (Migula) produces exotoxin which have been noted to be absorbed through the cuticle of insects and act on the heamolymph proteins (Kucera & Lysenko, 1971). In this present study, extracellular proteins of *Pseudomonas fluoresecens* isolates were analysed for larvicidal effect against dengue vector, *Aedes aegypti* (Linn). The late 3rd instar larvae of this vector remain highly susceptible to exoprotein of isolate PX03 (paddy rhizosphere soil) and are less susceptible to exoproteins of isolate PX01 (garden soil). This showed that *Pseudomonas fluorescens* strains are potential agents to assess the larvicidal activity.

CONCLUSION

In this preliminary study, all the isolates of *Pseudomonas fluorescens* remain effective against *Aedes aegypti* larvae. But few strains from rhizosphere region showed the highest mortality in minimum concentration of exoproteins. Therefore, these strains could be selected as suitable candidate for biocontrol efficacy of mosquito vectors. Further, identification of the toxic compound responsible for the larvicidal activity and molecular characterization has to be done to develop suitable effective strains. In this connection, the present work is in progress.

References

1. Haas D and Defago G 2005 Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 3:307-319.
2. Palleroni NJ 1992 Introduction to the Pseudomonadaceae. The Prokaryotes; in A Handbook on the Biology of Bacteria, Ecophysiology, Isolation, Identification and Applications, Vol. III,(2nd eds) A Balows, HG Truper, M Dworkin, W Harder and KH Schleifer, (Springer (New York) pp. 3071-3085.
3. WHO/CDS/WHOPES/GCDPP/2005-2013, Guidelines for laboratory and field testing of mosquito larvicides,3-36.
4. Rajan VV and Pandian RS 2008, Mosquitocidal properties of the natural isolates of *Pseudomonas fluorescens* Migula (Pseudomonadales: Pseudomonadaceae. *Current Biotica*, 2(2):220-229.
5. Prabakaran G, Paily KP, Padmanabhan V, Hoti SL and Balaraman K 2003 Isolation of a *Pseudomonas fluorescens* metabolite/exotoxin active against both larvae and pupae of vector Mosquitoes. *Pest Manag Sci*, 59:21-24.
6. Prabakaran G, Hoti SL, Rao SP and Vijjapu S 2015 Di-rhamnolipid is a mosquito pupicidal metabolite from *Pseudomonas fluorescens*(VCRC B426). *Acta Tropica*, 48:24- 31.
7. Pushpanathan M and Selvaraj Pandian R 2008 Management of dengue and chikungunya vectors *Aedes aegypti (Linn)* and *Aedes albopictus(Skuse)* (Diptera: Culicidae) by the exotoxin of *Pseudomonas fluorescens* Migula Pseudomonadales: Pseudomonadaceae). *Current Biotica*, 2(1):74-102.
8. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ 1951 Protein measurement with the Folin Phenol reagent. *J Biol Chem*, 193:265-275.
9. Abbott WS 1925 A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265-267.
10. Rodriguez A, Villegas E, Montoya-Rosales A, Rivas-Santiago B, Corzo G 2014 Characterization of Antibacterial and Hemolytic Activity of Synthetic Pandinin 2Variants and Their Inhibition against *Mycobacterium tuberculosis. PLoS ONE* 9(7): e101742. doi:10.1371/journal.pone.0101742.
11. Grayston SJ, Wang S, Campbell CD and Edwards AC 1998 Selective influence of plant species on microbial diversity in the rhizosphere. *Soil Biol Biochem* 30: 369-378.
12. Rangarajan S, Loganathan P, Saleena LM and Nair S 2001 Diversity of pseudomonads isolated from three different plant rhizospheres. *J. Appl. Microbiol*. 91, 742-749.
13. Torsvik V, Sorheim R, Goksoyr J 1996 Total bacterial diversity in soil and sediment communities - a review. *J. Ind. Microbiol*. 17, 170-178.
14. King EO, Ward MK and Rany DE 1954 Two simple media for the demonstration of pyocyanin and fluorescein. *J. Lab. Clin*. Med. 44, 301-307.
15. Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST 1994 Bergey's Manual of Determinative Bacteriology. Williams and Wilkins Press, London.
16. Broeckling CD, Broz AK, Bergelson J, Manter DK and Vivanco JM 2008 Root exudates regulate soil fungal community composition and diversity.*Appl Environ Microbiol* 74:738-744.
17. Raaijmakrs JM, Paulitz CT, Steinberg C, Alabouvette C and Moenne LY 2009 The rhizospher: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant Soil*, DOI: 10.1007/s11104-008-9568-6.
18. Berg G, Zachow C, Lottmann J, Gotz M, Costa R and Smalla K 2005 Impact of plant species and site on rhizoshere associated fungi antagonistic to *Verticillium dahlia Kleb. Appli Environ Micrbiol* 171: 4203-4213.

19. Padmanabhan V, Prabakaran G, Paily KP and Balaraman K 2005 Toxicity of a Mosquitocidal metabolite of *Pseudomonas fluorescens* on larvae & pupae of the house fly, *Musca domestica. Indian J Med Res*, 121:116-119.
20. Bronstad, K., Dronen, K., Ovreas, L and Torsvik, V 1996 Phenotypic diversity and antibiotic resistance in soil bacterial communities. *Journal of Industrial Microbiology* 17, 253-259.
21. Usharani B and Kummankottil P 2012 Chitinase like activity of metabolites of *Pseudomonas fluorescens* Migula on immature stages of the mosquito, *Culex quinquefasciatus* (Diptera:Culicidae). *African Journal of Microbiology Research,* 6(11), 2718-2726.
22. Pushpanathan M and Pandian RS 2008 Management of dengue and chikungunya vectors *Aedes aegypti*(Linn) and *Aedes albopictus* (Skuse) (Diptera:Culicidae) by the exotoxin of *Pseudomonas fluorescens* Migula (Pseudomonadales: Pseudomonadaceae). *Current Biotica*, 2(1), pp:72-103.
23. Prabakaran G, Hoti SL, Surya Prakash Rao H and Vijjapu S 2015 Di-rhamnolipid is mosquito pupicidal metabolite from *Pseudomonas fluorescens* (VCRC B426). *Acta Tropica* 148: pp:24-31.
24. Preethi VS and Pandian RS 2009 Evaluation of the larvicidal and pupicidal activities of the exotoxin of *Pseudomonas fluorescens* Migula against *Aedes aegypti* (L.) and *Culex quinquefasciatus* Say. *Current Biotica*, 3(3):416-438.
25. Kucera M and Lysenko O 1971 The mechanism of pathogenicity of *Pseudomonas aeruginosa*, Isolation of hemolymph proteins from *Galleria mellonella* larvae and their digestibility by the toxic protease. *J Invertebr Pathol* 17:203-210.

How to cite this article:

Sivakami V., Kannan S and Selvaraj Pandian R.2018, Selection of Suitable Bacterial Isolates For Their Usage As Biocontrol Agents Against Medically Important Pests. *Int J Recent Sci Res.* 9(2), pp. 24521-24524.
DOI: http://dx.doi.org/10.24327/ijrsr.2018.0902.1676

****