African Journal of Microbiology Research

Volume 12 Number 3 21 January, 2018
ISSN 1996-0808

Academic Journals

ABOUT AJMR

The African Journal of Microbiology Research (AJMR) is published weekly (one volume per year) by Academic Journals.

The African Journal of Microbiology Research (AJMR) provides rapid publication (weekly) of articles in all areas of Microbiology such as: Environmental Microbiology, Clinical Microbiology, Immunology, Virology, Bacteriology, Phycology, Mycology and Parasitology, Protozoology, Microbial Ecology, Probiotics and Prebiotics, Molecular Microbiology, Biotechnology, Food Microbiology, Industrial Microbiology, Cell Physiology, Environmental Biotechnology, Genetics, Enzymology, Molecular and Cellular Biology, Plant Pathology, Entomology, Biomedical Sciences, Botany and Plant Sciences, Soil and Environmental Sciences, Zoology, Endocrinology, Toxicology. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles are peer-reviewed.

Contact Us

Editorial Office: ajmr@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJMR

Submit manuscript online http://ms.academicjournals.me/

Editors

Editorial Board Members

Editorial Board Members

Editorial Board Members

Editorial Board Members

African Journal of Microbiology Research

Table of Contents: Volume 12 Number 3 21 January, 2018

ARTICLES

academicJournals

Vol. 12(3), pp. 46-51, 21 January, 2018
DOI: 10.5897/AJMR2016.8036
Article Number: 183D45355815
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Evaluation of various marine products against *Rhizoctonia solani* under *in vitro* condition

T. Suthin Raj[1]*, K. Hane Graff[1] and H. Ann Suji[2]

[1]Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Chidambaram, India.
[2]Centre for Advance Studies in Marine Biology, Annamalai University, Chidambaram, India.

Recieved 30 March 2016; Accepted 23 May, 2016

Rhizoctonia solani **is the causative agent of rice sheath blight, which has become a major problem in rice production. Seaweed provides a rich source of structurally diverse and biologically active secondary metabolite and is proved to be better in decreasing foliar fungal diseases which ultimately increase its fertility and help in the growth of plants. The use of natural products is the ultimate way of combating this disease. In this context, five different seaweeds such as *Sargassum wightii, Dictyota bartyrensiana, Ulva reticulate, Gelidiella acerosa* and *Odonus niger* were used together with the fish powder extract in the control of sheath blight disease in rice were studied. Evaluation of marine products against *R. solani* was carried out by paper disc assay; agar well method and mycelial dry weight. Among the five marine extracts tested, extracts of *S. wightii* [brown seaweed algae] at a high concentration (20%) was found to be the best in the reduction of spore germination (19.60%). The leaf extracts of *S. wightii* [brown seaweed algae] at highest concentration of 20% showed a maximum reduction in both paper disc method and agar well method with 44.65 and 45.90% zone of inhibition, respectively. The antifungal compounds were identified through gas chromatography mass spectroscopy. The results revealed that, 18 compounds were present in *S. wightii* and among that n-hexadecanoic acid which was closely related to 9, 12-octadecadienoic acid may be responsible for the inhibition of the growth of *R. solani.* The present study revealed that the efficacy of seaweed extracts against fungal pathogens may be due to higher levels and early accumulation of phenolics and phytoalexins, and the pot study proved that *R. solani* can be controlled by the application of marine products which may be further used for field study.**

Key words: Seaweeds, *Rhizoctonia solani,* antifungal compounds, rice.

INTRODUCTION

Rhizoctonia solani Kuhn is the causal agent of rice sheath blight, which has become a major constraint to rice production during the last two decades (Kobayashi et al., 1997). The intensification of rice cropping systems

*Corresponding author. E-mail: suthinagri@gmail.com.

with the development of new short stature, high tillering, high yielding varieties, high plant density and an increase in nitrogen fertilization (Gangopadhay and Chakrabarthi, 1982; Ou, 1985) has seen the “emergence of *R. solani* as an economically important rice pathogen”.

This pathogen can survive in soil for many years by producing small (1-3 mm diameter) irregular shaped, brown to black sclerotia in soil and on plant tissues. The ability of *R. solani* to produce sclerotia with a thick outer layer allows them to float and survive in water. *R. solani* also survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Ghaffar, 1988). The sclerotia present in the soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fibre crops.

Presently, sheath blight disease management is mainly achieved through systemic fungicides (Pal et al., 2005) and the bacterial bio-control agents like plant growth-promoting rhizobacteria (PGPR) offer a promising means of controlling plant diseases (Mew and Rosales, 1992). Brown seaweeds contain bio-control properties and contain many organic compounds and growth regulators such as auxins, gibberellins and precursor of ethylene and betaine which affect plant growth. Seaweed extracts have been reported to increase plant resistance to diseases, plant growth, yield and quality (Jolivet et al., 1991). Thus, seaweeds are bestowed with varied sources of bioactive natural products that exhibit biomedical and antimicrobial properties (Kumar et al., 2005; Karthikeyan and Shanmugam, 2016). Peres (2012) was the first to observe antifungal substances in seaweeds. The seaweed is commercially available and some reports have indicated enhanced plant yield and health in different crops following application, although the mechanisms of action have not been determined (Norrie et al., 2002; Colapietra and Alexander, 2006).

Application of seaweed extracts is proved to be better to decrease the foliar fungal diseases which ultimately increase its fertility and help the growth of plants (Jayaraj et al., 2008). Kumar et al. (2005) evaluated the bioactive potential of seaweeds against plant pathogenic bacterium *Xanthomonas oryzae* pv. *oryzae*. Kumar et al. (2008) tested crude seaweeds extracts against the phytopathogenic bacterium, *Pseudomonas syringae* causing leaf spot disease of the medicinal plant, *Gymnema sylvestris*. The use of anti-microbial drugs (Arioli et al., 2015) has certain limitations due to changing patterns of resistance in pathogens and side effects they produce.

Seaweeds provide a rich source of structurally diverse and biologically active secondary metabolites. The functions of these secondary metabolites are defense mechanism against herbivores, fouling organ Figures and pathogens for example, grazer-induced mechanical damage triggers the production of chemicals that act as feeding detergents or toxins in seaweeds (Ammirato, 1986). They contain all major and minor plant nutrients as well as biocontrol properties and many organic compounds such as auxins, gibberellins and precursors of ethylene and betaine which affect plant growth (Wu et al., 1997).

Seaweeds are benthic marine macro algae mainly used for the production of agar, alginate, liquid fertilizers and manures (Sivakumar, 2014). Most of the secondary metabolites are the bactericidal or the antimicrobial compounds derived from seaweeds which consist of diverse groups of bacteriostatic properties such as brominates phenols, oxygen heterocyclic; Terpenols, Sterols, Polysaccharides, dibutenolides peptides and proteins. Although, most of the antibiotics found from terrestrial sources are used as therapeutic agents to treat various diseases, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value (Anderson et al., 2006). In this context, the present study was carried out to evaluate the various marine products against *Rhizoctonia solani* under *in vitro* condition.

MATERIALS AND METHODS

Evaluation of marine products against *R. solani in vitro*

The efficacy of the marine products listed in Table 1 was tested against *R. solani*

Preparation of marine products

Preparation of crude seaweeds extracts (Vallianayagam et al., *2009)*

Each 1 kg of live, healthy and matured samples (Brown and red seaweeds) of each seaweed collected along the Coast of Pamban (Rameswaram (9°14`N; 79°14`E), Gulf of Mannar, Tamil Nadu, India) were washed thoroughly in seawater followed by tap water to remove extraneous particles and epiphytes. Then, they were air dried under shade in the aboratory for 3 days. The shade-dried samples were chopped and pulverized. Each 50 g powdered sample was separately extracted for 7 days thrice in 500 ml of 1:1 (v/v) chloroform: methanol using 1 L Erlenmeyer conical flask under dark condition. The extractants were pooled and concentrated by using flask evaporator under reduced pressure at 45°C and weighed stored at 0°C (Plates 3, 4 and 5).

Preparation of fish powder extracts (Suji, 2004)

Two marine fish species (trash fish and edible fish) were processed at a local processing plant, using 3.5% sucrose and 0.15% phosphate as cryoprotectants. The frozen blocks were transported to the laboratory and stored at 18°C until drying. A 500 g block of each fish was dried using a Labconco Freeze Dry System at a temperature of 40°C until the moisture content reached 5%. The samples were milled and sieved using a 40 mm screen mesh. The resulting powder was vacuum packed and stored at 4°C. Powdered

Table 1. List of seaweeds and the use of active compounds present.

Scientific name	Active ingredient	Common name	Collected from
Sargassum wightii	Fucoidan	Brown seaweed	Gulf of Mannar Coast
Dictyota bartyrensiana	-	Brown seaweed	Gulf of Mannar Coast
Ulva reticulate	Caccamese and Azzolina	Green seaweed	Gulf of Mannar Coast
Gelidiella acerosa	Dimethicone	Red seaweed	Gulf of Mannar Coast
Odonus niger	-	Trash fish	Paliyaru (Nagappattinam dist.)

samples were soaked in chloroform (1:4 w/v) and extracted for 2 days at room temperature and the extracts were collected and concentrated.

Evaluation of marine products against *R. solani*

Paper disc assay

Various concentrations like 5, 10, 15 and 20% of seaweed extracts and fish powder extracts were made. 20 ml of PDA medium was seeded with 3 ml of sclerotial suspension ($1x10^6$ sclerotia/ml) of the fungus and solidified. Sterile filter paper discs (10 mm) were dipped separately in known concentration of treatments and placed equidistantly over the seeded medium. Three replications were maintained. The plates were incubated at 28±2°C for 48 h. The inhibition zone of the fungal growth around the treated paper discs was measured and recorded. The paper disc dipped in sterile distilled water served as control (Plate 9) (Saha et al., 1995).

Agar well method

Seaweed extracts and Fish powder extracts like 5, 10, 15 and 20% individually (10 ml) were added to the sterilized potato dextrose agar medium and thoroughly mixed just before plating. 20 ml of these mixtures individually were immediately poured into sterilized Petri plates and were allowed to solidify. A 9 mm of PDA disc was removed by using cork borer to form wells; 1 ml of spore suspension was poured into the well. All these were carried out under aseptic conditions. The plates were incubated at 28±2°C for 10 days. Potato dextrose agar medium without natural product served as the control. Three replications were maintained. The radial growth of the colony was measured. The percent inhibition of the growth was calculated (Thongson et al., 2004).

Mycelial dry weight

Potato dextrose broth was prepared in 250 ml Erlenmeyer flasks and autoclaved. Seaweed extracts and Fish powder extracts at 2.5, 5, 7.5 and 10 ml concentrations were added to 47.5, 45, 42.5 and 40 ml broth in flasks so as to get final concentrations of 5, 10, 15 and 20% of the filtrate in the broth. Similar procedure has been followed for taking the mycelial dry weight as stated earlier.

Identification of antifungal compounds

Analysis of antifungal compound through gas chromatography mass spectroscopy (GCeMS) (NIST Version. 2.0, 2005)

Based on the growth inhibition studies, seaweed extract was selected and chemical constituents were determined with a GC Clarus 500 Perkin Elmer Gas chromatography equipped with a mass detector. Turbo mass gold containing an Elite-1 (100% dimethyl poly siloxane), 30 m × 0.25 mm ID employed were the following: Carrier gas, helium (1 mL/min); oven temperature program 110°C (2 min) to 280°C (9 min); injector temperature (250°C); total GC time (36 min). The water extract was injected into the chromatograph in 2.0 Ml aliquots. The major constituents were identified with the aid of a computer-driven algorithm and then by matching the mass spectrum of the analysis with that of a library (NIST Version. 2.0, year 2005). Software used for gas chromatography mass spectroscopy (GCeMS) was Turbo mass-5.1. This work was carried out in Indian Institute of Crop Processing Technology (IICPT), Thanjavur (Figures 1 and 2).

RESULTS

In vitro evaluation of marine products against *R. solani*

Paper disc method and well method

Various marine products were selected and evaluated for the antimicrobial activity by two methods, such as paper disc and agar well method. The leaf extracts of *Sargassum wightii* [brown seaweed algae] at the highest concentration (20%) was found to be the maximum reduction in both paper disc method and agar well method recorded was 44.65 and 45.90% inhibition zone, respectively. It was followed by a highest concentration (20%) of *Odonus niger* [trash fish] which recorded 39.00 and 34.33% inhibition zone in paper disc method and agar well method, respectively. All the concentrations of *G. acerosa* [red seaweed algae] recorded the minimum percent inhibition zone than all other extracts (Table 2).

The result of the experiment revealed the superiority of *Sargassum wightii*. Hence, the same was used for further studies.

Gas chromatography mass spectroscopy (GCeMS) analysis

On the basis of performance of marine products in the preceding *in vitro* studies, *S. wightii* (brown seaweed) was tested to determine the nature of chemical compound (s) present in the seaweed extract. The results revealed that 18 compounds were present in *S. wightii*.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	11.03	7-Octen-1-ol, 3,7-dimethyl-, (S)-	$C_{10}H_{20}O$	156	11.57
2.	11.27	E-2-Tetradecen-1-ol	$C_{14}H_{28}O$	212	4.69
3.	11.49	Butanoic acid, 3,7-dimethyl-6-octenyl ester	$C_{14}H_{26}O_2$	226	4.18
4.	12.57	2-Aminononadecane	$C_{19}H_{41}N$	283	0.90
5.	12.79	n-Decanoic acid	$C_{10}H_{20}O_2$	172	29.46
6.	12.96	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.78
7.	13.08	n-Capric acid isopropyl ester	$C_{13}H_{26}O_2$	214	3.27
8.	13.26	9,9-Dimethoxybicyclo[3.3.1]nona-2,4-dione	$C_{11}H_{16}O_4$	212	6.06
9.	19.16	3-Hexadecyloxycarbonyl-5-(2-hydroxyethyl)-4-methylimidazolium ion	$C_{24}H_{45}N_2O_3$	409	0.93
10.	20.54	Valeric acid, 3-tridecyl ester	$C_{18}H_{36}O_2$	284	1.47
11.	21.90	Methoxyacetic acid, tridecyl ester	$C_{16}H_{32}O_3$	272	2.47
12.	23.26	7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	$C_{14}H_{14}O_3$	230	2.60
13.	24.57	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	2.30
14.	27.01	Diazoprogesterone	$C_{21}H_{30}N_4$	338	1.43
15.	29.56	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	208	7.47
16.	30.05	5à-Androstan-16-one, cyclic ethylene mercaptole	$C_{21}H_{34}S_2$	350	13.94
17.	30.53	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3à,17à)-	$C_{22}H_{32}O_2$	328	1.84
18.	30.70	9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester	$C_{25}H_{38}O_2$	370	0.66

*Parameters tested are not covered under the scope of NABL accreditation

Figure 1. Components identified in the seaweed powder (Code No. 311) [GC MS study].

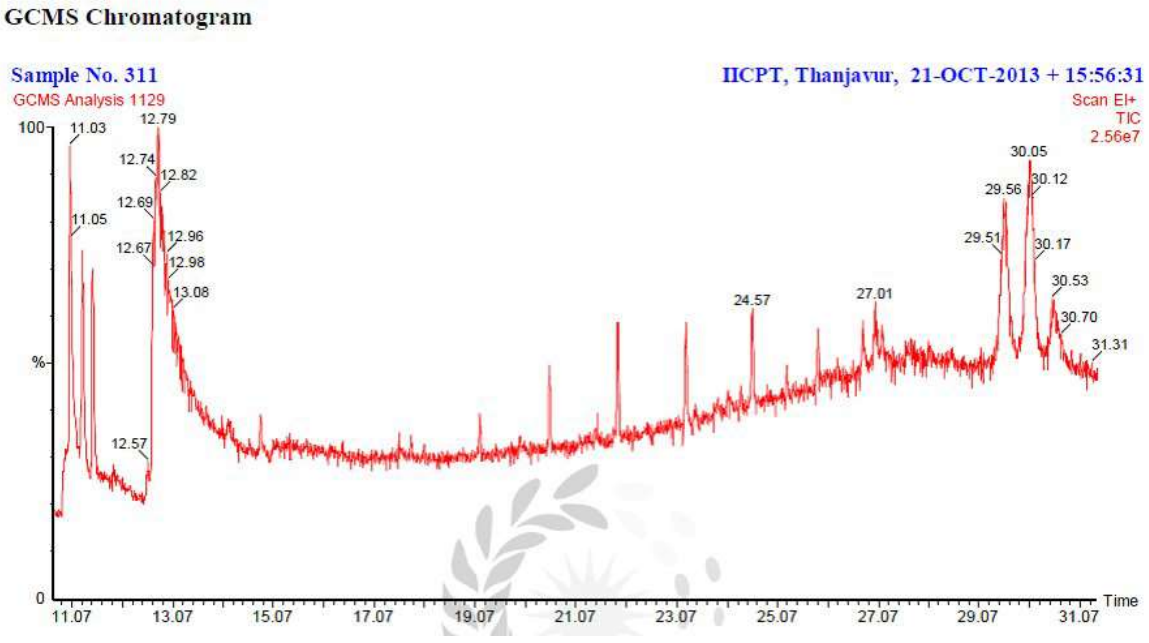

Figure 2. GC-MS analysis of sea weeds extract.

Table 2. Evaluation of various marine products against *R. solani* under *in vitro* condition.

Marine products	Agar well method					Paper disc method				
	5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
Sargassum wightii [Brown seaweed algae]	36.10	40.28	46.10	44.65	41.78 [a]	30.71	34.65	39.16	45.90	37.60 [a]
Dictyota bartyrensiana [Brown seaweed algae]	20.00	21.50	26.70	28.30	24.12 [c]	18.20	20.50	23.10	25.95	21.93 [c]
Ulva reticulate [Green seaweed algae]	12.25	14.50	18.80	27.10	1816 [d]	10.00	11.50	15.20	18.00	13.67 [d]
Gelidiella acerosa [Red seaweed algae]	12.00	14.21	16.28	19.72	15.55 [e]	10.66	11.92	14.00	17.00	13.39 [d]
Odonus niger [Trash fish]	24.00	25.60	30.00	39.00	29.65 [b]	20.00	22.67	28.67	34.33	26.41 [b]
Control	0.00	0.00	0.00	0.00	0.00 [f]	0.00	0.00	0.00	0.00	0.00 [e]

*****Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

The molecular weights, name of the compound, chemical formula, retention time and peak area percentage are given in Figure 1. Among these, n-Hexadecanoic acid which was closely related to 9, 12-Octadecadienoic acid may be responsible for the inhibition of the growth of *R. Solani* (Figure 2).

DISCUSSION

The seaweeds and the prepared marine products has significant role in the control of the *R. solani* in *in vitro* condition. Generally, all the marine products inhibited the mycelial growth of pathogen in the present study of which, *S. wightii* [brown seaweed algae] @ 20% exhibited the highest level of inhibition of *R. solani*. This statement has been confirmed by several workers. Sultana et al. (2007), reported that brown, green and red seaweeds were highly effective against *R. Solani* in- vitro and vivo conditions. Several workers have reported on the efficacy of seaweed extracts against fungal pathogens (Norrie et al., 2002; Jayara et al., 2008). This may be due to higher levels and early accumulation of phenolics and phytoalexins (Garcia-Mina et al., 2004). The above results lend supports to the present findings and helpful for further study in the treatment of sheath blight caused by *R. solani* in rice plant.

Conflict of interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors thank the authorities of the Science and Engineering Board (SERB) for their financial support and acknowledge the co-operation of the Department of Plant Pathology, Faculty of Agriculture, Annamalai University for the successful completion of the research work.

REFERENCES

Ammirato P (1986). Morphogenesis and clonal propagation. In: Plant tissue culture and its agricultural application. (Eds): Withers L, Alderson P. Butterworth, London. pp. 21-47.

Anderson MX, Kourtchenko O, Dangl JS, Mackey D, Elerstrom M (2006). Phospholipase dependent signaling during the AvrRpml and AvrRpt2-induced disease resistance response in *Arabidopsis thaliana.* Plant J. 47:947-949.

Arunkumar A, Vijayababu MR, Kanagaraj P, Balasubramanian K, Aruldhas MM (2005). Growth suppressing effect of Garlic compound Diallyldisulfide on prostate cancer cell line (pc.3) *in vitro.* Biol. Pharm. Bull. 28:740-743.

Baker KF (1990). Types of *Rhizoctina* diseases and their occurrence. In parameter, JB Jr (ed) *Rhizoctina solani*, biology and pathology pp125-48. Univ of Calif. Press, Berkeley, Los Angeles and London.

Colapietra M, Alexander A (2006). Effect of foliar fertilizer on yield and quality of table grapes. (proc. V[th]IS on mineral nutrition of fruit plants, Eds. J.B. Retamales and G.A.Lobos,) Acta Hort. 721:213-218.

Gangopadhay S Chakrabarti NK (1982). Sheath blight of rice. Rev. Plant Pathol. 61:451-460.

Garcia-Mina JM, Antolin MC, Sanchez-Diaz M (2004). Metal humic complexes and plant Micro-nutrient uptake: a study based on different plant species cultivated in diverse soil types. Plant Soil. 258:57-68.

Ghaffar A (1988). Soil borne disease research. Final Research Report. Department of Botany, University of Karachi 75270, Pakistan, 111 p.

Jayaraj J, Wan A, Rahman M, Punja ZK (2008). Seaweed extract reduces foliar fungal disease on carrot. Crop Protect. 27:1360-1366.

Jolivet I, Huchon P, Brun J, Pichon X, Chamot Rooke N, Thomas J (1991). Arc deformation and marginal basin opening: Japan sea as a case study. J. Geophys. Res. 96:1-10.

Karthikeyan K, Shanmugam M (2016). Development of a protocol for the application of commercial bio-stimulant manufactured from *kappaphycus alvarezii* in selected vegetable crops. J. Exp. Biol. Agric. Sci. 4:92-102.

Kobayashi T, Mew TW, Harshiba T (1997). Relationship between incidence of rice sheath blight and primary inoculum in the Philippines: mycelia in plant debris and sclerotia. Ann. Phytopathol. Sco. Jpn. 63:324-327.

Mew TW, Rosales AM (1992). Control of *Rhizoctonia solani* sheath blight and other diseases by rice seed bacterization. Biol. control plant dis. pp. 113-123.

Norrie J, Branson T, Kethley PE (2002). Marine plants extract impact on grape yield and quality. In: Int. Symp. Foliar Nutr. Perennial Fruit Plants 594:315-319.

Pal R, Chakrabarthi K, Chakraborthy A, Chowdhury A (2005). Dissipation of pencyeuron in rice plant. J. Zhejiang. Univ. Sci. 613(8):756-758.

Saha BP, Saha K, Mukherjee PK, Mandal SC, Pal M (1995). Antibacterial activity of leucas lavandulaefolia rees. Indian Drugs 32:

402-404.
Sivakumar SR (2014). Antimicrobial potential of white crystalline solid from red algae *Porteiriahornemanii* against the plant pathogenic bacteria. Afr. J. Agric. Res. 9(17):1353-1357.
Suji HA (2004). Microbiological, biochemical, biophysical and sensory assessment of quality in value added products developed from marine fish (*Sufflamen capistratus*). M.Phil Thesis. CMST. pp. 80.
Thongson C, Davidson PM, Mahakarrchanakul W, Weiss J (2004). Antimicrobial activity of ultrasound – assisted solvent – extracted species. Letters Appl. Microbial. 39:401-406.
Wu Y, Jenkins T, Bluden G, Von Mende N, Hankins SD (1997). Suppression of fecundity of the root knot nematode, *Melodogynejavanica* in monoxenic cultures of Arabidopsis thaliana treated with an alkaline extract of *Ascophyllum nodosum*. J. Appl. Phycol. 10:91-94.

.

academicJournals

Vol. 12(3), pp. 52-61, 21 January, 2018
DOI: 10.5897/AJMR2017.8696
Article Number: 6A1B9F655819
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

GC-MS and molecular analyses of monocrotophos biodegradation by selected bacterial isolates

Buvaneswari G.[1*], Thenmozhi R.[1], Nagasathya A.[2], Thajuddin N.[3] and Praveen Kumar D.[4]

[1]PG and Research Department of Microbiology, JJ College of Arts and Science (Autonomous), Sivapuram, Pudukkottai, Tamil Nadu, India.
[2]Goverment Arts College for Women, Pudukkottai, Tamil Nadu, India.
[3]Department of Microbiology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India.
[4]Contec Global Agro Limited (Contec Global Group), Abuja, Nigeria.

Received 31 August, 2017; Accepted 30 November, 2017

Soil treatment incubation method was carried out to analyze the monocrotophos (MCP) biodegradation using three selected bacterial isolates namely *Pseudomonas stutzeri* (KY287931), *Bacillus licheniforms* (KY287928), and *Bacillus sonorensis* (KY287930). After 45 days of incubation of pesticide exposed to soil samples, the MCP biodegradation by individual cultures and consortium culture was analyzed and conformed by gas chromatography–mass spectrometry (GC-MS) method. The three isolates individually degrade toxic MCP into non-toxic intermediate while the consortium cultures completely degrade the MCP into water-soluble compounds. PCR-based RFLP analysis of 16S rRNA was performed. *Pseudomonas* and *Bacillus* species have been phylogenetically analyzed by using 16S rRNA gene sequences. The isolated bacterial consortium culture proved a potential agent for MCP biodegradation.

Key words: Soil, MCP-monocrotophos, bacterial isolate, gas chromatography–mass spectrometry (GC-MS).

INTRODUCTION

Use of microorganisms for properly detoxifying, degrading and eradication of toxic compounds from contaminated soil and water has emerged as the best technique to clean up contaminated environments (Strong and Burgess, 2008). Monocrotophos (MCP) is still widely used in India for the protection of cash crops such as cotton, sugarcane, tobacco, maize, groundnut, soybeans, rice, and vegetables (Acharya et al., 2015). Microorganism plays a major role in agriculture as they increase the exchange of plant nutrients and make chemical fertilizers usage minimal as much as possible.

Beneficial plant-microbe interactions in the rhizosphere can improve the soil fertility (Dastager et al., 2011). Microbes plays a crucial role for changeover of phosphorous in water and sediments and the phosphate ions are reported strongly, absorbed by sediments with a high content of slit and clay (Chen et al., 2015). In order to accomplish an effective degradation, the

*Corresponding author. E-mail: dr.s.buvaneswari@gmail.com. Tel: +91 7395843404.

microorganisms were isolated from agricultural fields which were previously exposed to the pesticides (Jayanthi et al., 2014). Organophosphate hydrolase (opdA gene) wrapped up in starting biodegradation of monocrotophos in KPA-1 was quantitatively shown, which fundamentally indicates cytosolic enzyme; a catabolic pathway for MCP degradation has been proposed (Bhadbhade et al., 2002). Numerous problems associated with pesticides application are their possible persistence in the ecosystem and therefore, their possible integration into the food chain affects ecosystems and human population (Hill et al., 2003). In the recent years soil quality deterioration has caused a serious concern to our planet. Pesticide contaminations of soil pose a grave danger to human health as well as to the natural environment. The methods procurable today (physical or chemical) are on the other hand costly or incomplete. Bioremediation contribute a superior process or tools for the efficient detoxification of pesticides from the soil. Bioremediation is environmentally healthy, cost effective and has efficient method (Baba et al., 2016).

The pathway is initiated by the conversion of dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate and then to N-methylhydroxyl monocrotophos dimethyl phosphate; both steps are catalyzed dependently by dimethylphosphate. N-methylacetoacetamide is transformed to 3-hydroxy-N-methylbutyramide, and methyl phosphate is converted to phosphoric acid. Microorganisms contribute a great percentage in the conversion of phosphorous in water and the phosphate ions are actively taken by ground with major portion of clay and slit (Seema et al., 2013).

Pesticides can be degraded by microbial, chemical and photo degradation processes in the environment. Nonetheless, microbial degradation is considered as the major determining factor of the organophosphrus fate in the environment and is often the main process of pesticide degradation in soils, representing the safest, least disruptive and most cost-effective treatment method (Zrostlikova et al., 2003; Singh et al., 2004). Several researchers investigated the capabilities of bacteria to degrade MCP (Singh et al., 2009). All of these enzymes are found to induce the degradation of organophosphate compounds.

The aim of this work is to study the impact of MCP on soil microorganisms. Twenty-five safe bacterial species were isolated, purified and identified from soil with history pesticide application, then used to estimate their effect on the biodegradation rate of tested organophosphrus insecticides, to give a primitive indication about organophosphrus insecticides biodegradation.

MATERIALS AND METHODS

Bacterial samples

Three promising MCP degrading bacterial isolates-from our previous work (Buvaneswari et al., 2017), namely Pseudomonas stutzeri KY287931, Bacillus licheniformis KY287928, and Bacillus sonorensis KY287930 were selected for the present study.

Soil treatment incubation

The organophosphorus pesticide monocrotophos used at concentration of 1000 ppm was mixed with 250 g of soil already dried and sterilized. The autoclaved soil and water was used as control and the treatments made were as follows:

Control- Soil + Pesticide (1000ppm)
Treatment 1- Soil + Pesticide (1000ppm) + *P. stutzeri* (100ml)
Treatment 2- Soli + Pesticide (1000ppm) + *Bacillus licheniforms* (100ml)
Treatment 3- Soil + Pesticide (1000ppm) + *B. sonorensis* (100ml)
Treatment 4- Soil + Pesticide (1000ppm) +Consortium (100ml)

100 g of soil was added in 100 ml of sterilized water for the preparation of soil culture. Individual treatments of soil in triplicate were flooded with inoculum to keep them moist during incubation period. These treatments were kept at incubator (28 to 30°C) under darkness for 45 days. Two sets of flasks were prepared with the pesticides at 1000 ppm. After the completion of incubation, period flasks were taken out accordingly for extraction and analysis (Wasim et al., 2009).

Extraction and analysis

25 g of treated soil (samples) was air dried for extraction and homogenized with 0.5 g charcoal (activated) for 4 h at 120°C. Then added 1.0 g Florisil (activated for 4 h at 650°C) and 5 drops of 25% NH_3H_2O solution placed over a 2.5 cm layer of anhydrous sodium sulphate (Parthiban et al., 2015) (analytical grade) in a glass column with 34 cm length and 2.5 cm dia.

Extraction was done, using a solution of n-hexane (distilled) and acetone (distilled) in a ratio of 2:1 by the method described by Wasim Aktar et al. (2009). Eluted material was collected in a 250 ml conical flask (Pyrex) and later evaporated on rotary evaporator to almost dryness dissolved in 20 to 50 µl quantity n-hexane in small glass vials for Gc-ms determination.

Analysis of liquid samples on the Agilent GC-MS

Gas chromatographic determination

The samples were analyzed in GCMS (Gas Chromatograph- Mass Spectrophotometer) (5975c: Insert MSD with Triple Axis Detector). The limit of detection (LOD) of OCPs was determined, the blank. Before analysis, standards were run to check for the column performance, peak height and resolution. The extract was analyzed on GC (equipped with flame ionization detector) with the parameters as column temperature 230°C, injector temperature 250°C, detector temperature 300°C, hydrogen gas flow 4.5 ml/min and air flow 175 ml/min.

The parameter of the glass column was 1.5 % OV-17 + 1.75 % OV-210 Chrom W-HP 80/100 mesh 2 meters x ¼" x 2 mm ID (internal diameter). The retention time was 1.6 min, detection limit was 1µg and recovery percentage was 100%. An Agilent Gas Chromatography system 5975c with Mass Selective Detector 5973 was used. If the retention time, qualifying ions (molecular weights) and response (area on the graph giving the abundance corresponding to the concentration) values were in the correct range and the Q value (accuracy estimation from the machine in

percentage) was high then, the result was accepted and process (Mishra et al., 2017; Gilani et al., 2010).

Molecular characterization of MCP degrading bacterial isolates

Restriction fragment length polymorphism (RFLP) analysis

Extraction of genomic DNA from MCP degrading bacterial strains: The isolates were used to inoculate in nutrient broth (Hi-media) and incubated overnight at 37°C and 200 rpm. The resulting bacterial suspension was pelleted at 10,000 rpm for 5 min and the genomic DNA was extracted from the samples using phenol-chloroform extraction technique. The buffer in the micro centrifuge tube contains the DNA. The DNA samples were stored at -20°C until further use (Lori et al., 2005; John, 2014).

Purification of 16S rRNA PCR product

The partial 16S rRNA gene fragments were amplified from the cultures with PCR conditions as follows: the PCR products were precipitated with 9 µl of 3 M sodium acetate (pH 5.2) and 60 µl of isopropanol and resuspended in 50 µl of distilled water. Extraction of the PCR products with chloroform was carried out (Akhter et al., 2013).

Electrophoresis and enzymatic digestion of amplified DNA

Purified DNA was digested for 2 h in 10 µl volumes with restriction endonucleases. The following three restriction enzymes were used: ALU1, SAU3A1and HAE. Triple digestion with SAU3A1 and ALU1 and HAE was performed as follows: DNA was first digested for 1 h at 25°C with ALU1.

Following the addition of buffer recommended by the manufacturer, SAU3A1and HAE were added and the reaction mixture was incubated for 1 h at 37°C (Daniel et al., 2009). The resulting DNA fragments were electrophoresed in 1.5% (wt/vol) agarose gels in Trisacetate buffer (0.04 M Tris-acetate, 0.001 M EDTA; pH 8.3) at 100 V for 30 min. The gels were stained with ethidium bromide and DNA fragments were visualized by UV transillumination and photographed. Strains were categorized according to their distinct RFLPs (Henrik, 2012).

Phylogenetic analysis data

To assess the phylogeny of the isolates, a BLASTN analysis (16S rRNA sequence) was carried out through GenBank (Liu et al., 2001; Castresana et al., 2000; Dereeper et al., 2008).

Construction of phylogenetic trees

The computer assisted by data analysis was performed for RFLP profiles. Two numbers of alternative trees were constructed. Negative branch lengths were avoided or minimized in the best trees selected. Farris trees were constructed according to Archibald (2002) and UPGMA trees as described by Allman et al. (2017).

RESULTS AND DISCUSSION

In our previous study, a total of 121 bacteria isolates belong to three genera, capable of MCP degradation with particular concentration which were studied and conformed by FTIR (Gilani et al., 2010). All of the pure bacterial isolates were sequentially subcultures on mineral salt medium.

Similarly, several species of bacteria were isolated from different environment which degrade organophosphorus compound in laboratory cultures and soil (Buvaneswari et al., 2017). Monocrotophos degradation in soil treatment incubation method using selected bacterial isolates was analyzed and conformed by GC-MS method. After forty five days of inoculation with the selected bacterial species, the consortium is compared with uninoculated samples. The metabolic products of MCP degradation by strains *P. stutzeri* (KY287931), *B. licheniforms* (KY287928), and *B. sonorensis* (KY287930) filtrates were extracted and confirmed by GC-MS. Sasano et al. (2000) proposed the analysis of pesticides in water samples using the GC/MS technique (Singh et al., 2006).

Gas chromatographic mass-spectrum is generated by monocrotophos in control culture media (Figures 1 and 2). The control sample compared to NIST library identification program have a phosphoric acid, dimethyl1-methyl-3-(metylamino)-3-oxo-1-propenyl ester group compound ($C_7H_{14}NO_5P$). The degradation of organophosphate pesticide by microorganisms appears to be identical, where OPH or PTE catalyzes the first step of the degradation (Sasano et al., 2000). The peaks at 11.6 min indicate that 2-hydroxy-4-hydroxylaminopyrimidine is one of the most important compounds in monocrotophos and peaks at 12.86 min indicate phosphoric acid and dimethyl 1-methylpropenyl ester. Each of the peaks was identified on the basis of its mass spectra and the NIST library identification program. These compounds strongly evidence the presence of MCP in the extracted control samples. Anizio et al. (2009), proposed a method of analysis for six pesticides (imazethapyr, imazaquin, metsulfuron-methyl, bentazone, chlorimuron-ethyl and tebuconazole), by GC with mass spectrometer detector (Anizio et al., 2009).

After 45th day of incubation, the gas chromatographic mass-spectrum generated by KY287931 (*P. stutzeri*) culture media was analyzed (Figures 3 and 4). The peak at retention time 11.6 min (2-hydroxy-4-hydroxylaminopyrimidine) and 12.86 min (phosphoric acid and dimethyl 1-methylpropenyl ester) corresponded to control sample, this peak disappeared concomitantly with the formation of another new peak with a retention time of around 1.668 min. The new peak with a retention time of around 1.668 min indicate 2-pentanone, 4-hydroxy-4-methyl-$C_6H_{12}O_2$.The results demonstrated that *P. stutzeri* is a potent and easily acquired bioremediation agent that could be used to remove other pollutants from pesticide contaminated soils. A similar study was conducted by Aleem (2003), who investigated the determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by *Pseudomonas* strains.

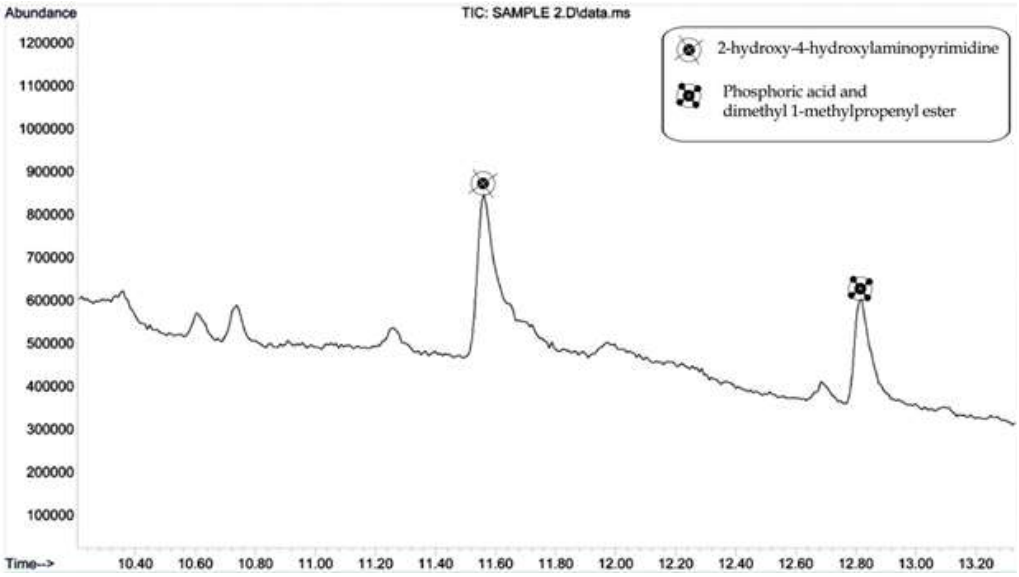

Figure 1. Gas chromatographic pattern of monocrotophos in control culture media.

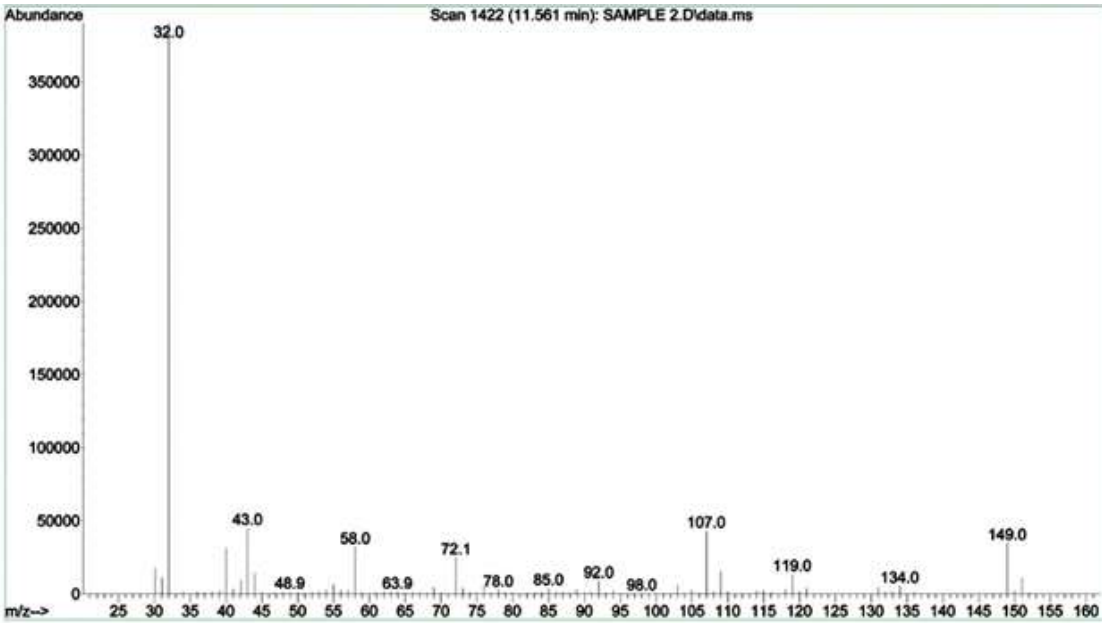

Figure 2. Mass-spectrum generated by pattern of monocrotophos in control culture media.

After 45th day of incubation, the gas chromatographic mass-spectrum generated by BAGN005 (*B. licheniformis*) culture media were analyzed (Figures 5 and 6). The peak at retention time of 11.6 min (2-hydroxy-4-hydroxylaminopyrimidine) and 12.86 min (phosphoric acid and dimethyl 1-methylpropenyl ester) corresponded to control sample. This peak disappeared concomitantly with the formation of another new peak through a retention time of around 1.676 min. Zhao et al. (2012) described the same method to study pesticides in vegetables. The graph shows peak with a retention time of around 1.676 min, which indicates the 2-pentanone 5-chloro C_5H_9ClO. This is one of the four stable isomers of butanediol. In biology, 1, 3-butanediol is used as a hypoglycemic agent.

After 45th day of incubation, the gas chromatographic mass-spectrum generated by BKGN007 (*B. sonorensis*) culture media were analyzed (Figures 7 and 8). The peak

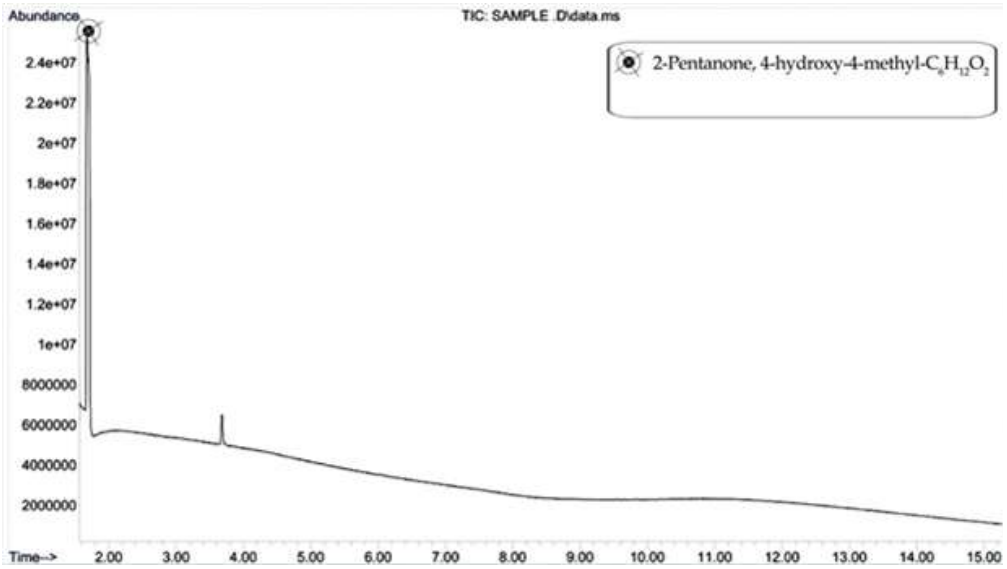

Figure 3. Gas chromatographic patterns of purified *Pseudomonas stutzeri* extracts obtained from the liquid culture.

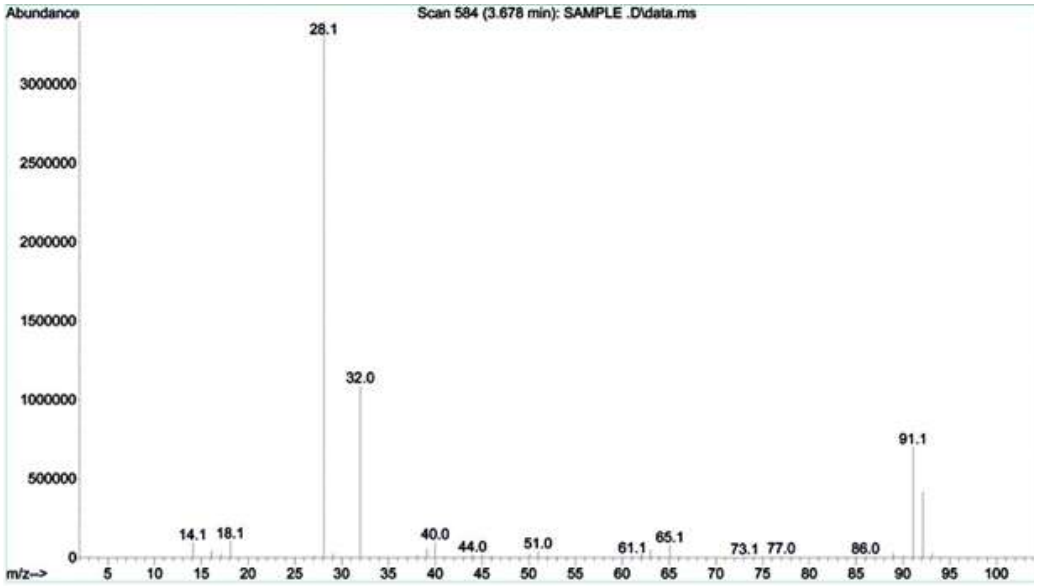

Figure 4. Mass-spectrum generated by pattern of *Pseudomonas stutzeri* extracts obtained from the mliquid culture.

at retention time of 11.6 min (2-hydroxy-4-hydroxylaminopyrimidine) and 12.86 min (phosphoric acid and dimethyl 1-methylpropenyl ester) corresponded to control sample. This peak disappeared concomitantly with the formation of another new peak through a retention time of around 1.495 min. The results demonstrated that *B. sonorensis* is a potent and easily acquired bioremediation agent that could be used to remove other pollutants from pesticide contaminated soils. The new peak with a retention time of around 1.495 min indicates the trimethylene oxide C_3H_6O and hydrazine H_4N_2. Hydrazine is a convenient reductant because the by-products are typically nitrogen gas and water. Thus, it is used as an antioxidant and an oxygen scavenger. After 45th day of incubation, the gas chromatographic mass-spectrum generated by mixed

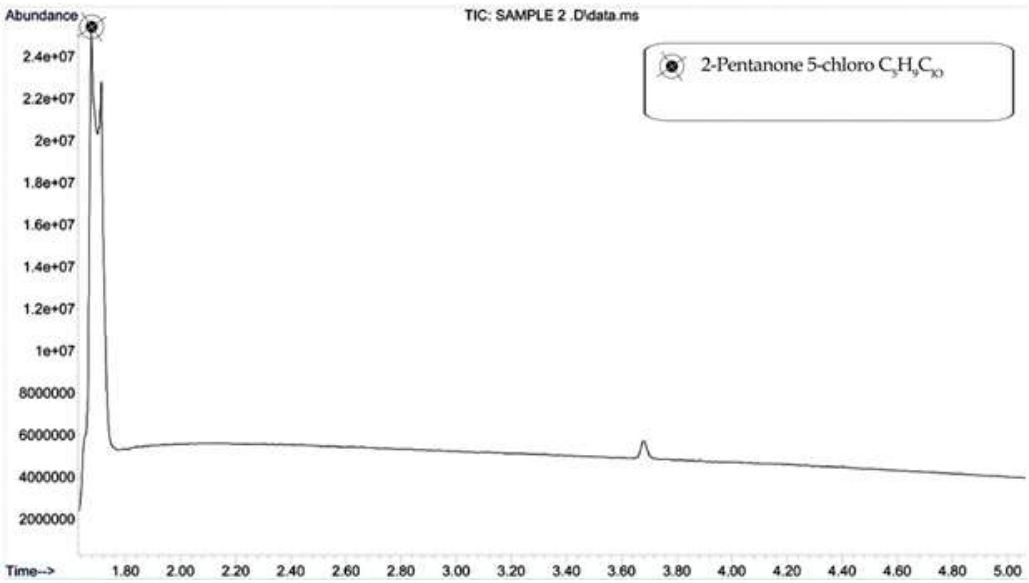

Figure 5. Gas chromatographic patterns of purified *Bacillus licheniformis* extracts obtained from the liquid culture.

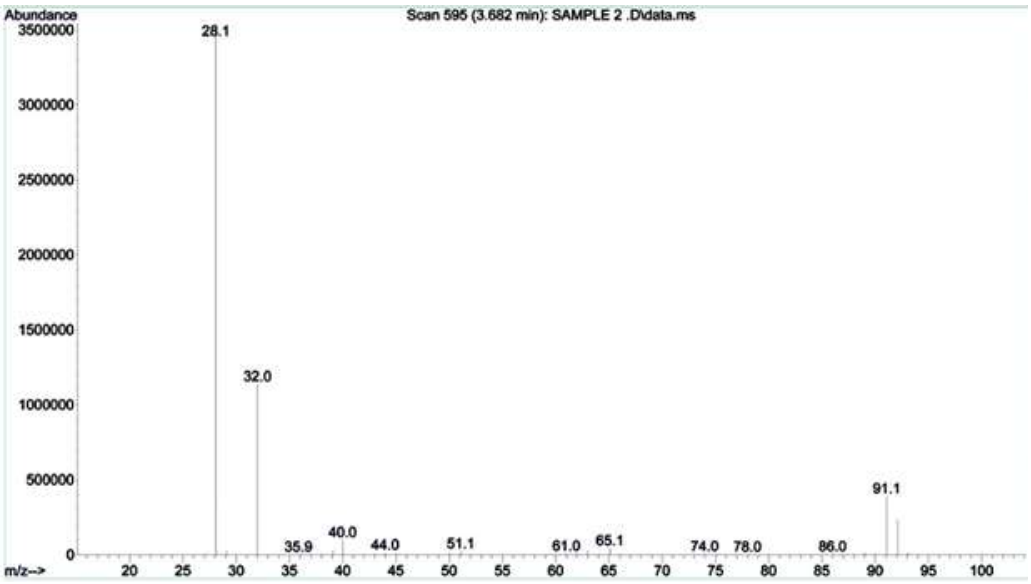

Figure 6. Mass-spectrum generated by patterns of *Bacillus licheniformis* extracts obtained from the liquid culture.

consortium culture media were analyzed (Figure 9 and 10). The peak at retention time of 11.6 min (2-hydroxy-4-hydroxylaminopyrimidine) and 12.86 min (phosphoric acid and dimethyl 1-methylpropenyl ester) corresponded to control sample, this peak disappeared concomitantly with the formation of another new peak through a retention time of around 3.60 min (Mishra et al., 2017; Gilani et al., 2010). The new peak with a retention time of around 3.60 min indicates the carbonic dihydrazide CH_6N_4O. The result presented good results for the microbial degradation of monocrotophos. Carbohydrazide is the chemical compound with the formula $OC(N_2H_3)_2$. It is a white and water-soluble solid. It decomposes upon melting. A number of carbazides are known where one or more N-H groups are replaced by other substituents. The accumulation of the products was not found by the

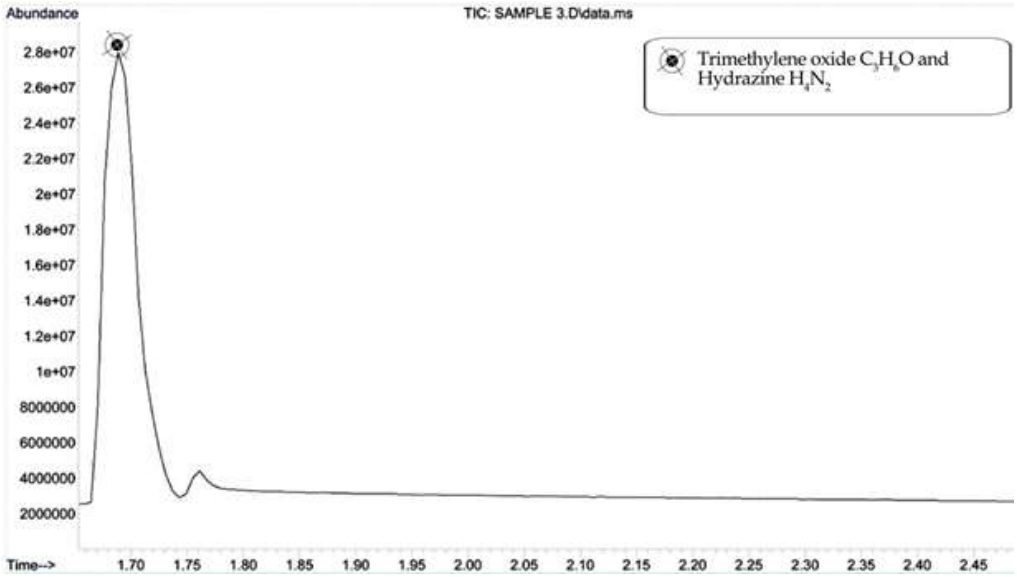

Figure 7. Gas chromatographic patterns of purified *Bacillus sonorensis* extracts obtained from the liquid culture.

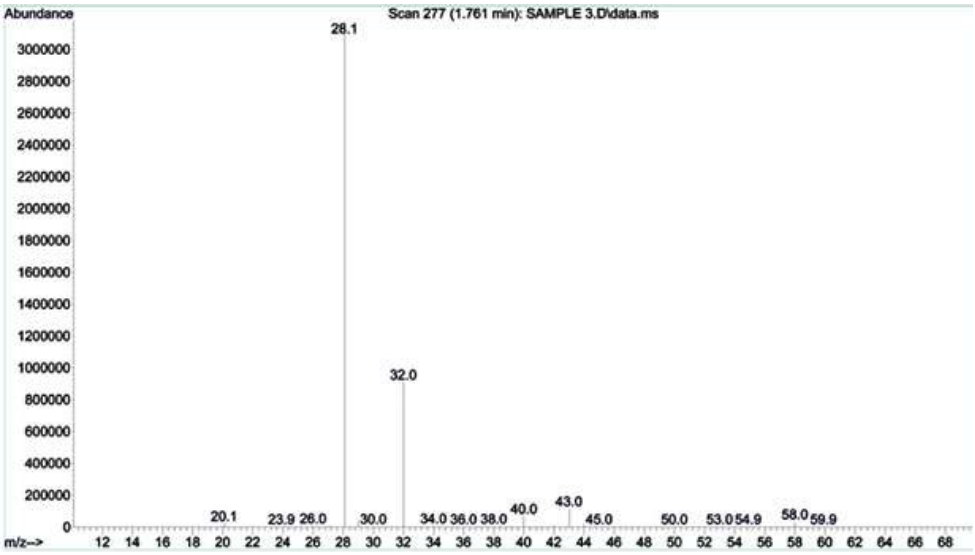

Figure 8. Mass-spectrum generated by patterns of *Bacillus sonorensis* extracts obtained from the liquid culture.

determination after incubation for 45 days. The degradation products were similar to those observed in previous studies (John et al., 2004; Horsfield et al., 2008; Singh et al., 2003). They occur widely in the drugs, herbicides, plant growth regulators, and dyestuffs. GC-MS analysis showed that consortium culture has more capacity to degrade the MCP than other isolated microorganisms. The ester metabolite is detected by GC-MS analysis; it is eco- friendly for soil environment. However, in the present study, degradation of organophosphorus pesticide was obtained and this could be the reasoned why bacterial strains consortium express the pesticide degrading enzymes (Singh et al., 2009).

In the present study, total DNA was extracted according to the procedures described by M. A. Akhter and R. Laz, (2013). The isolated chromosomal DNA was 1500 bp which compared with 1kb + DNA marker (Figure 11). The RFLPs of 16S rRNA were determined using

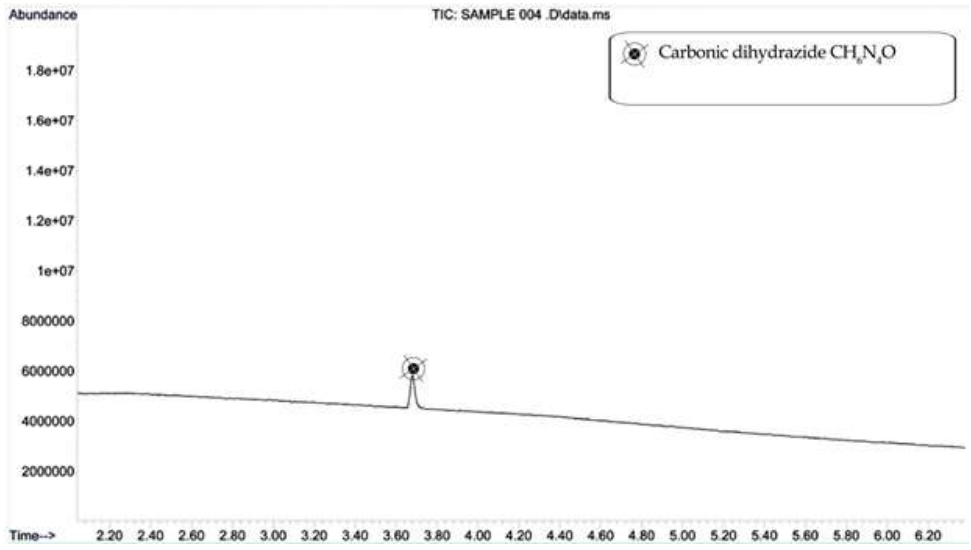

Figure 9. Gas chromatographic patterns of purified consortium extracts obtained from the liquid culture.

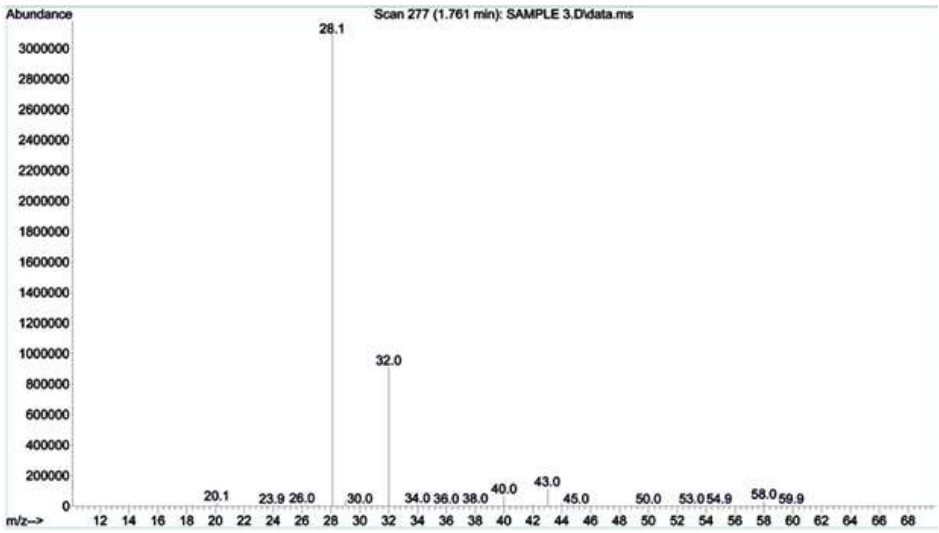

Figure 10. Mass-spectrum generated by patterns of consortium extracts obtained from the liquid culture.

three restriction enzymes, ALU1, SAU3A1 and HAE. PCR-based RFLP analysis of 16S rRNAs was performed. The genetic diversity assessment by RFLP shows genetic similarity between the isolates which were varied (Figure 11). *Pseudomonas* and *Bacillus* species have been phylogenetically analyzed by using 16S rRNA gene sequences (Bhadbhade et al., 2002).

The distinct RFLP pattern of the 3 strains could be due to increased mutation under stress, facilitation adaptation of the strains to stressful environments and also due to the different types of pesticide contamination and varying age of contamination at the sites. Phylogenetic analysis was done on the basis of partial 16S rRNA sequences (600 nucleotides) of 3 isolates. The 3 strains were grouped into two clusters, the blast similarity search and phylogenetic analysis of 16S rRNA sequence which revealed that, the isolated bacterial strain are closely similar to *Pseudomonas and Bacillus* groups (Figure 12). Dendrogram analysis Illustrate the relationship between strains of *P. stutzeri* KY287931, *B. licheniforms* KY287928 and *B. sonorensis* KY287930 bacteria. Based on the results of physiological, biochemical and molecular

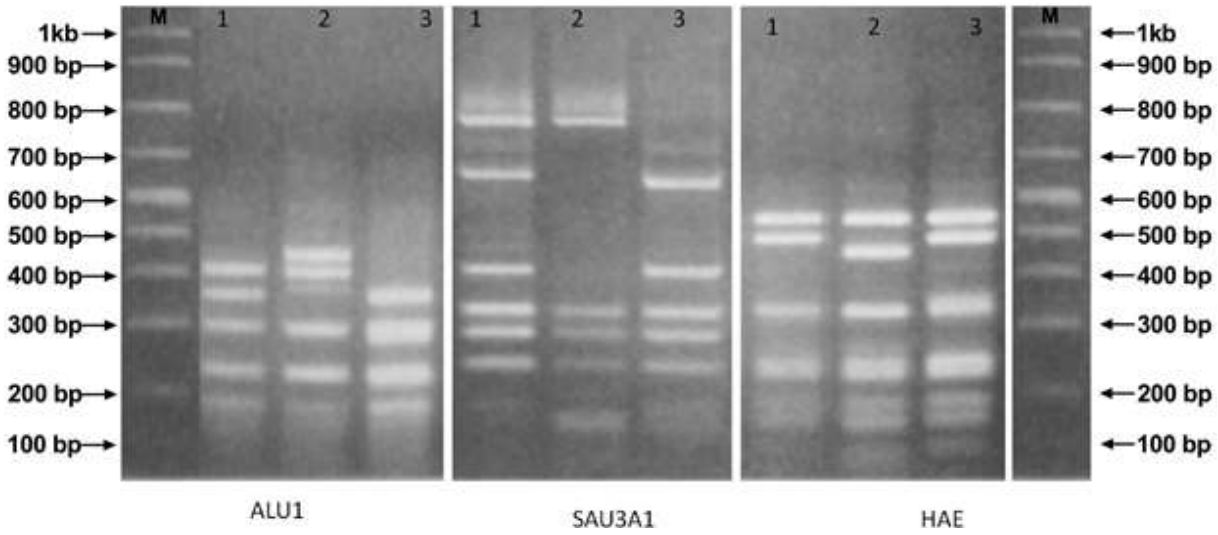

Figure 11. Restriction Fragment Length Polymorphism of 16S rRNA gene clusters digested with ALU1, SAU3A1 and HAE. Lane M: Marker is 100-bp DNA ladder. Lane Number 1: *Pseudomonas stutzeri* (KY287931), Lane Number 2: *Bacillus licheniformis* (KY287928) and Lane Number 3: *Bacillus sonorensis* (KY287930).

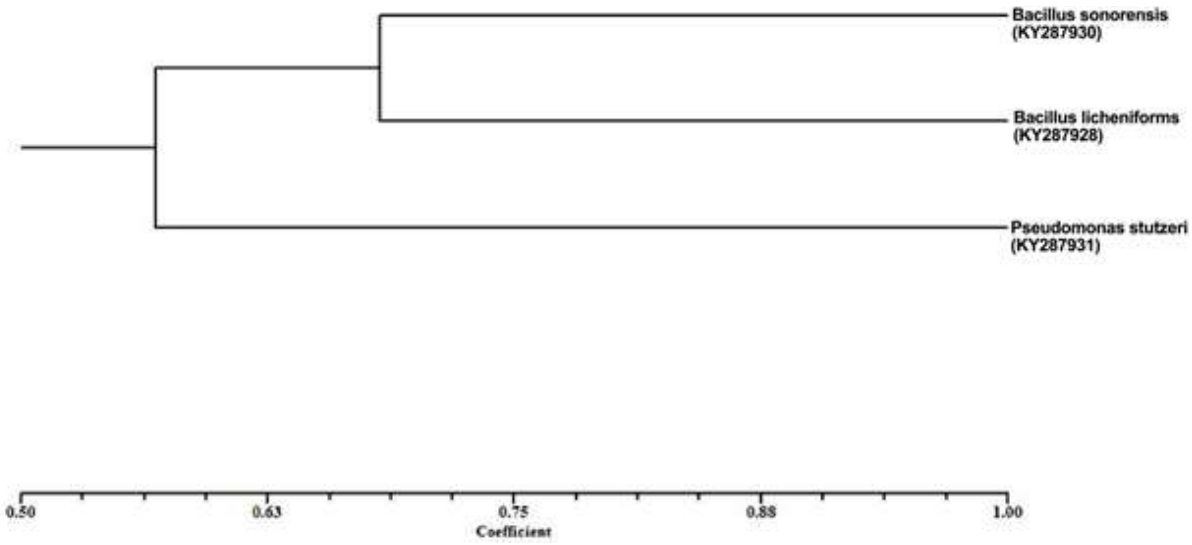

Figure 12. Dendrogram illustrated genetic relatedness between bacterial isolates, generated by UPGMA cluster analysis.

analysis, the isolates were designated. At present, more than 20 isolates of MCP-degrading strains have been reported and yielded a variety of microorganisms.

Conclusion

With this increased awareness, research has recently focused to the fate of pesticides in soils and health risk due to their potential transfer and accumulation in plants. Our study reveals that isolated bacterial strains growing in contaminated sites are able to with-stand high levels of monocrotophos in the soil. The consortium culture shows increased degradation activity in the MCP contaminated soil as compared to individual cultures.

MCP was highly adsorbed by the studied isolates. The bacterial samples were identified and confirmed as *P. stutzeri* KY287931, *B. licheniforms* KY287928, and *B. sonorensis* KY287930 through Restriction Fragment Length Polymorphism of 16S rRNA gene clusters.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are grateful to the Management and the Principal of J.J College of Arts & Science (A), Namunasamudram, Sivapuram, Pudukkottai, Tamilnadu, India for providing all the necessary facilities and encouragement. We would like to acknowledge the helpful and extensive recommendations of anonymous reviewers made to this paper. Dr. N. Thajuddin wish to acknowledge financial support from UGC-BSR One Time Grant (Ref.F.19-156/2015 (BSR) Oct.2015.

REFERENCES

Acharya KP, Shilpkar P, Shah MC, Chellapandi P (2015). Biodegradation of insecticide monocrotophos by *Bacillus subtilis* KPA-1, isolated from agriculture soils. Appl. Biochem. Biotechnol. 175(4):1789-804.

Akhter MA, Laz R (2013). Isolation and Molecular Characterization of Pesticide (Fenitrothion) Resistant Bacteria from Agricultural Field, IOSR J. Pharm. (e)-ISSN:2250-3013.

Aleem A, Malik A (2003). Determination of organochlorine pesticides in agricultural soil with special reference to γ-HCH degradation by Pseudomonas strains. Biores. Technol. 88(1):41-46.

Allman E, Degnan J, and Rhodes J (2017). Species tree inference from gene splits by unrooted star methods. IEEE/ACM Transactions on Computational Biology and Bioinformatics 99:1-1.

Anizio M, Faria Carol H, Collins and Isabel CSF, Jardim (2009). State-of-the-Art in Immobilized Polymer Stationary Phases for High-Performance Liquid Chromatography. J. Braz. Chem. Soc. 20(8):1385-1398.

Archibald J, Roger A (2002). Gene conversion and the evolution of euryarchaeal chaperonins: a maximum likelihood– based method for detecting conflicting phylogenetic signals. J. Mol. Evol. 55:232-245.

Baba Uqab, Syeed Mudasir, Ruqeya Nazir (2016).Review on Bioremediation of Pesticides. J. Bioremediat. Biodegrad.7:3.

Bhadbhade BJ, Sarnaik SS, Kanekar PP (2002). Biomineralization of an organophosphorus pesticide, Monocrotophos, by soil bacteria. J. Appl. Microbiol. 93:224-234.

Buvaneswari G, Thenmozhi R, Nagasathya A and Thajuddin N (2017). Screening of Efficient Monocrotophos Degrading Bacterial Isolates from Paddy Field Soil of Sivagangai District, Tamil Nadu, India. J. Environ. Sci. Technol. ISSN 1994-7887.

Castresana J (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Molec. Biol. Evol. 17:540-552.

Chen M, Xu P, Zeng G, Yang C, Huang D, and Zhang J (2015). Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting, Biotech. Adv. 33:745-755.

Daniel G Gibson, Lei Young, Ray-Yuan Chuang, Craig Venter J, Clyde A Hutchison and Hamilton O Smith (2009). Enzymatic assembly of DNA molecules up to several hundred kilobases. Nat. Methods 6:343-345.

Dastager SG, Deepa CK, and Pandey A (2011). Potential plant growth promoting activity of Serratia nematodiphila NII- 0928 on black pepper. World J. Microbiol. Biotechnol. 27:259-265.

Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM (2008). Phylogeny. fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36(suppl_2):W465-W469.

Gilani STS, Ageen M, Shah H and Raza S (2010). chlorpyrifos degradation in soil and its effect on soil microorganisms. J. Animal Plant Sci. Page:99-102. ISSN:1018-7081.

Henrik BR (2012). Restriction fragment length polymorphism analysis of PCR-amplified fragments (PCR-RFLP) and gel electrophoresis-valuable tool for genotyping and genetic fingerprinting. InGel Electrophoresis-Principles and Basics. InTech. pp.315-334, ISBN 978-953-51-0458-2.

Hill TCJ, Walsh KA, Harris JA, and Moffett BF (2003). Using ecological diversity measures with bacterial communities. FEMS Microbiol. 43:1-11.

Horsfield A, Sallam MN, Drummond FA, Williams DJ, Schultz RJ (2008). Role of climatic factors on damage incidence by Dermolepida albohirtum (Coleoptera: Scarabaeidae), in Burdekin sugarcane fields, Australia. J. Econ. Entomol.101 (2):334-40.

Jayanthi A, Sivagnanam S, Peter L (2014), Simultaneous degradation of organophosphorus and organochlorine pesticides by bacterial consortium. J. Taiwan Institute Chem. Eng., pp. 2590-2596.

John L, Fostera Bia R, KwanaTonyVancovb H (2004). Microbial degradation of the organophosphate pesticide. Ethion 240(1):49-53.

John TC (2014). Basic Molecular Protocols in Neuroscience: Tips, Tricks, and Pitfalls. Academic Press ISBN: 9780128014615.

Liu YH, Chung YC, Xiong Y (2001) Purification and characterization of adimethoate-degrading enzyme of Aspergillus niger ZHY256, isolated from sewage. Appl. Environ. Microbiol. 67:3746-3749.

Lori S, Eggert Jesus E, Maldonado Robert , Fleischer C (2005). Nucleic acid isolation from ecological samples animal scat and other associated materials. Methods Enzymol. pp. 73-82.

Mishra A, Jamaluddin and Pandey AK (2017).A Review on Microbial Degradation of Organophosphorous Pesticide: Methyl Parathion, Austin J. Biotechnol. Bioeng. 4(1):1074.

Parthiban K, Vignesh V, Nilmini V, Thirumurugan R (2015). A statistical approach on optimization of exopolymeric substance production by Halomonas sp. S19 and its emulsification activity. Bioresour. Bioprocess. 2:48.

Sasano R, Hamada T, Kurano M, Furuno (2000). On-line coupling of solid-phase extraction to gas chromatography with fast solvent vaporization and concentration in an open injector liner. Analysis of pesticides in aqueous samples. J. Chromatogr. 896:41- 49.

Seema BS, Sayyed RZ, Trivedi MH, Gobi TA (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus. 2(1):587.

Singh BK, Walker A (2006). Microbial degradation of organophosphorus compounds. FEMS Microbiol. Rev. 30:428-71.

Singh BK, Walker A, Morgan AW, Wright DJ (2003). Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl. Environ. Microbiol. 69:5198-5206.

Singh P, Dhanjal S, Singh KV, Suri CR, Cameotra SS (2009). Monitoring atrazine uptake by a new fluorometric assay. Current Sci. 25:268-271.

Singh P, Suri CR, Cameotra SS (2004). Isolation of a member of Acinetobacter species involved in atrazine degradation. Biochemical and biophysical research communications. 317(3):697-702.

Strong PJ, Burgess JE (2008). Treatment methods for wine-related and distillery wastewaters review. Bioremedy J. 12:70-87.

Wasim Aktar Md, Dwaipayan Sengupta, and Ashim Chowdhury (2009). Impact of pesticides use in agriculture their benefits and hazards. Interdiscip Toxicol. 1-12.

Zhao, Suli, Zhai, Andy (2012). A blind study of pesticides in vegetables by agilent bond elut quechers extraction kits and agilent 5975T LTM GC/MSD, Agilent Technologies, Inc. Publication number 5990-6323EN.

Zrostlikova J, Hajslova J, Cajka T (2003). Evaluation of two dimensional gas chromatography-time-of-flight mass spectrometry for the determination of multiple pesticide residues in fruit. J. Chromatogr. 1019:173-186.

academicJournals

Vol. 12(3), pp. 62-67, 21 January, 2018
DOI: 10.5897/AJMR2017.8757
Article Number: 835323055830
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Antibiotic susceptibility and serotype patterns of *Pseudomonas aeruginosa* from clinical isolates in Abidjan, Cote d'Ivoire

Clarisse Kouamé Elogne[1*], Julien Coulibaly Kalpy[1], Alain Yéo[1], Nathalie Guessennd[1], Jean Claude Anné[1], Tatianah Kangah Ngoran[1], Sofia Okpo Boyou[1], Adèle Kacou N'douba [2] and Mireille Dosso[1]

[1]Department of Bacteriology and Virology, Institut Pasteur de Côte d'Ivoire, 01 BP 490, Abidjan 01, Côte d'Ivoire.
[2]Laboratoire de bactériologie, Centre hospitalier universitaire de Cocody, 01 BP.V 13 Abidjan 01, Côte d'Ivoire.

Received 6 November, 2017; Accepted 14 December, 2017

Pseudomonas aeruginosa* (*P. aeruginosa*) has been found to be a common hospital acquired pathogen, responsible for several severe infections. The objective of this study was to describe the antibiotic resistance profile of *P. aeruginosa* strains from human sample. This descriptive study was carried out on 168 isolated strains of *P. aeruginosa* collected from January 2014 to December 2015 at the Pasteur Institute of Côte d'Ivoire. The strains identification was done according to the methods of conventional bacteriology. The antibiotic sensitivity tests were performed using the disc diffusion method in agar medium according to CA-SFM (Antibiogram committee of French society of microbiology) criteria. The serotyping of the strains was carried out by using the agglutination method slide test, with the aid of 4 specific polyvalent antisera. The most prevalent *P. aeruginosa* serotypes were O4 (24.4); O11 (14.6); and O6 (9.5%). The rate of antibiotic resistance to ticarcillin was 32.9%, ciprofloxacin 18.4%, ceftazidime 14.9%, Imipenem 11.3%, and amikacin 11.3%. Resistance to Imipenem was above 10% in an intensive care unit and in the pneumonology unit (PPH). Strains of O6 serotypes were the most multidrug-resistant followed by O11 with respective rates of 31.2 and 28% MDR (Multidrug Resistance). *P. aeruginsa* are microorganism capable of developing mechanisms of complex resistance which makes it difficult to manage. The attention of hygiene rules and the rational use of antibiotics are very important in other to prevent the spread of MDR *P. aeruginosa*.

Key words: *Pseudomonas aeruginosa*, serotype, multidrug-resistance, infection.

INTRODUCTION

Pseudomonas aeruginosa has become a major hospital acquired pathogen, responsible for several severe infections (Bertrand et al., 2013). Due to its ability to use different organic compounds as energy substrates,

*Corresponding author. E-mail: nzolecla@yahoo.fr. Tel: 225 01017548.

this strictly aerobic gram-negative bacillus lives in very humid environments (Fuentefria et al., 2011).

Hospital environment is suitable for this micro-organism because it contains numerous ecological niches (Bertrou et al., 2000). The intestinal carriage of the pyocyanic is rare in healthy subjects, but frequent among hospitalized patients, particularly in intensive care unit (Bertrand et al., 2013). Due to its natural resistance to many antibiotics and its ability to build up new resistance, the hospital environment favors the growth of *P. aeruginosa* despite multiple antibiotic selection pressure.

The emergence of multidrug-resistant strains of *P. aeruginosa* in hospital is often associated with some serotypes (Thrane et al., 2015). In order to find an epidemiological link between the strains of *P. aeruginosa* isolated in hospital, phenotypic markers such as antibiotype and serotype are used to differentiate strains before genetic characterization (Blot et al., 2013; Wolska et al., 2012). There is paucity of data on *P.aeruginosa* infections in Côted' Ivoire. The aim of this study was to evaluate the antibiotic resistance profile of *P. aeruginosa* and to compare these profiles with serotypes of isolated strains.

MATERIALS AND METHODS

This descriptive study was carried out on strains *P. aeruginosa,* isolated and collected from January 2014 to December 2015 at the Clinical Bacteriology Unit (CBU) of the Department of Bacteriology-Virology of Pasteur Institute of Côte d'Ivoire. The variables taken into account were: the origin of the strains, type of services, nature of the samples, antibiotic profile and the type of the serotype. Duplicates were not considered.

Strains of *P. aeruginosa*

A total of 168 strains of *P. aeruginosa* were isolated from various biological fluids: pus (87), sputum (320), urine (16), blood (13), material (13), Cerebrospinal fluid (6) and stools (1). Strains isolated from other media were examined for growth and pigmentation on *Pseudomonas* isolation agar. The strains were identified based on the standard bacteriological characteristics: appearance of colonies on King A and King B medium, presence for pyocyanine and pyoverdine, growth at 42°C, morphological appearance (gram negative bacillus, polar mobile), the positive oxidase reaction and other biochemical characteristics using the API 20 NE gallery (Bio-Mérieux®, France) according to Breed et al. (2000) and Garrity et al. (2005). The strains were stored in deep agar at room temperature.

Study of antibiotic sensitivity

Antibiotic susceptibility tests were performed using Kirby-Bauer disc diffusion method in agar medium (Kirby-Bauer, 1996). The reading and interpretation were done according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM 2015).

The following antibiotics were tested: ticarcillin (75 µg), ticarcillin + clavulanic acid (75/10 µg), ceftazidime (30 µg), imipenem (10 µg), amikacin (30 µg) and ciprofloxacin (5 µg). The internal quality control was carried out using the reference strain *P. aeruginosa* ATCC 27853.

Serotyping

The serotyping of the strains was performed using the slide agglutination technique with the aid of 4 polyvalent specific antisera PMA (O1 + O3 + O4 + O6), PMC (O9 + O10 + O13 + O14), PME (O5 + O15 + O16), PMF (O7 + O8 + O11 + O12) (Biorad ®) followed by specific monovalent antisera (Biorad®).

RESULTS

P. aeruginosa were isolated from different biological fluid at variable frequencies, pus (51.8%), followed by pulmonary secretions (19%), urine (9.5%), blood (7.7%), Invasive material (7.7%) and cerebrospinal fluid (3.6%).

Overall, the most prevalent serotypes were O4 (24.4%), O11 (14.6%), and O6 (9.5%). O4 serotype was most frequent in pus with 28%, pulmonary secretions (31%) and urine (30.7%). In urine and hospital materials, O6 and O11 serotypes were the most commonly found (Table 1).

In Table 2, the rate of sensitivity of 168 strains of *P. aeruginosa* to different antibiotics tested is summarized. The proportion of resistant strains was high for ticarcillin (34.5%) and ticarcillin-clavulanic acid (31.5%). The rate of strains resistant to cetazidime was 14.9 and 18.4% for ciprofloxacin. The resistance rate to Imipenem was 11.3%. Distribution of resistant strains according to their isolation origin showed a higher proportion from hospital strains compared to isolates from non-hospitalized patients. Among hospital services, the highest rates of resistance were observed in pneumonology unit and neurology for ticarcillin (50 and 58.3%, respectively) and ticarcillin-clavulanic acid (43.3 and 50%, respectively). In Intensive care unit, 28.6% is from resistant strain to imipenem. About 23% of strains isolated from pneumonology were resistant to ciprofloxacin (Table 3).

Serotypes O6, O11 and O2 were most associated with resistance to antibiotics. Serotype O6 strain has resistance rate of ticarcillin 62.5%, imipenem 31.2% and ciprofloxacin 37.4% to ceftazidime. For serotype O11, frequent association was observed with ticarcillin resitance (44%), ciprofloxacin resistance (28%) and piperacillin resitance (20%). For serotype O2, the predominant resistance was ticarcillin (35.7%), ceftazidime (21.4%) and imipenem (21.4%) (Table 4).

Multiresitance is associated with serotypes O11 (30.8%), O6 (19.2%), O16 (11.5%) and O2 (11.5%). The most common phenotypes were associated to ticarcallin, ceftazidime and ciprofloxacin resistance (Table 5).

DISCUSSION

P. aeruginosa is generally isolated from suppurative

Table 1. Distribution of serotypes of *Pseudomonas aeruginosa* according to the nature of the biological fluid collected at the Clinical Bacteriology Unit, 2014-2015.

Variable	Serotypes {No. (%)}														
Biological fluid	O1	O2	O3	O4	O5	O6	O7	O9	O10	O11	O12	O14	O15	O16	Not typable
Pus (n=87)	6 (5.2)	9 (7.8)	8 (6.9)	24 (20.9)	7 (6.1)	7 (6.1)	0	1 (0.9)	3 (3.4)	13 (14.9)	1 (0.9)	0	1 (0.9)	6 (6.9)	1 (0.9)
Pleuropulmo Naryfluid (n =32)	1 (3.1)	3 (9.3)	1 (3.1)	10 (31.2)	1 (3.1)	3 (9.3)	0	1 (3.1)	1 (3.1)	4 (12.4)	1 (3.1)	1 (3.1)	2 (6.2)	3 (9.3)	0
Urines (n=16)	1 (6.2)	0	1 (6.2)	2 (12.5)	1 (6.2)	4 (25)	1 (6.2)	0	3 (18.7)	2 (12.5)	0	0	1 (6.2)	0	0
Blood (n=13)	2 (15.4)	0	0	4 (30.7)	2 (15.4)	1 (7.7)	0	0	0	2 ( )	1 (7.7)	0	1 (7.7)	0	0
Invasive materials (n=13)	0	1 (7.7)	2 (15.4)	1 (7.7)	0	0	0	0	0	3 (23)	1 (7.7)	0	1 (7.7)	2 (15.4)	2 (15.4)
Cerebrospinal fluid (n=6)	1 (16.7)	1 (16.7)	0	0	0	0	0	0	2 (33.3)	1 (16.7)	0	0	1 (16.7)	0	0
Stools (n=1)	0	0	0	0	0	1(100)	0	0	0	0	0	0	0	0	0
Total (%) N=168	11 (6.5)	14 (8.4)	12 (7.1)	41 (24,6)	11 (6.5)	16(9.5)	1 (0,6)	2 (1.2)	9 (5.4)	25 (14.9)	4 (2.4)	1 (0.6)	7 (4.2)	11 (6.5)	3 (1.8)

Serotype O13 and O8 were not found.

collections as reported by Ben Abdallah et al. (2008) with the rate of 52.9% closer to 51.8%. The presence in other biological fluid is reported with variable proportions 19% in pleuropulmonary secretions and, 7.5% in urine and blood, respectively.

Authors reported 37.2% pus, 19.9% lungs and 30.7% urine (Mindolli et al., 2015) and the rate of 38.5% urine, 8.3% blood and 3.1% catheter (Kiana et al., 2016). The contamination of wounds by this bacterium is usual, however the use of invasive materials in particular the urinary catheters and catheters of intubation could explain their presence in a pleuropulmonary secretions and urines. The rates of *P. aeruginosa* isolated from hospitalized patients (47.6%) vs. outpatients (52.4%) could be a bias in recruitment, where the previous idea of hospitalization and invasive gestures are not well informed. In Morocco in 2002, the rate was 64.5% among inpatients (Louzi et al., 2003) while in Nigeria in 2012, it was 70% from hospitalized patients (Iregbu et al., 2015). The hospital service most frequently associated is the intensive care unit as reported by several authors (Tacconeli et al., 2002 ; Goncalves et al.,2017). Other services such as pneumology and neurology are also at-risk (Iregbu et al., 2015).

The serotypes of *P. aeruginosa* constitute epidemiological markers with the rate of 24.4, 14.9 and 9.5% for serotypes O4, O11 and O6, respectively. In Tunisia, a study carried out in 2012 reported the same serotypes with variable rates predominance of serotypes O11, followed by O6 and O4 (Zoghlami et al., 2012). Serotype O4 was predominant in prolong hospitalization units (19.8%) (Adjerald et al., 1999).

Regarding the resistance to antibiotics, the higher rates were recorded for Ticarcillin (34.5%), ciprofloxacin (15.5%) and ceftazidime (14.9%). In Algeria, similar rates were observed for ticarcillin (32.7%), ciprofloxacin (19.4%) and ceftazidime (14.7%) (Sefraoui, 2015) while In Nigeria in 2015, higher rates were reported for ceftazidime (46%) and ciprofloxacin (34%) (Iregbu et al., 2015). In Côte d'Ivoire, a study carried out in 2013 on hospital effluents showed very high rates of resistance to ceftazidime and ticarcillin, resistant strains of 100% (Guessennd et al., 2013). Amikacin and imipenem were the most active antimicrobial agent on *P. aeruginosa* isolates with resistance rates of 7.1 and 10.7%, respectively. Nigeria resistance rates were 13% for imipenem and 23% for amikacin (Iregbu et al., 2015).

In Côte d'Ivoire in 2008, *P. aeruginosa* isolated from infections which originate from surgical operation site shown a sensitivity of 98.5% to an imipenem, that is 1.5% resistance with lower rate (Faye-Kette et al., 2008) . A study carried out in 2013 on hospital effluents showed very high rates of resistance to imipemen with 80% of resistant strains (Guessennd et al., 2013).In Teheran, higher rate were found for imipenem (41.3%) and amikacin (28%) (Peymani et al., 2017).

The highest resistances were observed in strains isolated from the department of pulmonology with 50% resistance for ticarcillin, 23.3% for ciprofloxacin and 20% for ceftazidime. This observation is confirmed by Hamze et al. (2013) in Lebanon.

The serotypes O11 (30.8%) and O6 (19.2%) presented more multiresistant strains. Association of serotypes and multiresistance to antibiotics

Table 2. Antibiotic sensitivity of 168 strains of *Pseudomonas aeruginosa* tested at Clinical Bacteriology Unit, 2014-2015.

Antibiotics	S (%)	I(%)	R(%)	I+R(%)
Ticarcilline	110 (65.5)	0	58 (34.5)	58(34.5)
Ticarcilline+ clavulanic acid	115 (68.5)	0	53 (31.5)	53 (31.5)
Piperacillin	144 (85.7)	0	24 (14.3)	24 (14.3)
Imipenem	149 (88.7)	1	18(10.7)	19 (11.3)
Ceftazidim	143 (85.11)	0	25 (14.9)	25 (14.9)
Amikacin	147 (87.5)	9 (5,3)	12 (7.1)	21 (12.5)
Ciprofloxacin	137 (7.7)	5 (3)	26 (15.5)	31 (18.4)

S= Susceptible; R= Resistant; I= intermediate.

Table 3. Distribution of *Pseudomonas aeruginosa* resistant strains to antibiotics, isolated from the Clinical Bacteriology Unit, according to originating units, 2014-2015.

Resistance	TIC(%)	TCC(%)	PIP(%)	IMP(%)	CAZ(%)	AN(%)	CIP(%)
Outpatient (n=88)	29(32.9)	26(29.5)	12(13.6)	9(10.2)	12(13.6)	11(12.5)	16(18.2)
Hospitalized (n=80)	-	-	-	-	-	-	-
Operating Theatre (n=9)	2(22.2)	2(22.2)	1(11.1)	0	1(11.1)	0	1(11.1)
ORL (n=3)	1(33.3)	0	0	0	0	1(33.3)	1(33.3)
Neonatal.Pediatrics (n=14)	3(21.1)	2(14.3)	0	0	1(0.7)	0	1(0.7)
Neurology (n=12)	7(58.3)	6(50)	4(33.3)	2(16.7)	4(33.3)	0	0
PPH (n=30)	15(50)	13(43.3)	5(16.7)	5(16.7)	6(20)	0	7(23.3)
Intensive unit (n=7)	1(14.3)	1(14.3)	1(14.3)	2(28.6)	1(14.3)	0	0
Rhumatology (n=3)	1(33.3)	1(33.3)	0	0	0	0	0
Gynecology (n=1)	0	0	0	0	0	0	0
Hematology (n=1)	0	0	0	0	0	0	0

TIC= ticarcillin; PIP= piperacillin; CIP = ciprofloxacin; CAZ= ceftazidim, IMP= imipenem; AN= amikacin ;TCC=Ticarcilline+ acide clavulanic ; ORL= oto rhino laryngology; PPH= human pneumology.

Table 4. Distribution of the antibiotic resistance profile of *Pseudomonas aeruginosa* strains isolated in the Clinical Bacteriology Unit, according to serotype, 2014-2015.

Serotype	Resistance rates						
	PIP(%)	IMP(%)	TIC(%)	TCC(%)	AN(%)	CAZ(%)	CIP(%)
Non typable (n =3)	1(33.3)	0	2(66.6)	2(66.6)	0	1(33.3)	2(66.6)
O1 (n = 11)	2(18.2)	2(18.2)	2(18.2)	2(18.2)	0	1(9)	1(9)
O2 (n= 14)	0	3(21.4)	5(35.7)	4(28.6)	0	3(21.4)	2(14.3)
O3 (n = 12)	3(25)	1(8.3)	5(41.6)	3(25)	1(8.3)	2(16.6)	1(8.3)
O4 (n = 41)	5(12.2)	3(7.3)	9(21.9)	8(19.5)	1(2.4)	1(2.4)	4(9.7)
O5 (n = 11)	0	0	2(18.2)	1(9.1)	0	1(9.1)	0
O6 (n = 16)	5(31.2)	5(31.2)	10(62.5)	9(56.2)	3(18.7)	6(37.4)	5(31.2)
O7 (n = 1)	0	0	0	0	0	0	0
O9 (n = 2)	0	0	0	0	0	0	0
O10 (n = 9)	1(11.1)	0	3(33.3)	2(22.2)	2(22.2)	2(22.2)	0
O11 (n = 25)	4(25)	2(12.5)	11(44)	11(44)	5(20)	3(12)	7(28)
O12 (n = 4)	1(25)	0	1(25)	1(25)	0	1(25)	0
O14 (n = 1)	0	0	1(100)	1(100)	0	0	0
O15 (n = 7)	1(9.1)	1(9.1)	3(27.2)	3(27.2)	0	2(18,2)	1(9,1)
O16 (n = 11)	1(0.1)	1(0.1)	4(36.4)	5(45.4)	0	2(18.2)	3(27.2)
Total (n=168)	24	18	58	53	12	25	26

TIC= ticarcillin ; TCC=Ticarcilline+ acide clavulanic PIP= piperacillin ; CIP = ciprofloxacin ; CAZ= ceftazidim, IMP= imipenem; AN= amikacin.

Table 5. Distribution of serotypes of *Pseudomonas aeruginosa* according to the phenotype of multi-resistance to antibiotics, 2014-2015.

Phenotypes of the resistance (MDR)	O1	O2	O3	O4	O6	O10	O11	O15	O16	Not typable
ticR+ pipR+ cipR	0	0	0	0	0	0	0	0	1	0
ticR + cazR+ cipR	0	0	0	0	0	0	2	1	1	1
ticR + cazR+ impR	1	2	0	0	0	0	1	0	0	0
tccR+ cipR + AkR	0	0	0	1	0	0	5	0	0	0
caz R+cipR+ impR	0	0	1	0	2	0	0	0	1	0
tccR + cazR+ AkR	0	0	0	0	0	2	0	0	0	0
cazR + cipR+ impR+ AkR	0	0	0	0	3	0	0	0	0	0
Tcc R+ CazR + impR+ cipR	0	1	0	0	0	0	0	0	0	0
Total MDR N (%)	1(3.8)	3(11.5)	1(3.8)	1(3.8)	5 (19.2)	2(7.7)	8 (30.8)	1(3.8)	3(11.5)	1(3.8)

R= resistant, ticR= ticarcillin Resistant; tccR=Ticarcilline+ clavulanic acid Resistant; pipR= piperacillin Resistant; cipR = ciprofloxacin Resistant ; cazR= ceftazidim Resistant ; impR= imipenem Resistant ; AkR= amikacin Resistant MDR= multi drug resistant.

was reported by several studies, as in the case with serotype O11 (Sardelic et al., 2012). Approximately, 32% of the multi-resistant strains of this study belonged to serotype O11. Similarly, in Greece, serogroups O12 and O11 had multiresistance rates of 91 and 79%, respectively (Panayotis et al., 1998), whereas in Japan in 2007, out of 214 strains of *P. aeruginosa* of the serotype O11, 212 (99%) were MDR (Jun-Ichiro et al., 2007).

Conclusion

This study showed the importance of serotyping of *P. aeruginosa* isolated from clinical sources. The circulation of serotypes O4, O11 and O6 of *P. aeruginosa* was frequent. They were predominant in suppurative and pleuro-pulmonary secretions. In addition, serotypes O11 and O6 were the most multiresistant. The most common phenotype of MDR is Ticarcillin-Ceftazidime-Ciprofloxacin. Knowledge of serotypes can guide the choice of antibiotic therapy in 24 h before sensitivity test results. In addition, this study should alert health professionals to an increasing rate of *P. aeruginosa* resistant to useful carbapenems and Fluoroquinolones.

Regular monitoring of the antimicrobial resistance profile is essential to guide prescribing antibiotics and controlling the emergence of MDR *P. aeruginosa* strains.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adjerald L, Cattoen C, Levent T, Grandbastien B, Descamps D, Bouillet L, Coignard B, Beaucaire G (1999). Réseau de microbiologistes de l'ARECLIN. Observatoire régional Pseudomonas aeruginosa du Nord-Pas-de-Calais: Données épidémiologiques et microbiologiques. *Med Mal Infect* ; 29(3):160-166.

Bertrou A, Chapuis C, Hajjar J (2000). Relation entre contamination et environnement hospitalier. In vigilance environnementale: Contrôles microbiologique de l'environnement hospitalier. Hygiène 8 (3):143-146.

Blot S, Koulenti D, Dimopoulos G, Martin C, Komnos A, Krueger WA, Spina G, Armaganidis A, Rello J (2013). Prevalence, risk factors, and mortality for ventilator-associated pneumonia in middle-aged, old, and very old critically Ill patients. Crit. Care Med. (2014) Mar; 42(3):601-9

Breed Rs, Murray EGD, Smith Nr (2000). Bergey's Manual of Determinative Bacteriology, seventh edition, the Williams & Wilkins Co, Baltimore, 67p.

Faye-Ketté H, Kouassi M. Y, Akoua-Koffi G, Bakayoko S, Boni-Cissé C, Diallo-Touré K, Dosso M, Lambin Y (2008). Epidémiologie microbienne des infections de sites opératoires dans un service de traumatologie à Abidjan et sensibilité des germes aux antibiotiques. Revue Bio-Africa - N°6, pp. 25-31.

Fuentefria DB, Ferreira AE, Corcao G (2011). Antibioticresistant Pseudomonas aeruginosa from hospital wastewater and superficial water: Are they genetically related. J Environ Manage. 92:250.

Garrity Gm, Bell Ja, Lilburn T. (2005). Bergey's Manual of Systematic Bacteriology, second edition, vol. 2 (The Proteobacteria), part B (The Grammaproteobacteria), Springer-Verlag, New York, 323p

Goncalves I R, Dantas RCC, Ferreira ML, Deivid , Batistão DWF, Gontijo-Filho PP, Ribas RM (2017). Carbapenem-resistant *Pseudomonas aeruginosa*: association with virulence genes and biofilm formation. Braz j. microbiol. 4(8):211-217.

Guessennd NK, Ouattara MB, Ouattara ND, Nevry RK, Gbonon V, Tiekoura KB, Dosso M (2013). Étude des

bactéries multi résistantes des effluents hospitaliers d'un centre hospitalier et universitaire (CHU) de la ville d'Abidjan (Côte d'Ivoire). J. Appl. Biosci. 69:5456- 5464.

Hamze M, Mallat H, Dabboussi F, Achkar M (2013). Antibiotic susceptibility and serotyping of clinical Pseudomonas aeruginosa isolates in northern lebanon. Int. Arab. J. Antimicrob. Agents 4(2):720.

Iregbu KC, Eze SO (2015). *Pseudomonas aeruginosa* infections in a tertiary hospital in Nigeria. Afr. J. Cln. Exper. Microbiol. 16(1):33-36.

Jun-Ichiro S, Tsukasa A, Miyoshi-Akiyama T, Atsushi K, Yukie M, Minako A, Tomoko F, Hideko K, Satoru S, Hajime W, Tadashi K,Hiroshi M, Keiji K, Hiroyuki K, Yoshihiro K, Mitsuo K, Hiroshi Y, Tadatoshi K and Teruo K (2007). Outbreaks of Multidrug-Resistant *Pseudomonas aeruginosa* in Community Hospitals in Japan. J. Clin. Microbiol. 45(3):979-989.

Kiana S, Behrouz A, Fardad (2016). Antibiotic resistance pattern and evaluation of metallo-beta lactamase genes (VIM and IMP) in *Pseudomonas aeruginosa* strains producing MBL enzyme, isolated from patients with secondary immunodeficiency. Adv. Biomed. Res. 5:124.

Kirby-Bauer A. (1996) Antimicrobial sensitivity testing by agar diffusion method. J. Clin. Pathol. 44:493.

Louzi L, Boughalem M, Charra B, Jana M. (2003) *Pseudomonas aeruginosa*: profils de resistance aux antibiotiques à propos de 62 souches. *J. magh. A. Réa*. 2001-2002 vol x-P. 191.

Mindolli PB, Salami MP (2015); Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* from clinical isolates at a tertiary care center of in jivaypur karna. J. Chem. Phama. Res. 7(8):186-190.

Tassios PT, Gennimata V, Maniatis AN, Fock C, Legakis NJ (1998). Greek Pseudomonas aeruginosa Study Group. Emergence of multidrug resistance in ubiquitous and dominant Pseudomonas aeruginosa serogroup O: 11. J. Clin. Microbiol. 36(4):897-901.

Peymani A, Naserpour-Farivar T, Zare E, Azarhoosh Kh. (2017). Distribution of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing P. aeruginosa isolated from Qazvin and Tehran hospitals, Iran. J. Prev. Med. Hyg. 58:E155-E160.

Mindolli PB, Salami MP (2015). Antimicrobial susceptibility pattern of *Pseudomonas aeruginosa* from clinical isolates at a tertiary care centre of in jivaypur karna. J. Chem. Phama. Res. 7(8):186-190.

Rana Barakat (2012). Etude des propriétés biologiques et antimicrobiennes de la pyocyanine, pigment redox-actif produit par *Pseudomonas aeruginosa*. Sciences agricoles. Université de La Rochelle, Disponible : https://tel.archives-ouvertes.fr/tel-00825873/document

Sanda S, Branka B, Colinon-Dupuich C, Orhanovic S, Zrinka B, Plecko V, Cournoyer B and Gian M.R (2012). Infrequent Finding of Metallo-β-Lactamase VIM-2 in Carbapenem-Resistant Pseudomonas aeruginosa Strains from Croatia. *Antimicrob Agents Chemother*; 56(5):2746-2749.

Sefraoui Imane Epse Khelil (2015). Etude de la résistance aux antibiotiques de *Pseudomonas aeruginosa* au niveau de différents hôpitaux de l'Ouest algérien. Thèse de doctorat en Biologie présentée et soutenue le 29 janvier 2015.

Tacconelli E, Tumbarello M, Bertagnolio S, Citton R, Spanu T, Fadda G (2002). Multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections: analysis of trends in prevalence and epidemiology. Emerg Infect Dis. 8(2):220-221.

Valerie A, Navon-Venezia S, Seigman-Igra Y, Shaltiel C , and Yehuda C (2006). Multidrug-Resistant *Pseudomonas aeruginosa*: Risk Factors and Clinical Impact. Antimicrob Agents Chemother. 50(1):43-48.

Xavier B, Céline S, Pascal C, Talon D (2011). Épidémiologie des infections à *Pseudomonas aeruginosa*. Revue Francophone des Laboratoires. 435:35-40.

Zoghlami A, Kanzari L, Boukadida J, Messadi AA, Ghanem A (2012). Epidemiological profile and antibiotic resistance of *Pseudomonas aeruginosa* isolates in burn and Traumatology center in Tunisia over a three-year period. Tunis Med. 90 (11):803-806.

Thrane SW, Taylor VL, Freschi L, Kukavica-Ibrulj I, Boyle B, Laroche J, Pirnay JP, Lévesque RC, Lam JS, Jelsbak L (2015). The widespread multidrug-resistant serotype O12 *Pseudomonas aeruginosa* clone emerged through concomitant horizontal transfer of serotype antigen and antibiotic resistance gene clusters. MBio. 6(5):e01396-15.

Wolska K, Kot B, Jakubczak A (2012). Phenotypic and genotypic diversity of Pseudomonas aeruginosa strains isolated from hospitals in Siedlce (Poland). Brazilian J. Microbiol. 43(1):274-282.


African Journal of
Microbiology Research
Related Journals Published by Academic Journals
African Journal of Biotechnology
African Journal of Biochemistry Research
Journal of Bacteriology Research
Journal of Evolutionary Biology Research
Journal of Yeast and Fungal Research
Journal of Brewing and Distilling
academicJournals