Journal of Global Biosciences
ISSN 2320-1355
Volume 9, Number 1, 2020, pp. 6718-6736
Website: www.mutagens.co.in
DOI: www.mutagens.co.in/jgb/vol.09/01/090103.pdf

SCAN ME

Research Paper

PRODUCTION OF SALICYLIC ACID BY A PURPLE NON SULFUR BACTERIUM *Rubrivivax gelatinosus* STRAIN RASN4 FROM RHIZOSPERIC SOIL OF PADDY FIELD

Debamalya Gupta and Sankar Narayan Sinha

Environmental Microbiology Research Laboratory,
Department of Botany,
University of Kalyani-741235, West Bengal,
India.

Abstract

A considerable numbers of bacteria produce salicylic acid as their exogenous metabolite secreted in microbial culture. This characteristic can be exploited as a substantial quality to fight against plant and crop pathogens as a potential phytopathogenic controlling factor in agricultural fields. Our work deals with the isolation, purification and identification of a purple non sulfur bacterium (PNSB), tentatively identified as *Rubrivivax gelatinosus* (strain RASN4) and evaluation of its potentiality for salicylic acid production (*in vitro)* as a substantial phytopatho-remidiatory quality against a wide range of agricultural crop and plant pathogens in crop fields beside of its other plant growth promoting rhizobacterial (PGPR) features in order to formulate a potential microbial biofertilizer. For the said purpose, this purple non sulfur bacterium (PNSB) *Rubrivivax gelatinosus* (strain RASN4) was first screened qualitatively followed by quantitative determination of bacterial salicylic acid production (*in vitro)* in order to confirm this assessment. The maximum level of salicylic acid production (*in vitro*) by this rice rhizospheric isolated PNSB strain RASN4 was noted as 27.3 mg/l^{-1} through its quantitative estimation.

Key words: Purple non sulfur bacterium, PNSB, Salicylic acid, *Rubrivivax gelatinosus*.

INTRODUCTION

Salicylic acid (2-hydroxy benzoic acid) is a plant growth regulatory phenolic phytohormone[1,2] that also surves as an intermediate precursor in pyochelin siderophore biosynthesis[3]. Salicylic acid is also reported to act as an endogenous siderophore showing siderophore like activity as evident in case of *Pseudomonas fluoresccens* [4] and *P. Cepacia* [4,5] consisting of a salicyl substituted Cysteinyl

peptide[5,6,7]. Like *Pseudomonas*, many genera of rhizobacteria can synthesize salicylic acid as an intermediate of their pyochelin siderophore biosynthesis [8].

Salicylic acid plays a significant role in plant defense response against the antagonistic plant pathogens showing aggression. It can influence the plant defense regulation against phytopathogens and insects through signaling of the plant defense response [9,10,11,12] and thereby providing protection to the plants against various infections caused by numerous plant pathogens[13,14,15]. Accumulation of salicylic acid is a necessary criteria for basal plant host defense responses including expression of defense related genes [16].

Salicylic acid shows it's antifungal activity in different ways like reducing hyphal growth & biomass and inhibiting spore germination in different plant pathogenic fungi[17]. It can also down regulate some virulence factors in *Pseudomonas aeruginosa* influencing total proteases and elastase activities in order to attenuate it's virulence on *Arabidopsis thaliana* and *Caenorhabditis elegance*.

Salicylic acid can induce local and systemic defense responses in plants on its exogenous application[18,19]. Salicylic acid can induce accumulation of pathogen related protein (PRP) in order to induce Systemic Acquired Resistance (SAR) against antagonistic plant pathogens showing aggression and plays an essential role for inducing SAR[20] when applied exogenously to the plants[2] to protect them from plant pathogenic disease attack indirectly facilitating improved plant growth[1]. *Pseudomonas fluorescens* can produce salicylic acid as their siderophore[2] that can induce SAR resistance in plants when treated with [2].

On the other hand, salicylic acid is also an essential signal elicitor for inducing Induced Systemic Resistance (ISR) in host plants[21] as evident in case of tobacco[4,22]. in raddish [23] and beans[24].

Apart from its pivotal role in plant defense response and inducing ISR and SAR in plants, salicylic acid has its potential influences on plant growth and development, photosynthesis, transportation, iron uptake and transport, inducing specific alteration in chloroplast structures and leaf anatomy[2]in addition to its significant effects on seed germination, establishment of seedlings, cell growth, respiration, gene expression in association with senescence, nodulation, fruit yield and responses to various biotic and

abiotic stresses[25].[26] established that damage of pea seedlings due to Cadmium (Cd) can be overcomed by the salicylic acid treatment. They also reported salicylic acid has its significant role in mitigating various biotic, abiotic and environmental stress mediated responses in plants.

Many genera of bacteria can synthesise salicylic acid[27,3,28,29]. Zhang *et.al*, (2002) [30] reported the level of salicylic acid in plants become highly increased when treated with *Bacillus pumilas*. Indiragandhi et.at, (2008)[31] reported *Acinetobacter sp, Pseudomonas sp*, and *Serratia sp*, have their potentiality for salicylic acid production. In present work, an investigation was undertaken to assess the phytopathoremidiatory biocontrol potentiality of isolated rice rhizospheric PNSB strain RASN4, later identified as *Rubrivivax gelatinosus*, through evaluating their capability of salicylic acid (SA) production, followed by its quantification (*in vitro*).

MATERIALS AND METHODS

Isolation Purification and Characterization of Purple Non Sulfur Bacterium (PNSB)

The soil samples were collected aseptically from the rhizospheric soil of the rice fields of Ramnagar (Latitude 22.82N; Longitude 87.80E) located in Hooghly district of west Bengal, India and also transported aseptically to the laboratory in sterilized containers immediately after sampling for further studies.

The enrichment of soil samples were done with modified Biebl and Pfenning medium (1981)[32] by following standard protocol as described by Madigan *et. al,* (1984)[33] and Ponsano et.al,(2002 & 2008)[34].The growth obtained this way was further enriched with modified RCVBNP medium following the general enrichment protocol as described by Madigan and Gest (1982)[35] without altering the other growth parameters and factors except pH(=6.8).

Isolation of purple non-sulfur bacteria (PNSB) was made on modified Biebl and Pfenning's (1981)[32] agar medium under anaerobic condition following a method as described by Archna *et.al,* (2004)[36] followed by its purification after repetitive streaking and finally single bacterial colonies were picked up aseptically and maintained by sub culturing successively in modified RM2 medium [37,38].Then enrichment of PNSB was done following a standard protocol, described by Yasa *et.al,*

(2006) [39] and growth obtained in this way was further enriched with RCVBNP medium following a method as described by Madigan and Gest (1982)[35]

The stock cultures of PNSB were maintained under anaerobic condition on modified RM2 Medium[37, 38] at $4C^0$ for further experimentation.

The isolated PNSB bacterial strain was characterized through the method of Bergey's manual of systemic bacteriology (1986)[40] followed by automated BIOLOG™ microbial identification system.

Screening of Salicylic Acid (SA) Production Potential

The screening of bacterial potentiality for Salicylic acid production (*in vitro*) was performed by a method as described by Cook (1993)[41]. Freshly grown bacterial culture was centrifuged at 10000 rpm for 10 min in order to collect cell free bacterial culture supernatant. 4 ml of Supernatant was acidified with 1N HCl (pH 2.0) prior to the extraction of salicylic acid with chloroform (2×2 ml). Finally 4ml of distilled water & 5 ml of 2M $FeCl_3$ was added to the pooled chloroform extracts and subjected to observation.

Extraction and Purification

Extraction and purification of SA from bacterial culture was performed following the method as described by Shanmugam and Narayanaswamy (2008)[42].

50ml of bacterial culture supernatant was concentrated up to a volume of 20ml by evaporation in desiccators and then it was acidified at pH2 before it's extraction with double volume of chloroform.

Thin Layer Chromatography (TLC) of Bacterial Supernatant

Confirmation of the presence of SA in cell free bacterial culture supernatant was done by using the chloroform fractions for Thin Layer Chromatography (TLC). 20µl of cell free bacterial culture supernatant was spotted aseptically on TLC silica gel plates and run there after using chloroform : acetic acid : ethanol (95:2.5:2.5) (Pervin *et.al,* 2015)[43] mixture solvent. The TLC plates were then removed from the developing chamber prior to spray on them with Ehrlich's reagent before watching them finally under UV light at 365nm (Pervin *et.al,* 2015)[43]. The presence of SA from bacterial origin was confirmed by matching their *Rf* value with that of a known control SA

standard (Sigma). The *Rf* value was calculated by using the formula: *Rf* value = Distance spot travels (A) / Distance solvent travels (B).The *Rf* value 0.74 for SA from cell free RASN4 bacterial culture supernatant origin was matched with that of an authentic SA control standard (0.74) confirming the presence of SA in cell free RASN4 bacterial culture supernatant.

Detection of SA in bacterial supernatant was confirmed through TLC by visually examining the appearance of blue bands under UV-light (365nm) both in case of cell free RASN4 bacterial culture supernatant and its authentic standard control.

Appearance of blue bands both in case of samples and authentic SA standard control, showing same fluorescence postulated strongly the capability of *in vitro* bacterial (RASN4) SA production potentials.

The blue bands on TLC silica plates under UV-light (365nm), confirmed the existence of SA in cell free RASN4 bacterial culture supernatant also confirming the potentiality of its *in vitro* SA production.

Both qualitative screening and quantitative estimation suggested the existence of SA in cell free RASN4 bacterial culture supernatant confirming the potentiality for *in vitro* SA production by PNSB bacterial strain RASN4.

Quantitative Estimation of Salicylic Acid (SA) Production (In vitro)

The quantitative assay of bacterial salicylic acid (SA) production (*in vitro*) was determined following the method of Meyer *et al* (1992)[4]. Freshly grown bacterial culture was centrifuged at 10000 rpm for 10 min in order to collect cell free bacterial culture supernatant. 4 ml of Supernatant was acidified with 1N HCl (pH 2.0) prior to the extraction of salicylic acid with chloroform (2×2 ml) [41].Concentration of salicylic acid was determined by addition of 5 µl of 2 M $FeCl_3$ and 3 ml of water in to 1ml of concentrated extract of salicylic acid containing bacterial supernatant. Absorbance of the purple iron-SA complex, developed in the aqueous phase, was measured at 527 nm and the total amount of salicylic acid was estimated with the help of a standard curve prepared with the salicylic acid dissolved in ethyl acetate (HPLC grade, Sigma)[4].

RESULTS AND DISCUSSION

Plant pathogenic microorganisms can reduce 25-100% crop yields by causing various diseases in agricultural crops [44]. Phytotoxicity and accumulation of chemical residues after chemical fungicide treatments are the two major issues leading to the environmental pollution as well as serious human health hazards [45]. So, biological control through some chemical inducing microorganisms are considered to be a potentially effective non hazardous strategy to mitigate the crop damage by agricultural plant pathogens[46] /MT. use of microbes and microbial metabolite products based formulations can be used to achieve the cumulative effects and sustainability in the arena of agriculture[47,48] in addition to mitigate chemical pollution hazards occurring from fungicide residue. In present years, salicylic acids (SA) of microbial origin are being used as an extensive strategy in order to protect the plants from plant pathogens to mitigate plant disease for its control. Biological control of soil born crop pathogens are primarily attributed to improvement of boosting up host plants defense or to direct inhibition of growth and activities of agricultural crop pathogens[49].

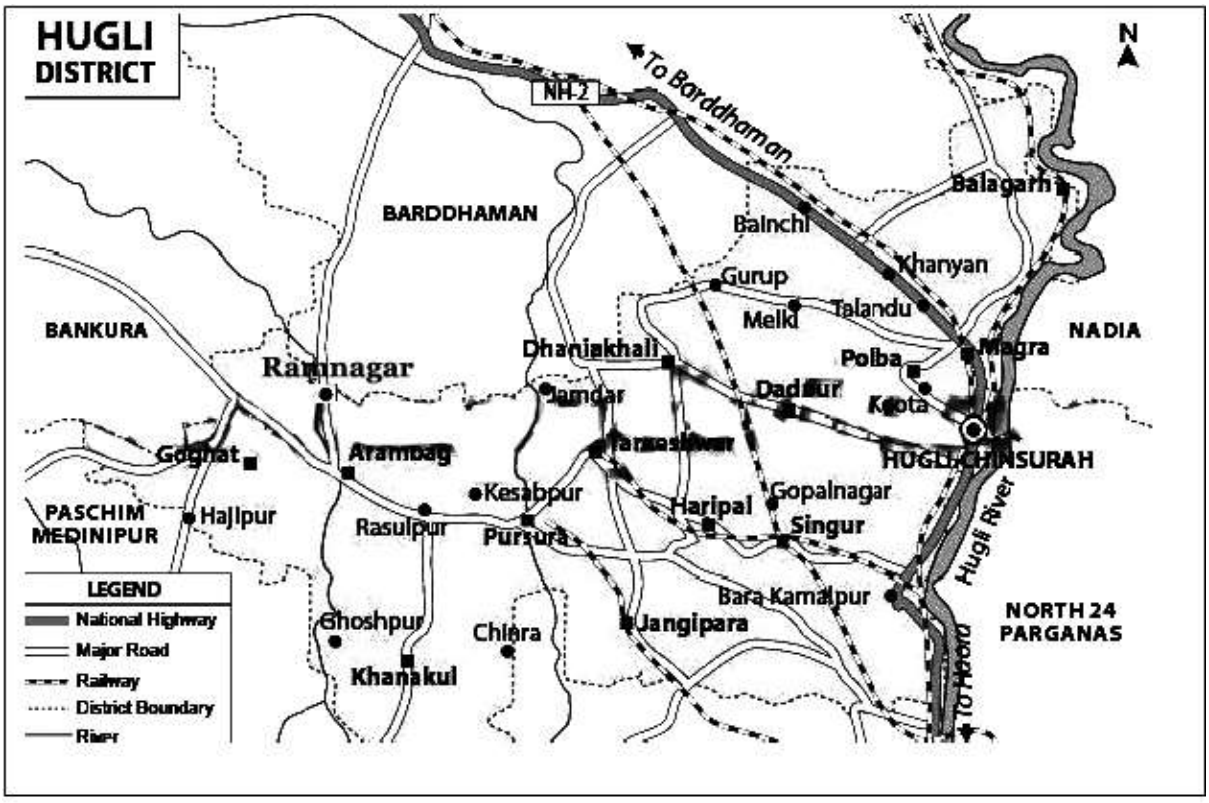

Fig: 1. Map showing location of bacterial sampling site of rice fields at Ramnagar, Hooghly, West Bengal, India (Latitude 22.85"; Longitude 88.39")

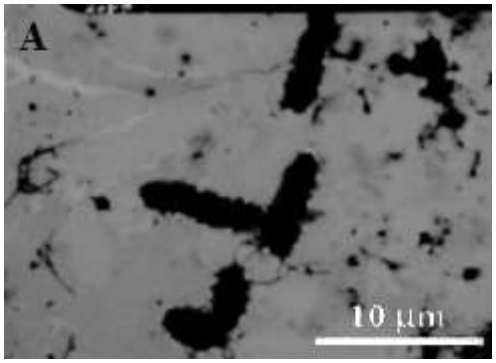

Fig: 2. SEM of *Rubrivivax gelatinosus* Strain RASN4

Table-1: Morphological, physiological and biological characteristics of the isolated bacterial strain *Rubrivivax gelatinosus* (RASN4)

Sl. No.	Characteristics	Bacterial Isolates *Rubrivivax gelatinosus* (RASN4)
1.	Color of Anaerobic Culture	Pale Peach to Red
2.	Cell Shape	Curved Rod
3.	Size (Width × Length)	(0.3 - 0.7 × 1.3 – 2.1) µm
4.	Formation of Sheaths	-
5.	Gram Staining	Gram negative
6.	Motility	+
7.	Flagellation	Polar, Monotrichous
8.	Slime Production	+
9.	Growth	Photoautotrophic
10.	Salt Tolerance Level	5%
11.	Growth Temperature Range (°C)	5 – 45°C
12.	Nitrogen Fixation Ability	+
13.	Oxidase Activity	+
14.	Catalase Activity	+
15.	Urease Activity	-
16.	NO_3^- Reduction Test	+
17.	Gelatine Liquefaction (Gelatinase)	+
18.	Starch Hydrolysis	-
19.	Casein Hydrolysis	+
20.	**IMViC Test** Indole production Test Methyl Red Test Vogese Proskauer Test Citrate Utilization Test	 + - - +

Sl. No.	Characteristics	Bacterial Isolates *Rubrivivax gelatinosus* (RASN4)
21.	Hugh-Leiffson (O/F)Reaction Test	O/F
22.	Carotenoid	+
23.	Bacteriochlorophyll	+
24.	Exo-Polysachharides (EPS)	+
25.	Poly-Beta-Hydroxybutyrate (PBHB)	+
26.	Growth Accelerators	Biotin, Thiamine

Table-1: Morphological, physiological and biological characteristics of the isolated bacterial strain *Rubrivivax gelatinosus* (RASN4) contd……

Sl. No.	Characteristics	Bacterial Isolates *Rubrivivax gelatinosus* (RASN4)
	Utilization of Carbon Source	
		+
	Acetate	-
	Butyrate	+
	Citrate	+
	Formate	+
	Fumarate	+
	Glutamate	+
	Lactate	+
	L-Malate	+
	Propionate	+
	Pyruvate	+
	Succinate	+
	Tartrate	
		+
1.	Arabinose	-
	Cellbiose	+
	Fructose	-
	Galactose	+
	Glucose	+
	Lactose	-
	Maltose	-
	Raffinose	-
	Rhamnose	-
	Ribose	+
	Sucrose	+
	Xylose	
		-
	Glycerol	-
	Mannitol	-
	Sorbitol	-

+ Indicates presence of positive reaction; - Indicates absence or negative reaction; O = Oxidation; F = Fermentation;

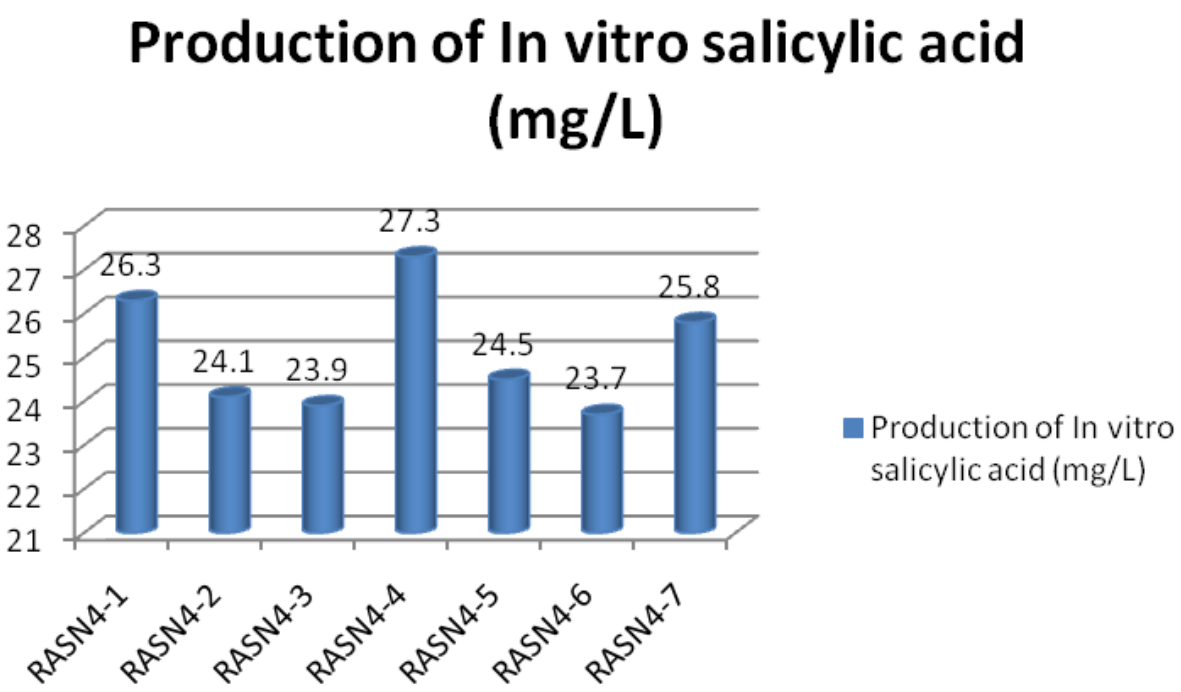

Fig 3. Quantification of Salicylic acid (*In vitro)* production by *Rubrivivax gelatinosus* Strain RASN4

Salicylic acid (SA) is a phenolic phytohormone formed from phenyl alamine via cinamic acid, then to benzoic acid or coumaric acid through SA biosynthetic pathway[50] .It can influence a series of biochemical and molecular process of events involved in disease resistance induction.

Earlier, some previous studies reported the influence and role of salicylic acid for protection of plants under various biotic and abiotic stresses including salinity[51,52,53] .Later on, many works had been done in order to substantiate the role of SA during plant-pathogen interactions[54,55,19] and to substantiate the fact SA plays an important role in SAR induction in cucumber and tobacco plants [13,9,19]. Further; many researchers established the prime role of SA in induced systemic resistance (ISR) induction[24,56,57]. SA plays its key role in inducing both local and systemic induced resistance initiated after an immediate plant pathogenic attack[58,59]. De-meyer and Hofte (1997)[24] showed *Pseudomonas aeruginosa* can induce systemic resistance through SA production. They assumed that PGPR can trigger on activation of phenyl alamine ammonia-layase (PAL) activity leading to increased SA biosynthesis and suggested that SA may be indirectly involved in introducing ISR

induced by PGPR with special reference to cucumber root disease [24]. So, Meyer and Hofte (1997)[24] considered SA as an important translocated signal that gets accumulated in PGPR treated host plant roots and greatly involved in inducing ISR. Siddiqui and Shaukat (2003) [60]showed *Pseudomonas aeruginosa* enhanced defence mechanism by inducing systemic resistance in tomato plants through production of SA.

Like all the other PGPR bacterial strains like *Pseudomonas aeruginosa* [60,24], *Bacillus licheniformis* [42], *Bacillus cereus, B.Mycoides, B.pumilus, B.sphaericus and B.subtilis* [46, 59]*Pseudomonous fluorescens* , S*erratia marcesceus* [30], many PNSB rhizobacterial strains can show the capability of SA production. Parvin *et.al,* (2015) [43]confirmed the potentiality of PNSB bacterial strain *Burkholderia cepacia* for synthesizing salicylic acid (SA) in vitro and also proved their role in oil palm seedling growth and development.

In present work, prime focus was emphasized on a rice rhizobacterial purple non sulfur bacterium (PNSB) strain RASN4; later identified as *Rubrivivax geatinosus* to assess its potentiality for in vitro salicylic acid (SA) production as it otherwise show promising plant growth promoting rhizobacterial activity in order to bioformulate a potent microbial biofertiliser bioinoculant together with some phytopathoremidiatory traits.

The PGPR activity of PNSB and its potential capability of salicylic acid production for sustainable plant protection is well established[43]. In this background perspective, the antagonistic potential of the isolated PNSB rhizobacterial strain *Rubrivax gelatinosus* RASN4 was tested in terms of their potentiality of SA production (in vitro), which showed a strong positive indication through screening prior to their quantification.

In Thin Layer Chromatography (TLC), appearance of blue bands both in case of samples and authentic SA standard control, showing same fluorescence postulated strongly the capability of *in vitro* bacterial SA production potentials of isolated rice rhizospheric PNSB RASN4 *Rubrivax gelatinosus* strain.

Further, during estimation, the amount of in vitro SA production (*in vitro*) by PNSB rhizobacterial strain RASN4 was determined up to 27.3 mg/l^{-1} as maximum highest level.

CONCLUSION

So, it can be concluded that the purple non-sulfur bacterial strain (RASN4) isolated from the rice rhiozospheric soils from the rice fields of Ramnagar of Hooghly district of west Bengal, India (Latitude 22.82 N; Longitude 87.80 E which was later been identified as *Rubrivivax gelatinosus* , has its potentiality of producing exogenous salicylic acid as becomes evident through the qualitative screening followed by its confirmation through quantification.

Salicylic acid, exogenously produced by the bacterial micro organisms, plays a pivotal role in plant growth and development together with the enhancement of their crop productivity through their phytopathoremediatory effects inducing system resistance against a wide range of fungal and bacterial phytopathogens. Such capability of exogenous Salicylic acid production by isolated PNSB strain RASN4 might have been exploited for bio formulating an efficient microbial biofertiliser with additional potentiality of phytopathoremediation in addition to their other PGPR activity traits. This will provide an additional synergistic advantage for bio formulating a potential microbial bioinoculant for promising agricultural crop productivity in Indian subcontinent.

ACKNOWLEDGEMENT

The authors are grateful to DST PURSE II for providing financial assistance. They are extending their thanks to Head of the Departments of Botany, University of Kalyani for providing DST PURSE II central equipment facility. Thanks are also extended to Dr. Rudradeb Banerjee for his kind help in preparing manuscript.

REFERENCES

[1]Khan, N., Bano, A., & Zandi, P.,2018, Effects of exogenously applied plant growth regulators in combination with PGPR on the physiology and root growth of chickpea (Cicer arietinum) and their role in drought tolerance. *Journal of plant interactions*, *13*(1), 239-247.

[2]Renga Ramanujam, J., Kulothungan, S., Prabhu, S. S., Kumaran, E., Shanmugaraju, V., & Arun, P.,2015, A study on synergism between Pseudomonas fluorescens and Parthenium hysterophorus as a biocontrol agent to Alternaria alternata (leaf

spot) in Lycopersicon esculentum (Tomato). *International Research Journal of Microbiology and Bio Technology*, *2*(1).

[3] Ankenbauer, R. G., & Cox, C. D.,1988, Isolation and characterization of *Pseudomonas aeruginosa* mutants requiring salicylic acid for pyochelin biosynthesis. *Journal of bacteriology*, *170*(11), 5364-5367.

[4] Meyer, J. M., Azelvandre, P., & Georges, C.,1992, Iron metabolism in Pseudomonas: salicylic acid, a siderophore of Pseudomonas fluorescens CHAO. *BioFactors (Oxford, England)*, *4*(1), 23-27.

[5] Sokol, P. A., 1984, Production of the ferripyochelin outer membrane receptor by Pseudomonas species. *FEMS microbiology letters*, *23*(2-3), 313-317.

[6] Cox, C. D., & Graham, R.,1979, Isolation of an iron-binding compound from Pseudomonas aeruginosa. *Journal of Bacteriology*, *137*(1), 357-364.

[7] Cox, C. D., Rinehart, K. L., Moore, M. L., & Cook, J. C.,1981,. Pyochelin: novel structure of an iron-chelating growth promoter for Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *78*(7), 4256-4260.

[8] Press, C. M., Wilson, M., Tuzun, S., & Kloepper, J. W.,1997, Salicylic acid produced by Serratia marcescens 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. *Molecular Plant-Microbe Interactions*, *10*(6), 761-768.

[9] Métraux, J. P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, & Inverardi, B.,1990, Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science*, *250*(4983), 1004-1006.

[10] Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, & Ryals, J.,1993),Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*, *261*(5122), 754-756.

[11] Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, & Ryals, J.,1994, A central role of salicylic acid in plant disease resistance. *Science*, *266*(5188), 1247-1250.

[12] Shulaev, V., León, J., & Raskin, I.,1995, Is salicylic acid a translocated signal of systemic acquired resistance in tobacco?. *The Plant Cell*, *7*(10), 1691-1701.

[13] Malamy, J., Carr, J. P., Klessig, D. F., & Raskin, I., 1990, Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, *250*(4983), 1002-1004.

[14] Cokmus, C., & Sayar, A. H.,1991, Effect of salicylic acid on the control of bacterial speck of tomato caused by Pseudomonas syringae pv. tomato. *J. Turkish Phytopathol*, *20*, 27-32.

[15] Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, & Ryals, J.,1992, Acquired resistance in Arabidopsis. *The Plant Cell*, *4*(6), 645-656.

[16] Jones, A. M., Lindow, S. E., & Wildermuth, M. C.,2007, Salicylic acid, yersiniabactin, and pyoverdin production by the model phytopathogen Pseudomonas syringae pv. tomato DC3000: synthesis, regulation, and impact on tomato and Arabidopsis host plants. *Journal of bacteriology*, *189*(19), 6773-6786.

[17] Forchetti, G., Masciarelli, O., Izaguirre, M. J., Alemano, S., Alvarez, D., & Abdala, G.,2010, Endophytic bacteria improve seedling growth of sunflower under water stress, produce salicylic acid, and inhibit growth of pathogenic fungi. *Current microbiology*, *61*(6), 485-493.

[18] White, R. F., 1979, Acetylsalicylic acid (aspirin) induces resistance to tobacco mosaic virus in tobacco. *Virology*, *99*(2), 410-412.

[19] Yalpani, N., Silverman, P., Wilson, T. M., Kleier, D. A., & Raskin, I., 1991, Salicylic acid is a systemic signal and an inducer of pathogenesis-related proteins in virus-infected tobacco. *The Plant Cell*, *3*(8), 809-818.

[20] Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y., & Hunt, M. D., 1996, Systemic acquired resistance. *The plant cell*, *8*(10), 1809.

[21] Mayers, C. N., Lee, K. C., Moore, C. A., Wong, S. M., & Carr, J. P.,2005, Salicylic acid-induced resistance to Cucumber mosaic virus in squash and Arabidopsis thaliana: contrasting mechanisms of induction and antiviral action. *Molecular plant-microbe interactions*, *18*(5), 428-434.

[22] Visca, P., Ciervo, A., Sanfilippo, V., & Orsi, N., 1993, Iron-regulated salicylate synthesis by Pseudomonas spp. *Microbiology*, *139*(9), 1995-2001.

[23] Leeman, M., Den Ouden, F. M., Van Pelt, J. A., Dirkx, F. P. M., Steijl, H., Bakker, P. A. H. M., & Schippers, B.,1996, Iron availability affects induction of systemic resistance to Fusarium wilt of radish by Pseudomonas fluorescens. *Phytopathology*, *86*(2), 149-155.

[24] De Meyer, G., & Höfte, M., 1997, Salicylic acid produced by the rhizobacterium Pseudomonas aeruginosa 7NSK2 induces resistance to leaf infection by Botrytis cinerea on bean. *Phytopathology*, *87*(6), 588-593.

[25] Rajjou, L., Belghazi, M., Huguet, R., Robin, C., Moreau, A., Job, C., & Job, D., 2006, Proteomic investigation of the effect of salicylic acid on Arabidopsis seed germination and establishment of early defense mechanisms. *Plant physiology*, *141*(3), 910-923.

[26] Popova, L. P., Maslenkova, L. T., Yordanova, R. Y., Ivanova, A. P., Krantev, A. P., Szalai, G., & Janda, T., 2009, Exogenous treatment with salicylic acid attenuates cadmium toxicity in pea seedlings. *Plant Physiology and Biochemistry*, *47*(3), 224-231.

[27] Hudson, A. T., & Bentley, R.,1970, Utilization of shikimic acid for the formation of mycobactin S and salicyclic acid by Mycobacterium smegmatis. *Biochemistry*, *9*(20), 3984-3987.

[28] Lebuhn, M., & Hartmann, A., 1994, Production of auxin and L-tryptophan related indolic and phenolic compounds by Azospirillum brasilense and Azospirillum lipoferum. In *Improving plant productivity with rhizosphere bacteria. Proceedings of the 3rd International Workshop on Plant Growth-Promoting Rhizobacteria. Commonwealth Scientific and Industrial Research Organisation, Adelaide, Australia* (pp. 145-147).

[29] Buysens, S., Heungens, K., Poppe, J., & Hofte, M.,1996, Involvement of pyochelin and pyoverdin in suppression of Pythium-induced damping-off of tomato by Pseudomonas aeruginosa 7NSK2. *Appl. Environ. Microbiol.*, *62*(3), 865-871.

[30] Zhang, S., Moyne, A. L., Reddy, M. S., & Kloepper, J. W.,2002, The role of salicylic acid in induced systemic resistance elicited by plant growth-promoting rhizobacteria against blue mold of tobacco. *Biological Control*, *25*(3), 288-296.

[31] Indiragandhi, P., Anandham, R., Kim, K., Yim, W., Madhaiyan, M., & Sa, T.,2008, Induction of defense responses in tomato against Pseudomonas syringae pv. tomato by regulating the stress ethylene level with Methylobacterium oryzae CBMB20 containing 1-aminocyclopropane-1-carboxylate deaminase. *World Journal of Microbiology and Biotechnology*, *24*(7), 1037-1045.

[32] Biebl, H., & Pfennig, N.,1981, Isolation of members of the family Rhodospirillaceae. In The prokaryotes (pp. 267-273). Springer, Berlin, Heidelberg.

[33] Madigan, M. I. C. H. A. E. L., Cox, S. S., & Stegeman, R. A.,1984, Nitrogen fixation and nitrogenase activities in members of the family Rhodospirillaceae. Journal of bacteriology, 157(1), 73-78.

[34] Ponsano, E. H. G., Lacava, P. M., & Pinto, M. F.,2002, Isolation of Rhodocyclus gelatinosus from poultry slaughterhouse wastewater. *Brazilian Archives of Biology and Technology*, *45*(4), 445-449.

[35] Madigan, M., & Gest, H.,1982, Nitrogen fixation by photosynthetic bacteria. *Handbook of biosolar resources*, *1*(part 1), 245-250.

[36] Archana, A., Sasikala, C., Ramana, C. V., & Arunasri, K.,2004, "Paraffin wax-overlay of pour plate", a method for the isolation and enumeration of purple non-sulfur bacteria. Journal of microbiological methods, 59(3), 423-425.

[37] Sinha, S.N., 1992, Microbial mobilization of sulfur in aquatic ecosystem and effect of pollution on each activities. Ph.D. Thesis. University of Kalyani, Kalyani, Nadia.

[38] Haldar, S., 2001, Ecology of purple non sulfur bacteria in paddy field and assessment of the potentiality of a suitable strain as biofertiliser in paddy cultivation. Ph.D. Thesis. University of Kalyani, Kalyani, Nadia.

[39] Yaşa, İ., Çadırcı, B. H., Koçyigit, A., & Öztürk, T., 2006, Enrichment and Isolation of Anoxygenic Phototrophic Bacteria in Winogradsky Column. *Su Ürünleri Dergisi*, *23*(1).

[40] Bergey's manual of systematic bacteriology, 1986, Baltimore, London, Los Angeles, Sidney: Williams and Wilkins, Vol. 2, pp. 965–1599.

[41] Cook, R. J. ,1993, Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual review of phytopathology*, *31*(1), 53-80.

[42] Shanmugam, P., & Narayanasamy, M. A. T. H. I. V. A. N. A. N. 2009, Optimization and production of salicylic acid by rhizobacterial strain Bacillus licheniformis MML2501. *Int J Microbiol*, *6*(1), 94-98.

[43] Parvin, W., Othman, R., Jaafar, H., Rahman, M., & Mui, W. 2015, Chromatographic detection of phytohormones from the bacterial strain UPMP3 of Pseudomonas aeruginosa and UPMB3 of Burkholderia cepacia and their role in oil palm seedling growth. *International Journal of Biotechnology Research*, *3*(5), 073-080.

[44] Frisvad, J. C., & Samson, R. A, 1991, Filamentous fungi in foods and feeds: ecology, spoilage and mycotoxin production. *Handbook of applied mycology*, *3*, 31-68.

[45] Mandal, S., Mallick, N., & Mitra, A, 2009, Salicylic acid-induced resistance to Fusarium oxysporum f. sp. lycopersici in tomato. *Plant Physiology and Biochemistry*, *47*(7), 642-649.

[46] Abdel-Monaim, M. F, 2017, The role of salicylic acid and Pseudomonas fluorescens in systemic resistance against Rhizoctonia solani Kühn in guar (Cyamopsis tetragonoloba (L.) Taub.). *International Journal of Phytopathology*, *6*(1), 1-15.

[47] Arora, N. K.,Ed., 2015, *Plant microbes' symbiosis: Applied facets* (pp. 1-381). New Delhi: Springer.

[48] Arora, N. K., & Mishra, J., 2016, Prospecting the roles of metabolites and additives in future bioformulations for sustainable agriculture. *Applied Soil Ecology*, *107*, 405-407.

[49] Abdel-Monaim, M. F., 2013, Improvement of biocontrol of damping-off and root rot/wilt of faba bean by salicylic acid and hydrogen peroxide. *Mycobiology*, *41*(1), 47-55.

[50] Lee, H. I., León, J., & Raskin, I.,1995, Biosynthesis and metabolism of salicylic acid. *Proceedings of the national academy of sciences*, *92*(10), 4076-4079.

[51] Wang, D., Pajerowska-Mukhtar, K., Culler, A. H., & Dong, X., 2007, Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. *Current Biology*, *17*(20), 1784-1790.

[52] Horváth, E., Szalai, G., & Janda, T., 2007, Induction of abiotic stress tolerance by salicylic acid signaling. *Journal of Plant Growth Regulation*, *26*(3), 290-300.

[53] Dempsey, D. M. A., & Klessig, D. F.,2017, How does the multifaceted plant hormone salicylic acid combat disease in plants and are similar mechanisms utilized in humans?. *BMC biology*, *15*(1), 23.

[54] Conti, G. G., Pianezzola, A., Violini, G., Maffi, D., & Arnoldi, A.,1996, Possible involvement of salicylic acid in systemic acquired resistance ofCucumis sativus againstSphaerotheca fuliginea. *European journal of plant pathology*, *102*(6), 537-544.

[55] Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, & Ryals, J.,1993, Requirement of salicylic acid for the induction of systemic acquired resistance. *Science*, *261*(5122), 754-756.

.[56] Chen, C., Bélanger, R. R., Benhamou, N., & Paulitz, T. C.,1999, Role of salicylic acid in systemic resistance induced by Pseudomonas spp. against Pythium aphanidermatum in cucumber roots. *European Journal of Plant Pathology*, *105*(5), 477-486.

[57] Audenaert, K., Pattery, T., Cornelis, P., & Höfte, M., 2002, Induction of systemic resistance to Botrytis cinerea in tomato by Pseudomonas aeruginosa 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. *Molecular Plant-Microbe Interactions*, *15*(11), 1147-1156.

[58] Hammer schmidt, R., Smith-Becker, J. A.,2000 , The role ofsalicylic acid in disease resistance. In Mechanisms ofResistance to Plant Diseases (Eds: Slusarenko, A., Fraser,R. S. S., Van Loon, L. C.), Pp. 37 – 53, Kluwer Academic Publisher.

[59] Saikia, R., Singh, T., Kumar, R., Srivastava, J., Srivastava, A. K., Singh, K., & Arora, D. K., 2003, Role of salicylic acid in systemic resistance induced by Pseudomonas fluorescens against Fusarium oxysporum f. sp. ciceri in chickpea. *Microbiological Research*, *158*(3), 203-213.

[60] Siddiqui, I. A., & Shaukat, S. S., 2003, Endophytic bacteria: prospects and opportunities for the biological control of plant-parasitic nematodes. *Nematologia Mediterranea*, *31*(1).