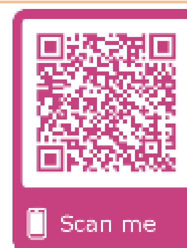
Journal of Global Biosciences
ISSN 2320-1355
Volume 8, Number 5, 2019, pp. 6219-6235
Website: www.mutagens.co.in



Research Paper

INDUCED ANTIVIRAL RESISTANCE INDUCTION AND GROWTH PROMOTION BY THE RHIZOBACTERIUM *Pantoea agglomerans*

Ashish Kumar Gupta, Jyoti Verma, Aparana Srivastava, Shalini Srivastava and Vivek Prasad

Molecular Plant Virology Laboratory,
Department of Botany, Lucknow University,
Lucknow-226007,
India.

Abstract

Application of beneficial rhizobacteria induces resistance against pathogens and promotes growth in plants. UN1, a rhizobacterial isolate recovered from the rhizosphere of healthy *Azadirachta indica* plants, could induce systemic antiviral resistance against *Tobacco mosaic virus* (TMV) in *Nicotiana tabacum* cv. Xanthi-nc and *Nicotiana glutinosa*, when applied on the leaves 24h prior to challenge inoculation with TMV. Reduction in the number of local lesions was evident on leaves away from the treated leaves. Isolate UN1did not exhibit antimicrobial activity *in vitro*. The isolate shared a 99 % sequence identity in its 16S rRNA partial gene sequence with *Pantoea agglomerans*, and also matched it in its morphological and biochemical profile, hence appears to be a strain of *P. agglomerans*. Isolate UN1 produced auxin (16 µg mL^{-1}), cellulase, lipase and solubilised phosphate. It significantly ($P \leq 0.05$) promoted the growth of the hypocotyl and radicle in germinating *Cyamopsis tetragonoloba* seeds. *P. agglomerans* is known to suppress bacterial and fungal growth in plants and several strains are active ingredients of biopesticide formulations. Induced antiviral resistance, auxin production and phosphate solubilisation could make this isolate potentially useful in plant growth promotion and biological control of plant viruses.

Key words: ***Induced systemic resistance, PGPR, Pantoea agglomerans, antiviral, phosphate solubilisation, auxin production***.

INTRODUCTION

Induction of systemic resistance in plants against pathogens can prevent major agronomic losses. In this context, systemic acquired resistance (SAR) by biotic agents has been explored with some success, and a few chemicals like salicylic acid (SA), benzodiathiazol-S-methyl ester (BTH) and β-aminobutyric acid (BABA) have provided

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resistance to pathogen infection in susceptible hosts [1, 2]. In a similar way, effective induction of resistance has been achieved through application of phytoproteins [3] and plant growth promoting rhizobacteria (PGPR) [4, 5]. The most studied PGPR genera include *Pseudomonas*, *Bacillus* and *Serratia*, and their formulations have been commercially employed in biological control, an arm of integrated pest management (IPM) meant to reduce the use of chemical fertilizers and pesticides.

There are several direct and indirect ways through which the PGPR exert their influences, depending upon the bacterial species and strain as well as the host and the environment [6]. Phosphate solubilisation, nitrogen fixation, production of volatile organic compounds (VOCs) and auxins by rhizobacteria, all directly promote plant growth [7, 8]. Antagonistic substances such as HCN and a variety of antibiotics produced by the PGPR strain can arrest pathogen development in soil or plant surfaces [9], while siderophores can chelate iron and interfere with pathogen growth. PGPR application primes a plant to respond quickly and effectively to various stresses, by causing changes at the molecular and physiological levels and a chemical interplay between the host and the microbe leads to ISR [5, 10].

Individual strains of PGPR effectively control both bacterial and fungal pathogens [11]. Thus, broad spectrum disease suppression is rather common, but there are fewer reports of plant virus control by rhizobacterial isolates. *Tobacco necrosis virus* (TNV) and *Tobacco mosaic virus* (TMV) were effectively controlled by *P. fluorescens* strain CHA0 and *P. aeruginosa* strain 7NSK2, on tobacco [12, 13, 14]. *Bacillus amyloliquefaciens* strain MB1600 induced resistance in tomato against *Tomato spotted wilt virus* and *Potato virus Y* [15], while *B. pumilus* SE34 and *B. subtilis* 937b could prevent accumulation of *Cucumber mosaic virus* (CMV) and *Tomato mottle virus* in tomato [16, 17, 18]. *Serratia marcescens* was reported to protect *Arabidopsis thaliana* from CMV infection [19]. This paper deals with the isolation and molecular identification of an antiviral resistance inducing bacterial strain from the rhizosphere of *Azadirachta indica*, its biochemical characteristics, and growth promotion activity.

MATERIAL AND METHODS

Raising of test hosts

Seeds of the test hosts, *Nicotiana tabacum* cv Xanthi-nc and *Nicotiana glutinosa* L., reacting hypersensitively to *Tobacco mosaic virus* (TMV), were sown in clay pots and seedlings were raised in a glasshouse. The seedlings were transplanted in clay pots filled with manure and soil. Both *Nicotiana* spp. were used at a 5-6 leaf stage, when they were nearly two months old.

Maintenance of virus culture and preparation of the inoculum

TMV was maintained in its systemic host, *Nicotiana tabacum* cv White Burley, and the inoculum was prepared using virus infected leaves of *N. tabacum* cv White Burley. Fresh leaves were weighed and homogenized in 10 mM phosphate buffer, pH 7.0 (1:1 w/v). The leaf tissue homogenate was squeezed through a double layered muslin cloth and the filtrate was centrifuged at 5000×g for 15 min. The supernatant was used as the inoculum, and the dilution was adjusted so as to produce 100-300 local lesions on Xanthi-nc tobacco and between 50-100 lesions on *N. glutinosa*, following mechanical inoculation.

Isolation of rhizobacteria

The bacteria were isolated from the rhizosphere of healthy *Azadirachta indica* (neem) growing in the Lucknow University campus. Microbes colonizing the rhizosphere were cultured on nutrient agar using spread plate technique. Following an overnight incubation of the plates at 37°C, single colonies were picked and subsequently streaked on nutrient agar plates. The resulting colonies were inoculated onto agar slants. Pure cultures were maintained at 4°C, sub-cultured every 7-15 days to retain their viability.

Induction of virus resistance in the test hosts

Nutrient broth (5 mL) supplemented with CM cellulose (0.5% w/v) was inoculated with a loopful of the isolates and incubated at 37°C. The overnight cultures were used either in a foliar application, applied with the help of a cotton swab on the leaf surface, or as a soil drench. Un-inoculated broth, also supplemented with CM-cellulose, was used to treat the control set of plants. The treatment was administered only once on the test hosts.

Four isolates, viz., UN1, UN2, UN3 and UN4 were tested for their ability to induce systemic antiviral resistance in tobacco Xanthi-nc and *N. glutinosa*. The strains were applied at 1x 10^8 CFU mL^{-1} onto the lower two leaves of tobacco and *N. glutinosa* plants, and the plants were challenge-inoculated with TMV on all the leaves after 24 hours of treatment. Induction of resistance was determined by counting the number of local lesions which developed on the test hosts, and percent reduction in lesion number was calculated for both treated (basal) and non-treated remote (upper) leaves by the formula:

Percent reduction in lesion number = C-T/C x 100, where C and T represent average number of lesions on leaves of the control and treated set, respectively. Experiment on each host was performed in triplicate, and a minimum of three plants were used per treatment. Standard error mean was calculated for the average lesion numbers from each treatment.

Isolation of genomic DNA of UN1 isolate

Genomic DNA was isolated from a 24h nutrient broth culture of UN1 isolate, using bacterial genome DNA Mini Prep kit (Chromous Biotech, India) as per manufacturer's instructions. Isolated genomic DNA was electrophoresed on a 1% agarose gel and ethidium bromide stained bands were visualized on a UV-transillumінator.

Amplification of the 16S rRNA gene and nucleotide BLAST

Amplification of the rRNA gene was performed through PCR using the frequently used universal primer pair 27f (5'AGAGTTTGATCCTGGCTCAG3') and 1492r (5'TACCTTGTTACGACTT3') [20]. PCR was carried out in a final volume of 50 µL using the Dream Taq PCR Master mix, 2X (Thermo Fisher Scientific). Reaction mixture included 25 µL master mix (2X PCR buffer, 1.0 Unit Taq polymerase, 200 mM of each dNTP, 50 mM $MgCl_2$), 4 µL (10 µM) each of forward and reverse primer, 4 µL DNA template (25 ng), and 13 µL RNase-free water. The T100 Thermal cycler (BioRad) was programmed as follows: Initial denaturation at 95°C for 5 min, 30 cycles of amplification with denaturation at 94°C×45 s, annealing at 52°C×45 s, extension at 72°C×45 s, followed by a final extension at 72°C for 10 min. The PCR amplicon (1494

bp) was separated on a 1% agarose gel in Tris-borate-EDTA buffer, stained with ethidium bromide and visualised on a UV trans-illuminator.

PCR product purification, sequencing and BLAST search

PCR product was purified using GeneJET PCR purification kit (Thermo Fisher Scientific) and submitted for sequencing to Chromous Biotech, India. The amplicon was sequenced by the Sanger method using the ABI 3500 Genetic Analyzer (Thermo Fisher Scientific). The partial 16S rRNA sequence was subjected to nucleotide BLAST analysis (NCBI) and the sequence aligned with other sequences obtained from the NCBI database by using CLUSTAL 2.1 Multiple Sequence Alignment tool.

Test for Phosphate solubilisation

The qualitative estimation of phosphate solubilisation by isolate UN1 was carried out by pouring Pikovyskaya agar medium containing calcium phosphate in the petri plates [21]. Sterile discs soaked either in an overnight nutrient broth culture of UN1 isolate or broth alone (control) were placed in the centre of the plate. Both plates were incubated for 72 hours at 37°C. The production of a clear zone around the disc indicated phosphate solubilisation by the isolate.

Detection of Indole acetic acid production

Production of the auxin, Indole acetic acid (IAA), by UN1isolate, was detected following a modified method of Bric et al [22]. A loopful of the isolate was incubated for 24 hours at 37°C in 5 ml nutrient broth. The inoculum (100 µL) was transferred to King's B broth (50 ml) containing tryptophan (500 µg mL^{-1}) and incubated for an additional 48 hour at 28^0C in a shaker incubator. Cultures were centrifuged at 10000 rpm for 15 min and 1 ml of supernatant was transferred to a fresh tube to which 50µL of 10 mM orthophosphoric acid and 2 ml of salkowski's reagent (1ml of 0.5M $FeCl_3$ in 50 ml of 35% $HClO_4$) were added. Development of red colour confirmed the isolate as positive for IAA production. IAA was quantified as per Gordon and Weber [23].

Test for HCN and Siderophore production

Rhizobacterial isolate UN1 was streaked on nutrient agar medium containing glycine (4.4 g L^{-1}). Whatman filter paper No.1 soaked in picric acid solution was placed inside

the petri lid [24]. The plates were sealed with parafilm and incubated at 30°C for 4 days. The change in colour of the filter paper from yellow to orange brown indicated HCN production.

Production of siderophore by the isolate UN1 was tested as per Schwyn and Neilands [25]. The 24 hr old broth culture was inoculated on chrome-azurol S (CAS) agar medium. Development of orange colour around the culture indicated production of siderophore.

Effect of UN1 on *in vitro* seed germination of *Cyamopsis tetragonoloba*

Cyamopsis tetragonoloba seeds were soaked for 5 minutes in 0.2% (w/v) $HgCl_2$ for surface sterilization. Seeds were briefly rinsed in sterile water and then soaked in a suspension of UN1 isolate ($1x10^8$ CFU mL^{-1}) for thirty minutes, finally being transferred to a petri dish containing Murashige and Skoog medium in 0.8% agar. They were left in the dark for three days, and their germination pattern monitored. The control set of seeds, not treated with UN1 isolate, were handled similarly. The experiment was conducted in triplicate, and each petridish was plated with 10 seeds. The t-test was applied at 95% confidence to determine the significance of the data obtained, using IBM SPSS statistics software, version 20.0.

Antimicrobial activity of UN1

Rhizobacterial isolate UN1 was tested for inhibition of two bacteria viz., *Escherichia coli* and *Bacillus subtilis*, and three fungi viz., *Aspergillus niger*, *Fusarium oxysporum* and *Rhizopus stolonifer*. The bacterial strains were procured from HiMedia, while the fungal species were isolated in the laboratory from contaminated food. In brief, a sterile disc dipped in the UN1 inoculum ($1x10^8$ CFU mL^{-1}) was placed at the centre of a lawn culture of *E. coli* and *B. subtilis*, and the petri plates incubated for 24h at 37°C. To detect antifungal activity in UN1 isolate, nutrient agar plates were inoculated with the isolate at one end, while the fungal gel plug (7 mm) was placed at the other end of the plate. The plates were incubated at 37°C for two days and the inability of the fungus to grow around the isolate was taken to indicate antifungal potential of UN1 isolate.

Morphological and physiological characterization of the isolate UN1

Isolate UN1 was subjected to a Gram stain reaction and its response to the stain and morphology was studied using light microscopy. KB002 HiAssorted Biochemical Test Kit (Himedia Labs) was used for a preliminary identification of UN1 isolate through a battery of 12 biochemical tests, some conventional and some based on carbohydrate utilization. Each well was inoculated with 50 µL of the overnight bacterial culture and incubated overnight at 37°C. Additional reagents were added for phenylalanine deamination and Nitrate reduction tests, and colour changes noted. The result was compared with an identification index provided by the manufacturer, and the bacterial species determined. Production of lipase and cellulase by the isolate was determined by adding 2% Tween and methyl red to 2.5% agar and 0.2% Carboxymethyl cellulose (CMC) to 1% agar, respectively [26, 27]. Following inoculation and a 24h incubation, a change in colour around the streak indicated lipase activity, while hydrolysis of CMC was visualized by flooding the plate with 0.1% Congo red and rinsing with 1M NaCl.

RESULTS

Induction of systemic resistance

Out of the four isolates tested, only UN1 expressed the ability to induce systemic antiviral resistance. None of the hosts responded to a soil-drench treatment in terms of induction of resistance (data not shown). Foliar application of isolate UN1 on the basal leaves of *N. tabacum* cv. xanthi-nc and *N. glutinosa*, inhibited TMV infection by nearly 95-98% as compared to the plants treated with broth alone. On the un-treated remote site leaves of the test hosts, the inhibition of TMV infection was between 85-96% (Fig. 1, Table 1). Thus, compared to the controls, TMV was inhibited effectively on both hosts, but only when isolate UN1 was used in a foliar application and not as a soil drench.

16S rRNA gene sequencing

Genomic DNA of isolate UN1 was used for 16S rRNA gene amplification as described in Materials and Methods (Fig. 2). The amplicon (1494 bp) was purified and sequenced. The sequenced data (551bp) was submitted for nucleotide BLAST (NCBI). With an E value of 0.0, query cover of 98% and percent identity of 99.64, it showed maximum identity with several strains of *Pantoea* listed either as *Pantoea* sp. or *Pantoea agglomerans,* formerly called *Erwinia herbicola or Enterobacter agglomerans* (family

Enterobacteriaceae, Phylum Proteobacteria). Percent sequence identity with representative *P. agglomerans* strains and other species of *Pantomea*, is given (Table 2). Aligned sequences of UN1 isolate and *Pantoea agglomerans* strain S31_BM2T 16S rRNA gene, partial sequence, is shown in Table 3.

Growth promotion by UN1

The germination percentage along with the length of the radicle and hypocotyl was noted for *C. tetragonoloba* seeds, in both UN1-treated and un-treated sets. Treatment did not inhibit seed germination and differences in the lengths of the radicle and hypocotyl were found to be statistically significant ($P \leq 0.05$), compared to the non-bacterized control set ((Figs. 3a and 3b).

Antifungal and antibacterial activity

No detectable antifungal or antibacterial activity was noted with respect to the fungal and bacterial cultures used (data not shown). UN1 isolate could not inhibit the growth of *E. coli*, *B. subtilis*, *A. niger*, *F. oxysporum* and *R. stolonifer*, *in vitro*, under the given conditions.

Morphological and Biochemical characteristics of isolate UN1

Isolate UN1 produced pale yellow coloured colonies on nutrient agar and consisted of Gram negative straight rods. It could produce IAA (16 µg mL^{-1}), cellulase and lipase, and solubilise calcium phosphate (Figs. 4a, 4b, 4c, 4d). It lacked the ability to produce HCN or siderophore. It could, however, reduce nitrate and utilize citrate, glucose and arabinose as sources of carbon, but showed negative ability to utilize various other metabolites. The spectrum of various activities of isolate UNI is summarized in Table 4.

Table 1: Induction of systemic antiviral resistance by the bacterial isolate UN1*

Test Host	Treatment	Average no. of lesions ±SEM		Induced Resistance (%)	
		Site	Remote-site	Site	Remote-site
Xanthi-nc	Control	232.66 ±23.87	243±29.42	---	---
	UN1	3.5 ±1.08	8.66 ±1.22	98.49	96.43
NG	Control	96.66±5.12	64.44±3.70	---	---
	UN1	4±0.91	9.55±1.77	95.86	85.17

*An overnight culture of the bacterial isolate UNI in nutrient broth supplemented with CM cellulose (0.5% w/v) was applied at 1 x 10^8 CFU mL^{-1}, on the surface of the basal leaves of the test hosts. The control set was similarly treated with the broth + CM cellulose only. The treated (site) as well as un-treated (remote-site) leaves

of *Nicotiana tabacum* cv. Xanthi-nc (Xanthi-nc) and *N. glutinosa* (NG) were challenge-inoculated with *Tobacco mosaic virus* (TMV) 24h post treatment. Local lesions were counted on both site and remote-site leaves of control and treated plants and expressed as percent reduction in lesion number or induced resistance (%). Each treatment was in triplicate and the experiment was repeated thrice with similar results. Standard error mean (SEM) was calculated for number of local lesions/leaf/treatment.

Table 2: Molecular identification of bacterial isolate UN1*

Bacterial species and strain	Accession ID	% Identity
Pantoea agglomerans S31_BM2T	MK883112.1	99.0
Pantoea agglomerans SXAU-S1	MK875137.1	99.0
Pantoea agglomerans BQC01	MH101508.1	99.0
Pantoea brenneri LMG 5343	NR_116245.1	98.0
Pantoea anthophila LMG 2558	NR_116113.1	98.0
Pantoea stewartii subsp. *Indologenes* CIP 104006	JN175332.1	98.0
Pantoea stewartii subsp. *stewartii*	U80208.1	98.0
Pantoea ananatis JCM 6986	LC462185.1	98.0
Pantoea vagans LMG 24199	NR_116115.1	97.0
Pantoea dispersa LMG 2603	NR_043883.1	96.0

*Genomic DNA was used for the amplification of the 16S rRNA gene using the universal primers 27f and 1492r. The PCR product was purified and sequenced. Isolate UNI shared 99.0 % sequence identity with *Pantoea agglomerans* strains and a lesser identity with other *Pantoea* spp, when the sequences were aligned using CLUSTAL 2.1 Multiple Sequence Alignment tool.

Table 3: Isolate UN1 ribosomal RNA gene, partial sequence*

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UN1             CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG
MK883112.1      CCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCG
                ************************************************************

UN1             GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAG
MK883112.1      GTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAG
                ************************************************************

UN1             CGAACTTAGCAGAGATGCTTTGGTTCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTG
MK883112.1      CGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACGCTGAGACAGGTGCTGCATGGCTG
                ************************ ***********************************

UN1             TCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT
MK883112.1      TCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCT
                ************************************************************

UN1             TTGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAG
MK883112.1      TTGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACTGCCGGTGATAAACCGGAGGAAG
                ************************************************************

UN1             GTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATG
MK883112.1      GTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATG
                ************************************************************

UN1             GCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCACAAAGTGCGTCGTAGT
MK883112.1      GCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCACAAAGTGCGTCGTAGT
                ************************************************************

UN1             CCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAG
MK883112.1      CCGGATCGGAGTCTGCAACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCGTGGATCAG
                ************************************************************

UN1             AATGCCACGGTGAATACGTTCCCGGGCCTTGTACACCCCGCCCGTCACACCATGGGAGTG
MK883112.1      AATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTG
                ************************************ ***********************

UN1             GGTTGCAAAAGTACAAGGTA
MK883112.1      GGTTGCAAAAGAAGTAGGTA
                *********** *  *****
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* *Pantoea agglomerans* strain S31_BM2T 16S (Sequence ID: MK883112.1) is compared with the 551 bp sequence of isolate UN1 by using CLUSTAL 2.1 Multiple Sequence Alignment tool, and gave a sequence alignment score of 99. The differences between the two are represented by gaps in the asterisks.

Table 4: Spectrum of biological and biochemical activity of the isolate UN1*

Biological/Biochemical activity of isolate UN1	
Positive	**Negative**
Antiviral	Antifungal[a]
Phosphate solubilisation	Antibacterial[b]
Auxin production	Siderophore production
Growth promotion[c]	HCN production
Lipase production	Lysine utilization
Cellulase production	H_2S production
Citrate utilization	Adonitol utilization
Nitrate reduction	Lactose utilization
Glucose utilization	Sorbitol utilization
Arabinose utilization	Phenylalanine deamination[d]

*The isolate UN1 did not exhibit any antimicrobial activity against *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* (a), *E.coli* and *Bacillus subtilis* (b). *Cyamopsis tetragonoloba* seedlings showed enhanced development of radicle and hypocotyl as compared to the non-bacterized controls (c). Based on the biochemical tests, the characteristics strongly matched the profile of *Pantoea agglomerans*, except for phenylalanine deamination for which UN1 isolate was negative (d), but some strains are positive.

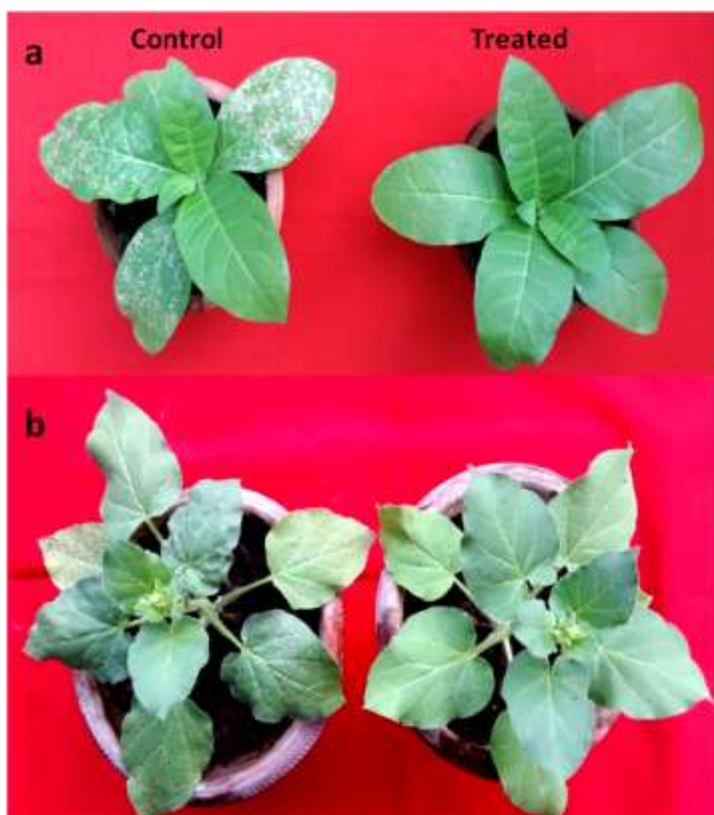

Fig. 1: Induction of systemic antiviral resistance. PGPR isolate UN1 was applied onto two basal leaves of the test hosts *Nicotiana tabacum* cv. Xanthi-nc (a) and *Nicotiana glutinosa* (b). The basal treated (site) and the upper un-treated (remote-site) leaves were challenge with *Tobacco mosaic virus* (TMV) 24h post treatment. The treated set of plants showed systemic resistance induction, with very few lesions (Treated) while the control set, treated with broth alone, developed numerous local lesions (Control).

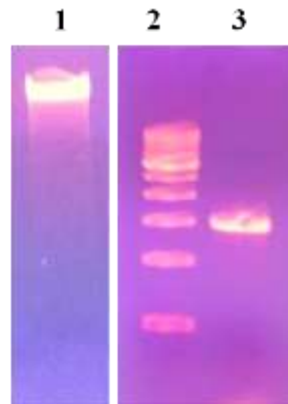

Fig. 2: Isolation of genomic DNA from isolate UN1 and amplification of the 16S rRNA gene. Genomic DNA (lane 1) was amplified through PCR with 16S rRNA gene universal primers as detailed in Materials and Methods. The DNA ladder (500 bp) and the amplicon of 1494 bp is seen in lanes 2 and 3, respectively.

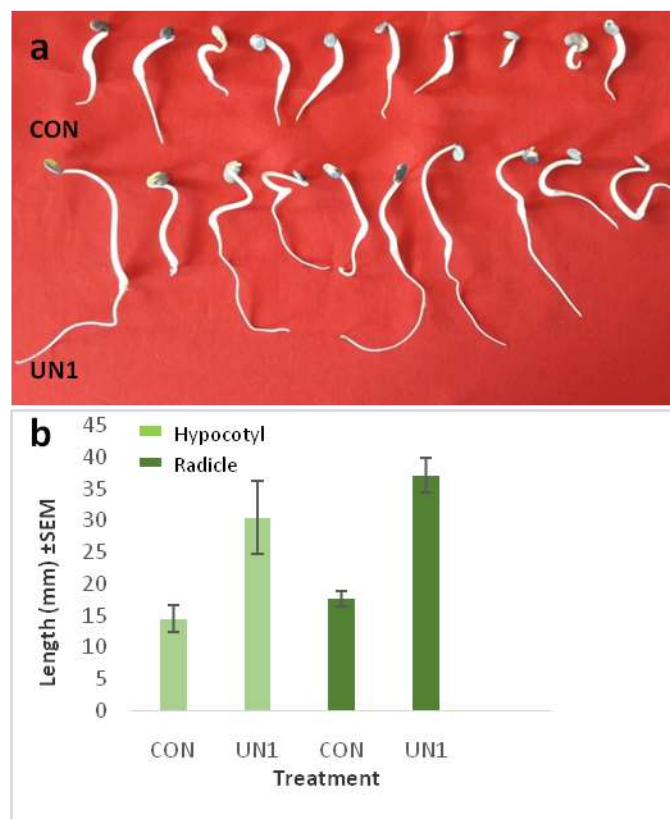

Fig. 3: Growth promotion by PGPR isolate UN1. *Cyamopsis tetragonoloba* seeds were soaked in a 24h culture of the isolate UN1 in nutrient broth (1 x 10^8 CFU/mL) and placed on MS medium for growth *in vitro* (3a). The lengths of the radicle and hypocotyl were estimated at day 3, and Standard Error Mean (SEM) calculated for 10 seeds per treatment (3b). The control set (CON) was treated with broth alone. The experiment was repeated thrice and the difference in length was found to be significant (P= <0.05), at 0.001 and 0.003 for radicle and hypocotyl, respectively.

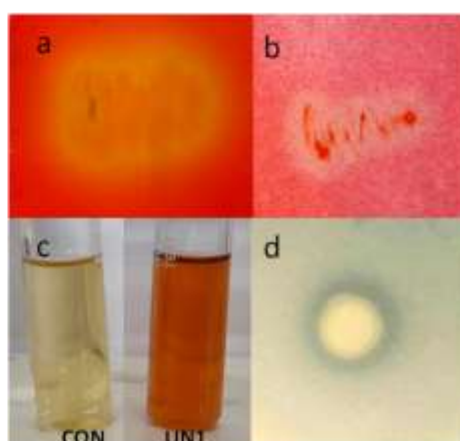

Fig. 4. Biochemical characterization of the bacterial isolate UN1. The isolate UN1 could produce lipase (a), cellulase (b), auxin (c, UN1), and solubilize phosphate (d). CON represents a negative control in Fig. 4c.

DISCUSSIONS

Rhizobacteria exert beneficial effects on plants, protecting them under biotic and abiotic stress conditions. Out of the four isolates, only UN1 could induce resistance against TMV in *N. tabacum* cv. Xanthi-nc and *N. glutinosa*. This strain was characterized at the biochemical and molecular levels and identified as *Pantoea agglomerans*. *Pantoea* species are opportunistic human pathogens, or plant pathogens [28], while some are associated with plants mostly as epiphytes and endophytes. The taxonomy and biochemical identification of *Pantoea* sp. at the strain level is rather complex, and its nomenclature has shifted periodically [29, 30]. Strains of *P. agglomerans* produce antibiotics that can negatively impact the growth of *Erwinia amylovora*, and hence suppress the fire blight of apple and pear. Strain Eh252 produces a single antibiotic and has been used as a biological control agent against *E. amylovora* in field trials [31]. *P. agglomerans* strain 48b/90, an epiphyte isolated from soybean leaves, produces peptide antibiotic effective against several phytopathogenic bacteria, similar to that produced by the strain C9-1, an established biocontrol agent [32]. A mixture of strains PTA-AF1

and AF2 of *P. agglomerans* were most effective in reducing symptoms of the grey mold, *Botrytis cinerea*, on grapevines in vineyard conditions through induction of systemic resistance in the host [33]. Thus, there are several reports of *P. agglomerans* suppressing bacterial and fungal pathogens. However, isolate UN1 could not inhibit bacterial and fungal pathogens under observation. Unlike a number of rhizobacterial strains used in biological control, this strain neither produced HCN nor siderophores, both exert antimicrobial effects, and the latter is known to play a role in plant growth promotion as well [34]. Production of cellulase could be of assistance in colonizing plants, while lipase producing strains are important in biodiesel production [35].

Widely used biochemical tests, designed for clinical identification of the microorganism, were performed using the isolate UN1 and the analytical index profile was in accordance with that listed for *P. agglomerans*, with the exception of phenylalanine deamination test, for which the studied strain was negative [36]. Based on 16S rRNA partial gene sequences, it showed a 99.0 % identity with *P. agglomerans*. It has been suggested that an identity above the threshold sequence similarity of 98.65% should be considered for species similarity [37]. Based on sequence identity and biochemical tests, the isolate UN1 appears to be a strain of *P. agglomerans*.

P. agglomerans strain UN1 does not produce HCN, and does not exhibit antimicrobial activity *in vitro*. It, however, induces resistance against TMV in Xanthi-nc tobacco and *N. glutinosa,* upon foliar application. It is entirely possible that strain UN1 uses ISR and not antibiosis as a mechanism of biological control on these hosts. *Pseudomonas* sp. strain WCS417 used ISR against fungal pathogens, and was dependent on jasmonic acid and ethylene signalling in plants for induced resistance [38]. ISR can also be induced by bacterial cell wall components and lipopolysaccharides.

P. agglomerans strain UN1 produced a fair amount of IAA in tryptophan-enriched medium. IAA is a plant hormone that is known to regulate and enhance plant growth, elicit transcriptional changes and induce resistance. The obvious increase in the lengths of the radicle and hypocotyl could be attributed to auxin production. It also solubilises phosphates and hence could function as a biofertilizer, improving plant growth. With

hardly any reports of antiviral resistance induction by *P. agglomerans*, this strain is important as it can be studied for its potential in biological control of plant viruses.

ACKNOWLEDGEMENT

Financial assistance from CSIR UGC to AKG (No. 2061630848) and RGNF to JV (F1-17.1/2017-18/RGNF-2017-18-SC-UTT-47935) is gratefully acknowledged.

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