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Gene expression profiling through microarray analysis in *Arabidopsis thaliana* colonized by *Pseudomonas putida* MTCC5279, a plant growth promoting rhizobacterium

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Abbreviations: MTCC5279, *Pseudomonas putida* MTCC5279; PGPR, plant-growth-promoting rhizobacteria; IAA, indole-3-acetic acid; ACCS, 1-aminocyclopropane-1-carboxylase synthase; TopII, topoisomerase II; HDH, histidinol dehydrogenases; ABA, abcissic acid; ET, ethylene; JAR1, jasmonate responsive gene; PRH75, plant RNA helicase 75; MAD, median absolute deviation; ISR, induced systemic resistance; JA, jasmonic acid; SA, salicylic acid

Plant growth promotion is a multigenic process under the influence of many factors; therefore an understanding of these processes and the functions regulated may have profound implications. Present study reports microarray analysis of *Arabidopsis thaliana* plants inoculated with *Pseudomonas putida* MTCC5279 (MTCC5279) which resulted in significant increase in growth traits as compared with non-inoculated control. The gene expression changes, represented by oligonucleotide array (24652 genes) have been studied to gain insight into MTCC5279 assisted plant growth promotion in *Arabidopsis thaliana*. MTCC5279 induced upregulated *Arabidopsis thaliana* genes were found to be involved in maintenance of genome integrity (*At5g20850*), growth hormone (*At3g23890* and *At4g36110*), amino acid synthesis (*At5g63890*), abcissic acid (ABA) signaling and ethylene suppression (*At2g29090*, *At5g17850*), Ca^{+2} dependent signaling (*At3g57530*) and induction of induced systemic resistance (*At2g46370*, *At2g44840*). The genes *At3g32920* and *At2g15890* which are suggested to act early in petal, stamen and embryonic development are among the downregulated genes. We report for the first time MTCC5279 assisted repression of *At3g32920*, a putative DNA repair protein involved in recombination and DNA strand transfer in a process of rapid meiotic and mitotic division.

Introduction

Plants are in continuous contact with soil micro biota, pathogenic or beneficial in nature. Beneficial free-living root colonizing bacteria, directly or indirectly supporting plant growth and yield are collectively termed as plant growth promoting rhizobacteria (PGPR).[1] In past years several mechanisms of plant growth promotions by PGPR including (1) bacterial production of phytohormone like indole-3-acetic acid,[2,3] gibrellins[4] cytokinin[5] (2) triggering induced systemic resistance (ISR)[6] (3); breakdown of plant produced ethylene by bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase[7] (4); suppression of plant pathogens[8] and (5) increased availability of soil nutrients[9] to plants have been discussed. In addition to plant growth promotion, PGPR also increases plant tolerance against abiotic environmental factors like drought, extreme temperature, high soil salinity, nutrient deficiency and metal toxicity.[10] Bacterial ACC deaminase activity and regulation of ACC production is considered to be an important beneficial mechanism for abiotic stressed plants.[11]

Plant-microbe interactions with PGPRs commonly result in a phenomenon called Induced systemic resistance (ISR) which prepares the host plant to resist a wide range of stresses and plant pathogens.[12–14] However mechanism of ISR is very diverse. These mechanisms may be, ethylene and jasmonic acid (JA) or salicylic acid (SA) dependent.[15,16] *Pseudomonas fluorescens* has been reported to induce ISR, independent of SA and dependent of JA but could also induces ISR even in ethylene insensitive mutant *Arabdiopsis thaliana* plants.[17] Moreover some specific strains trigger ISR by SA signaling indicates that the pathways for ISR are not universal and are very much dependent on plant species and bacterial partners. Most of the studies investigating plant-microbe interaction mechanisms are worked out with bacterial strains with traits like niche specificity, PGPR and biocontrol activity etc.[18–20] However reports of elucidating mechanisms of PGPR with abiotic

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stress tolerant bacterial strains is scanty. Hence the current study was taken up with the objective of to (1) understand the plant phsiological alteration under the influence of a abiotic stress tolerant plant growth promoting rhizobacteria *Pseudomonas putida* MTCC5279 (MTCC5279), (2) microarray analysis was performed for a broader understanding of the molecular determinants involved in plant growth promotion, which can serve as a viable strategy for promoting plant growth.

Results

Plant growth promotion by *Pseudomonas putida* MTCC5279. MTCC5279 was isolated from desert regions of Rajasthan, India and identified by fatty acid methyl ester analysis by CABI Biosciences and 16SrRNA sequencing as *Pseudomonas putida* and submitted to Microbial Type Culture Collection at IMTECH, Chandigarh; India. The strain was characterized for its abiotic stress tolerance and plant growth promotional attributes summarized in **Figure 1**. MTCC5279 shows growth in presence of up to 60% PEG (**Fig. 1C1**) and 500 mM NaCl (**Fig. 1C2**), produced Auxin, 70.23 μg ml^{-1} (**Fig. 1A2**) and solubilized insoluble tri-calcium phosphate (TCP) (**Fig. 1B1 and B2**). The PGPR activity of MTCC5279 was determined using *Arabidopsis thaliana (Col-0)* as host plant. Inoculation with MTCC5279 resulted in significant increase in vegetative growth of *Arabidopsis thaliana* plants (**Fig. 1D**). Significant increase in plant height by 43.2%, number of branches by 91.3% and dry weight by 79.8% was noted in MTCC5279 inoculated *Arabidopsis thaliana* in comparison to non-inoculated plants (**Table 1**). Significant increase in number of branches per plant resulted in higher silique formation (110.2% as compared with control), which ultimately gave better seed yield of about 32.3% in comparison to uninoculated control. Colonization in terms of mean colony-forming units (CFU) mg^{-1} of the MTCC5279 treated *Arabidopsis thaliana* plants showed 5.6×10^5 CFU mg^{-1} in rhizosphere and 7.0×10^5 CFU mg^{-1} in phyllosphere after 45 d of inoculation, whereas control plant roots did not show any colonization.

Microarray analysis of *Arabidopsis thaliana* leaves after inoculation with *Pseudomonas putida* MTCC5279. To obtain a global picture of the genes differentially expressed on colonization of MTCC5279, microarray analysis was performed in *Arabidopsis thaliana* using *25K OciChip*™. After filtration 24,652 probes were retained, which were used for downstream statistical and biological analysis and the comparison across samples was performed by using the median absolute deviation (MAD). To determine the differentially expressed candidate genes multivariate outlier detection was also performed. This approach provides fold-change value, 520 genes were found to be overexpressed and 364 were repressed using 3-fold change as a criterion. The functional classification of the differentially expressed genes were performed using Genowiz™, a gene expression analysis and tracking tool that enables to determine biological significance of differentially expressed genes and facilitates expression pattern analysis and gives an insight into metabolic pathways. Gene ontology data shows that in a biological process 15.58%, 13.62% in cellular component and 13.88% in molecular process were getting upregulated while the downregulated genes in terms of biological function, molecular function and cellular component were 21.30%, 13.62% and 18.20% respectively.

Pathway analysis data showed that the genes involved in glyoxylate and dicarboxylate metabolism, fructose and mannose metabolism, oxidative phosphorylation, carbon fixation, glycerolipid metabolism, lysine biosynthesis, glycine, serine and threonine metabolism, glycan biosynthesis and metabolism, one carbon pool by folate, biosynthesis of secondary metabolites, ribosome, SNARE interactions in vesicular transport, inositol phosphate metabolism and behavior are upregulated in treated with respect to control. Genes involved in butanoate metabolism, galactose metabolism, starch and sucrose metabolism, pentose and glucuronate interconversions, oxidative phosphorylations, nucleotide metabolism, histidine, tryptophan, glutathione, alanine and aspartate metabolism and phenyl propanoid biosynthesis, styrene degradation, caprolactam degradation, proteasome, and phosphatidyl inositol signaling system were downregulated in treated plants when compared with control plants.

Functional characterization of majority of the genes getting differentially expressed by MTCC5279 showed that genes upregulated in biological process were mainly involved in cellular process, 5.6%; biological regulation 3.2%; response to stimulus 6.2% and metabolic process 3.8% and 20.8% in cell part and 11.45% in cell in cellular process and 10.96% in catalytic activity and 11.7% in binding in terms of molecular function. Downregulated genes on functional characterization in terms of biological process showed 5.47% in biological regulation, 7.49% in cellular process, 5.15% in response to stimulus and 4.99% involved in metabolic process. Among cellular process 15.79% in cell part and 16.6% in cell and in molecular function class catalytic activity (12.57%) and genes responsible for binding (11.57%) were downregulated.

At gene level the genes getting upregulated by the colonization of MTCC5279 have shown 3- to 17-fold change factor in their expression value (supplementary data). The majority of genes getting upregulated shows similarity to the plant growth related traits such as carbohydrate metabolism (*At1g05030*, 5.71-fold), nucleoside transferase (*At1g63730*, 6.59-fold), kinases (*At1g06730*, 5.32-fold; *At1g53730*, 4.69-fold), transcription factor (*At1g12890*, 5.83-fold; *At1g01260*, 7.66-fold), defense and abiotic stress related genes, induction of amino acid biosynthetic pathway and nutrient uptake (*At5g63890*, 3.89-fold), hormone synthesis (*At3g23890*, 3.66-fold), ABA signaling and ethylene suppression (*At2g29090*, 3.17-fold), signal transduction through calcium signaling components (*At3g57530*, 3.60-fold) induction of induced systemic resistance (*At2g46370*, 3.87-fold) and induction in the mitotic and meiotic division (*At5g20850*, 3.02-fold change).

Validation of the microarray experiments through semiquantitative RT-PCR. To verify the GeneChip results, a semiquantitative RT-PCR analysis was performed on selected genes, including up- and downregulated genes identified in the microarray analysis. The upregulated genes selected for semiquantitative RT-PCR were of biological process, related to functions, DNA metabolic process in response to gamma radiation (*At5g20850*),

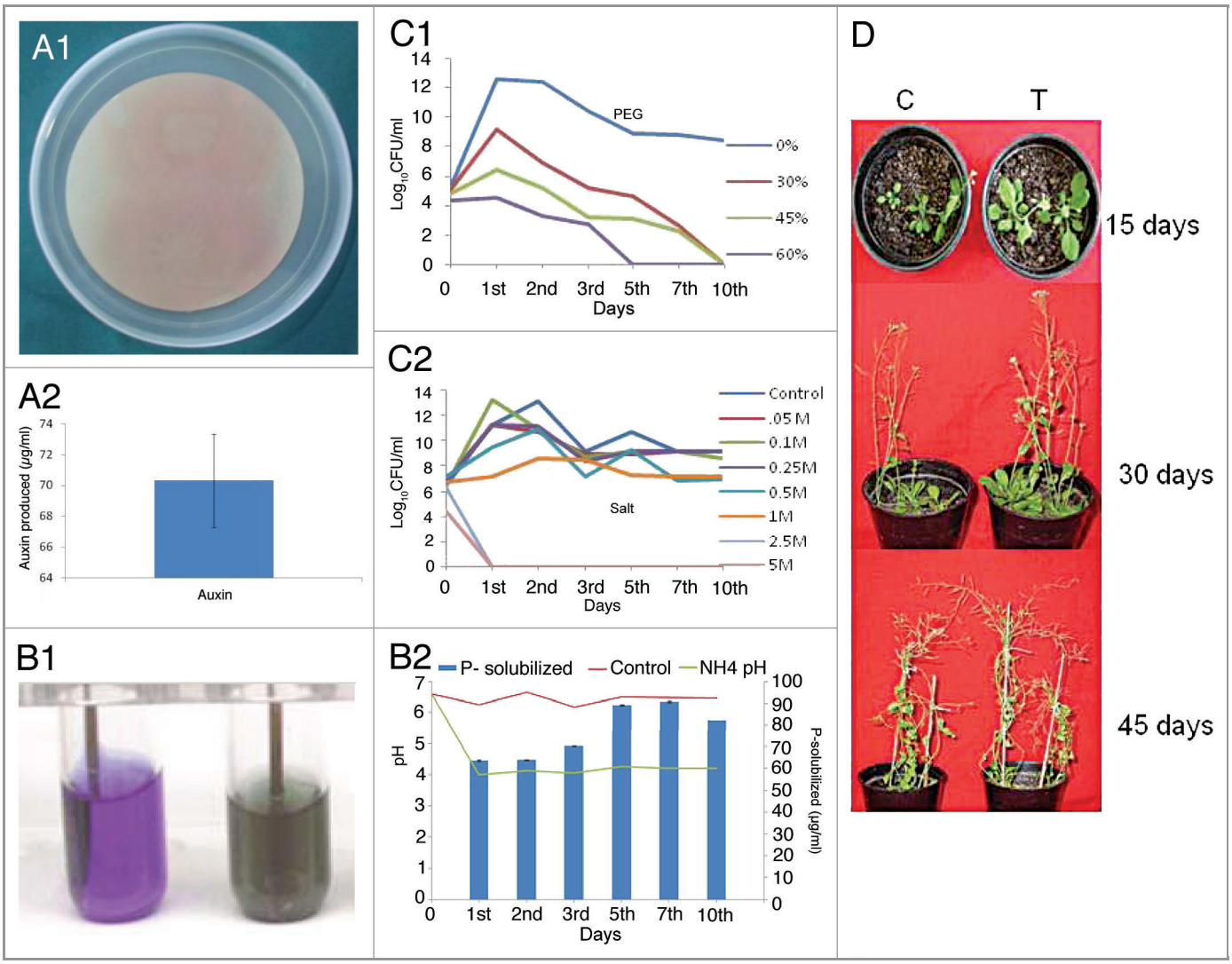

Figure 1. Auxin production by *Pseudomonas putida* MTCC5279. (A) Auxin production in *Pseudomonas putida* MTCC5279 was performed as per the protocol of Bric et al.[63] Phosphate solubilization by *Pseudomonas putida* MTCC5279. *Pseudomonas putida* MTCC5279 was inoculated in NBRI-BPB and NBRIP media and P-solubilization at different time interval was performed as described by Mehta and Nautiyal. (B1 and 2).[64] Abiotic stress tolerance of *Pseudomonas putida* MTCC5279 was performed by growing the culture in presence of different concentration of polyethylene glycol (PEG-6000) and salt (NaCl) and CFU ml^{-1} was determined at different time interval (C1 and 2).[65] Effect of *Pseudomonas putida* MTCC5279 inoculation on the growth of *Arabidopsis thaliana*.

Table 1. Plant growth promoting effect of *Pseudomonas putida* MTCC5279 using *Arabidopsis thaliana* as a host plant after 45 d of inoculation

Plant growth parameters	Control	Treated	CD at	
			5%	1%
Root length (cm)	2.46 ± 0.15	2.64 ± 0.21	0.29	0.40
Shoot length (cm)	23.10 ± 0.71	33.08 ± 1.24	3.64	5.13
No. of branches	5.79 ± 0.13	11.08 ± 0.72	1.92	2.71
No. of siliques	29.75 ± 1.75	62.54 ± 3.46	11.62	16.39
Fresh weight(mg)	630.00 ± 39.83	1245.00 ± 114.79	240.62	339.56
Dry weight (mg)	156.66 ± 8.43	281.66 ± 21.35	47.97	67.69
Seed weight (mg/plant)	5.10 ± 0.43	6.75 ± 0.56	0.82	1.12

histidinol dehydrogenase activity in response to UV (*At5g63890*), abcisic acid metabolic process responsive (*At2g29090*), induced systemic resistance responsive (*At2g46370*), *At3g57530* responsible for calcium- and calmodulin-dependent protein kinase activity, *At3g23890* in a DNA topological change in response to hormones, DEAD/H- box RNA helicase (*At5g62190*) involved in RNA metabolic process and *At5g17850* involved in cation transport. *At2g15890*, *At4g36110*, *At1g69490*, *At5g17220*, *At2g16660* were chosen among upregulated candidate genes based on prior report by Wang et al.[20] Some of the downregulated genes chosen for revalidation are *At3g32920*, involved in SOS response; *At1g74930* involved in regulation of transcription and *At2g44840* involved in ET mediated signaling pathway. The RT-PCR experiments were performed using RNA from mock (control) and MTCC5279 supplemented (treated) plants grown for 45 d initially and after 15, 30 and 45 d of inoculation to get relative expression of the selected genes in an independent experiment from the microarray analysis. The PCR reaction products obtained using gene specific primers with 28 cycles of amplification were analyzed by gel electrophoresis. The results of RT-PCR analysis clearly showed increased expression in treated leaves, whereas the mRNA level of the selected downregulated genes were lower in MTCC5279 treated plants (**Figure 4**). As expected, transcript levels of the selected genes are in accordance with the microarray results and thus confirm the data from the microarray experiments. Results showed that among all the targeted eight genes two were significantly downregulated in MTCC 5279 treated *Arabidopsis thaliana* as compared with untreated control; however differences were more prominent at 45 d in *At4g36110, At5g17850, At5g20850, At5g63890, At2g29090, At3g23890* genes respectively whereas two *At2g15890* and *At3g32920* were upregulated at 15 and 30 d followed by their downregulation at 45 d (**Fig. 4C**).

Discussion

Many plant-associated soil microorganisms interact with plant roots resulting in mutualistic and communalistic interactions, which plays a pivotal role on plant growth and development.[21,22] However, plant growth and production is continually decreasing owing to abiotic and biotic stress factors, which is increasingly becoming a severe global problem for agriculture production.[23,24] Therefore the methods to improve growth, development and stress tolerance in plants have become more and more important for crop production. One of the ways to evoke the above problem in crops is the exploitation of plant-associated microorganisms, which live in reciprocally beneficial relationships with plants.[25] *Pseudomonas putida* MTCC5279 is an abiotic stress tolerant, auxin producing, P-solubilizing, potent plant growth promoting strain and has a potential to be exploited in P translocation in the plants, especially in P limiting and abiotic stress conditions as a further extension of the work. The present study presents a global view on gene expression and physiological processes involved in MTCC5279 induced PGP in *Arabidopsis thaliana*. The expression profiling of *Arabidopsis thaliana* response to MTCC5279 assisted changes has allowed us to identify transcripts differentially expressed confirming that plant growth promotion is a multigenic process. Some of the genes identified are similar to general plant growth promotional response while others may be specific to plant growth promotional response of PGPR to *Arabidopsis thaliana*. However, we have been able to characterize previously uncharacterized genes involved in this process. The genes identified in this study, induced or repressed (3.0- to 6.0-fold) suggest they have very important roles and are worthy of further functional analyses.

Many of the genes induced in MTCC5279 assisted plants reported for the first time are involved in maintenance of genome integrity concerned with DNA metabolic process, auxin responsive growth stimulus, induction of amino acid biosynthetic pathway and nutrient uptake, ABA signaling and induction of induced systemic resistance. The new report of downregulated gene has been so far suggested to act early in petal, stamen and embryonic development ending in dormancy. Other important gene associated with plant growth promotion which is being reported for the first time by MTCC5279 assisted repression is *At3g32920*. Role of MTCC5279 in inducing plant growth promotion has been discussed in following heads.

Auxin responsive growth stimulus. Indole-3-acetic acid (IAA), cytokinins and gibberellins are important determinants of PGPRs[26–28] of which IAA is most frequently reported.[29–31] Overexpression of *At4g36110* has been correlated with IAA production during plant-microbe interaction in Arabidopsis in earlier reports.[20] Accordingly it is speculated that *Pseudomonas putida* MTCC5279 induces *At4g36110* which results in increased IAA production. Topoisomerases, which catalyzes a transient breakage and reunion of double-stranded DNA was reported to be overexpressed in hormone treated pea plants supports the overexpression of *At3g23890* (*TopII*) in MTCC5279 treated plants and speculate the view that MTCC 5279 treatment

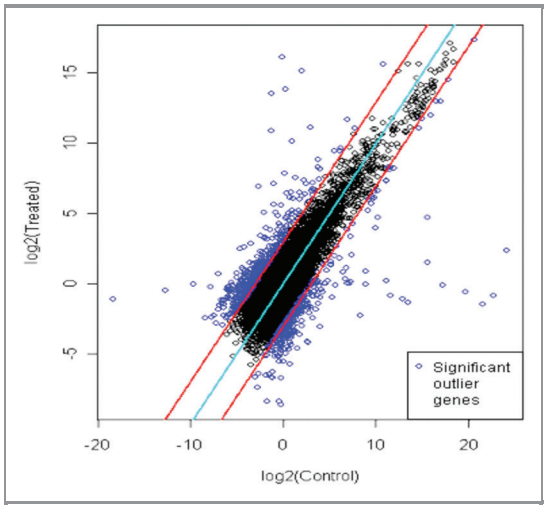

Figure 2. Scatter plot of differentially expressed genes in Arabidopsis plants with and without inoculation with MTCC5279. The upper and lower lines demarcate the limits of 3-fold differences in expression levels, dots outside the lines identify 882 genes differentially expressed between with and without inoculation of *Pseudomonas putida* MTCC5279 in *Arabidopsis thaliana* plants.

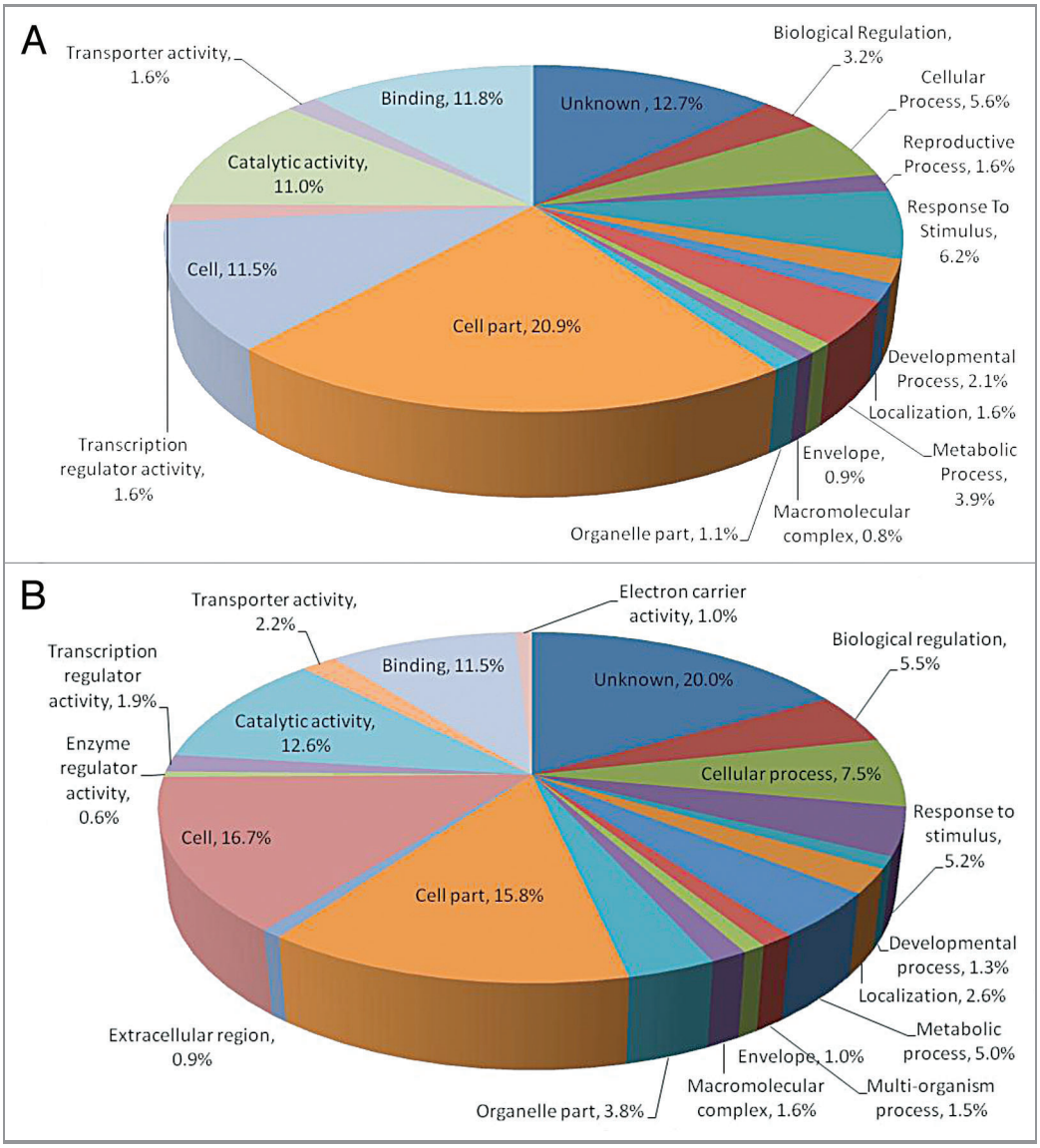

Figure 3. Functional classification of the upregulated and downregulated *Arabidopsis thaliana* genes in terms of their geneontology (GO terms), relative to their representation in the genome involved in plant growth promotion of *Arabidopsis thaliana* by *Pseudomonas putida* MTCC5279.

augment the hormonal level of the plant and enhance the catalysis step of transient breakage and reunion.[32,33]

Induction of amino acid biosynthetic pathway and nutrient uptake. Histidine is essential for plant growth and development, especially in root development in higher plants.[34] Overexpression of histidinol dehydrogenases (*At5g63890*, *HDH*), responsible for the conversion of histidinol to histidine in histidine biosynthetic pathway is correlated with more greenish plants and better root development in MTCC5279 treated *Arabidopsis thaliana* plants is being reported for the first time in this study to the best of our knowledge. Higher expression of HDH in presence of MTCC5279 suggests that the plant is using histidine as a ET receptor and play role in transmembrane signaling through His-Asp phosphorelay.[35] Overexpression of *At2g16660*, similar to nodulin-like protein is in accordance with the prior result suggesting that nodulin-like gene could be involved in signal perception or enhancement of plant mineral and nutrient uptake induced by PGPR colonization as evident by phosphate transportation by a PGPR *Piriformospora indica*.[20,36]

Signal transduction through calcium signaling components. The Ca^{2+} is one of a second messenger and is crucial for signal transduction processes during many metabolic interactions.[24] Expression of some of calcium signaling components e.g., calcium-dependent protein kinase (*At3g57530, CPK32*) were found to be increased in *Arabidopsis thaliana* colonized with *Pseudomonas putida* MTCC 5279 (**Table 3**) which is in accordance with earlier report by Verhagen et al.[37] The regulation of gene expression by elevations of cellular Ca^{2+} is crucial for plant defense against various stresses and interaction between *Piriformospora indica* and *Arabidopsis thaliana*.[38,39] Upregulation of *CPK32*, an ABA signaling component is also in accordance with the prior result suggesting that MTCC5279 mediated

Table 2. Genes showing significant increased /decreased expression in Arabidopsis plants colonized by *Pseudomonas putida* MTCC5279

Upregulated pathways	Probe set	Putative function	Fold change
Carbohydrate metabolism 2.185%			
Glyoxylate and dicarboxylate metabolism	*At4g17360*	Formyl tetrahydrofolate deformylase	4.65
Fructose and mannose metabolism	*At1g73250*	3, 5-epimerase-4-reductase	3.94
Energy metabolism 3.32%			
Oxidative phosphorylation	*At1g15690*	Inorganic pyrophosphatase (h(+)-Ppase	4.80
Carbon fixation	*At2g45290*	Transketolase	4.087
	At5g08530	NADH dehydrogenase	3.460
	At3g53620	Pyrophosphatase	3.461
	At1g78900	Vacuolar ATP synthase	3.147
Nitrogen metabolism	*At3g47340*	Glutamine-dependent asparagine synthase 1	3.080
	At3g10340	Ammonia ligase	3.041
Lipid metabolism 4.83%			
Glycerophospholipid metabolism	*At3g05630*	Phospholipase D activity	3.339
Glycerolipid metabolism	*At4g31780*	Udp-glycosyltransferase activity	3.048
Amino acid metabolism 4.34%			
Glycine, serine and threonine metabolism	*At4g13890*	Glycine hydroxymethyl transferase	3.836
Lysine biosynthesis	*At3g11710*	Aaspartate-t-RNA ligase	3.765
Alanine and aspartate metabolism	*At3g47340*	Glutamine-dependent asparagine synthase 1	3.080
Glycan biosynthesis and metabolism 18.18%			
N-glycan degradation	*At5g66150*	Alpha-mannosidase	3.559
Metabolism of cofactors and vitamins 16.6%			
One carbon pool by folate	*At4g17360*	Formyl tetrahydrofolate deformylase	4.647
Biosynthesis of secondary metabolites 2.469%			
	At5g19880	Peroxidase	4.663
Phenylpropanoid biosynthesis	*At3g53280*	Cytochrome p450 monooxygenase	3.802
	At2g41480	Peroxidase	3.193
Benzoate degradation via CoA ligation	*At1g01140*	CBl-interacting protein kinase	4.410
Translation 1.33%			
Ribosome	*At5g02960*	Structural constituent of ribosome	6.082
	At4g30800	Structural constituent of ribosome	4.389
	At3g62250	Protein binding	3.411
Aminoacyl-tRNA biosynthesis	*At3g11710*	Aspartate-tRNA ligase/ lysine-tRNA ligase	3.765
Sorting and degradation 10.4%			
Snare interactions in vesicular transport	*At1g15880*	Intra-Golgi vesicle-mediated transport	4.469
	At3g61450	Protein transporter activity	4.105
	At3g58170	SNAP-receptor activity	3.481
	At3g52400	Negative regulation of defense pathways	3.137
	At1g32270	intra-Golgi vesicle-mediated transport	3.011
Signal transduction 0.91%			
Phosphatidylinositol signaling system	*At3g57530*	Calcium-dependent protein kinaseC activity	3.607
Behavior 5.71%			
Circadian rhythm	*At1g01060*	Myb-related putative transcription factor	4.214
	At1g56650	Anthocyanin metabolism and radical scavenging	3.040

Table 2. Genes showing significant increased /decreased expression in Arabidopsis plants colonized by *Pseudomonas putida* MTCC5279 (continued)

Downregulated pathways	Custom id	Gene description	Fold change
Carbohydrate metabolism 5.88%			
Butanoate metabolism	*At5g06580*	Electron transport	-3.621
Galactose metabolism	*At5g17310*	UTP-glucose-1-phosphate uridylyl transferase	-3.236
Starch and sucrose metabolism	*At1g02790*	Polygalacturonase activity	-3.0245
	At1g02640	Beta-xylosidase	-3.672
	At3g43190	UDP-glycosyl transferase	-3.183
Pentose phosphate pathway	*At1g09420*	Glucose-6-phosphate dehydrogenase activity	-3.674
Pentose and glucuronate interconversions	*At1g02790*	Polygalacturonase activity	-3.025
Energy metabolism 1.626%			
Oxidative phosphorylation			
	At3g60330	Hydrogen-exporting ATPase activity	-3.247
	At4g09650	Hydrogen-transporting ATP synthase	-3.685
Nucleotide metabolism 2.65%			
Purine and pyrimidine metabolism	*At1g08260*	DNA polymerase epsilon catalytic subunit.	-3.775
	At4g12440	Adenine phosphoribosyl transferase	-4.103
Amino acid metabolism 2.83%			
Histidine metabolism	*At3g46100*	Histidyl-tRNA aminoacylation	-3.998
Phenylalanine metabolism	*At2g39040*	Peroxidase	-3.312
Alanine and aspartate metabolism	*At5g14760*	L-Aspartate oxidase	-3.052
Tryptophan metabolism	*At5g22300*	3-Cyanoalanine hydratase activity	-4.382
Metabolism of other amino acids 5.55%			
Glutathione metabolism	*At1g09420*	Glucose-6-phosphate dehydrogenase activity	-3.673
Cyanoamino acid metabolism	*At1g02640*	Beta-xylosidase	-3.672
Biosynthesis of secondary metabolites 1.92%			
Phenylpropanoid biosynthesis			
	At1g51680	4-Coumarate-coA ligase activity	-3.028
	At2g39040	Peroxidase activity	-3.312
Xenobiotics biodegradation and metabolism 4.05%			
Styrene degradation	*At5g22300*		-4.382
Caprolactam degradation	*At5g56650*	IAA conjugate hydrolases	-4.598
Translation 0.77%			
Ribosome	*At5g41520*	Structural constituent of ribosome	-3.081
Sorting and degradation 2.27%			
Proteasome	*At2g27020*	Endopeptidase activity	-4.884
Signal transduction 0.91%			
Phosphatidyl inositol signaling system	*At1g68000*	CDP-diacylglycerol-inositol 3-phosphatidyl transferase activity	-4.694

regulation of ABA responsive genes via ABF4 in a ABA signaling pathway through Ca^{+2} elevations.[40] ABA manipulation by endogenous signal is a promising means to improve productivity, performance and plant architecture.[41–43] The overexpression of *At5g17850* in MTCC5279 treated plants is involved in upregulated level of ABA (has regulatory roles in stress responses). Interestingly MTCC5279 overexpresses *At2g29090* (*CYP707A2*) which is responsible for catabolic repression of ABA,[45–47] hence suggesting a balanced level of ABA. Besides overexpression of At2g29090 (CYP707A2) is associated with upregulated level of nitrate as reported by Matakiadis et al.[44] which probably increases nitrate uptake in plants which results in the enhanced vegetative growth.

Induced systemic resistance (ISR). Overexpression of jasmonate responsive gene (*At2g46370, JAR1*) in the present study demonstrate that MTCC5279 mediates ISR. The mechanisms leading to ISR seem to be highly dependent on the bacterial partners and the majority of genes were predicted to be regulated by either JA or ET, or both.[6,48] *Pseudomonas fluorescens* WCS417r, elicited ISR via a salicylic acid (SA) independent pathway.[49,50]

Table 3. List of primers used for the validation of the microarray data by semiquantitative RT-PCR analysis

Gene(s)/locus tag	Up or downregulated	Accession number	Primer sequence	Annealing temperatures (°C)
At5g20850	Upregulated (This study)	NM_122092	Left Primer: 5′-GGCCATGTACATTGATGCTG-3′ Right Primer: 5′-TTGCGCAACTACTTGGTTTG-3′	55
At5g63890	-do-	NM_125784	Left Primer: 5′-AATCGATATGCCTGCTGGTC-3′ Right Primer: 5′-AGCCTGCGTTCTCAATCAGT-3′	55
At2g29090	-do-	NM_128466	Left Primer: 5′-TAAATGGTGGTTGCACTGGA-3′ Right Primer: 5′-ATGGTATGGACCTTGGTGGA-3′	63
At2g46370	-do-	NM_180122	Left Primer: 5′-GTCGTGGAGAATTCGGTGTT-3′ Right Primer: 5′-GTTTCAGGTCCCTGTGCATT-3′	55
At3g23890	-do-	NM_113294	Left Primer: 5′-CAACGGCTTGCTCAATACAA-3′ Right Primer: 5′-TTCTCGCTGCTCTCCTCTTC-3′	52
At5g62190	-do	NM_125613	Left Primer: 5′-GGCTCGGTTGATTCCTGATA-3′ Right Primer: 5′-GTTGCCAGCTCTTCCTGTTC-3′	52
At3g57530	-do-	NM_115613	Left Primer: 5′-GTCGTGGAGAATTCGGTGTT-3′ Right Primer: 5′-GTTTCAGGTCCCTGTGCATT-3′	63
At5g17850	-do-	NM_121791	Left Primer: 5′-TGGGCTATCACTCTGCCTCT-3′ Right Primer: 5′-CGCAACTAGCTCTTGTGCTG-3′	52
At2g15890	Upregulated (Wang et al.[20])	NM_127149	Left Primer: 5′-GTTCGACGCTTGAAGGTGAT-3′ Right Primer: 5′-GGTCAGGAGCGTGAGAGAAC-3′	55
At4g36110	-do-	NM_119778	Left Primer: 5′-TCAACACCGAAGTCGCTATG-3′ Right Primer: 5′-CATTTGCTTCCGTCTTCTT-3′	55
At1g69490	-do-	NM_105616	Left Primer: 5′-AATTCGACCCATGGCAATTA-3′ Right Primer: 5′-TCCATGAAACCCTCTTGCTC-3′	55
At5g17220	-do-	NM_121728	Left Primer: 5′-CTCTAGAGCACCGAGCCATC-3′ Right Primer: 5′-CTACCCCGAGCCTTAACCAT-3′	55
At2g16660	-do-	NM_099989	Left Primer: 5′-GTGAGATCCCACCAGCATCT-3′ Right Primer: 5′-TTCGGAGAGAGTACCGGAG-3′	52
At3g32920	Downregulated (This study)	NM_114061	Left Primer: 5′-ATCGGGAAAGACAGCACTTG-3′ Right Primer: 5′-CTGTTGGACCTCCAAATCGT-3′	60
At1g74930	Downregulated (Wang et al.[20])	NM_106151	Left Primer: 5′-TTACGACACTCCCGAGAAGG-3′ Right primer: 5′-TCCTCCGTAATCTTCGATGG-3′	55
At2g44840	-do-	NM_130048	Left Primer: 5′-GTTCCTCCCGTTACCTCTCC-3′ Right Primer: 5′-TCTGCTCCGACGTTAACTGA-3′	55
At3g46520	Wang et al.[20]	-	Left primer: 5′-GATATGGAAAAGATCTGGCATCAC-3′ Right primer: 5′-TCATACTCGGCCTTGGAGATCCAC-3′	57

MTCC5279 lead to ISR in a SA independent but JA dependent manner like *Pseudomonas fluorescens* strain, CHA0.[15,16] Suppression of *At2g44840*, an ET-responsive transcription factor 13, which binds to the GCC-box of pathogenesis-related promoter in a stress dependent manner is in accordance with previous report[37] and also supports ISR induction in a SA independent pathway without activating the PR proteins.[51] Systemic induced resistance in Arabidopsis conferred by *Piriformospora indica* require JA signaling has also been reported by Stein et al.[52]

Induction of mitotic and meiotic division. Overexpression of a RNA helicase (*PRH75, At5g62190*) essential for cellular processes, regulate plant growth and development and expressed mainly in young and rapidly developing tissues is in accordance with the prior reports.[37,53–55] Wang et al.[20] has reported overexpression of *At2g15890* (responsible for specifying petal and stamen identity in *Arabidopsis thaliana*) in presence of PGPR. On the contrary repression of *At2g15890* was observed in the present study which may be due to sampling after maturity.[56,57] *RAD51* (*At5g20850*), a *RecA* functional homolog plays important role in mitotic and meiotic recombination and its mutant produce aborted siliques and anthers having immature pollens, it's overexpression in MTCC5279 treated plants resulted in more silique formation as evident by physiological data (**Table 2**).[58,59] Repression of *AT3g32920*, a putative DNA repair protein involved in recombination and DNA strand transfer in a process of rapid meiotic and mitotic division has been found. Overexpression of *At1g69490*, a gene responsible for leaf senescence in the present study is in accordance with the prior report, suggest the early maturation of the plant on MTCC5279 treatment.[20,60,61] The overexpression of glutathione S-transferase (*At5g17220*) is in accordance with the prior result and supports prior reports of defense enzyme induction by the colonization of rhizobacteria. Overexpression of H^+ cation exchanger (*At5g17850*) and the repression of *At1g74930*, which codes for ET suppression

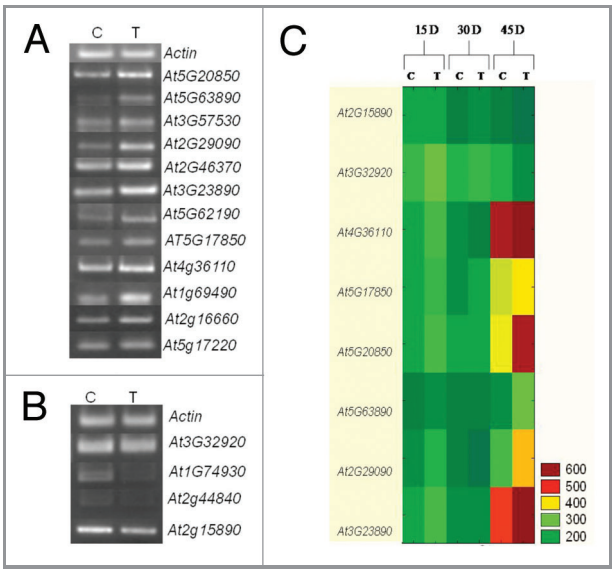

Figure 4. Semiquantitative RT-PCR analysis of genes showing significant differential expression. Increased (A) and decreased (B) expression in *Arabidopsis thaliana* plants colonized by *Pseudomonas putida* MTCC5279 after 6 weeks. (C) Relative expression of selected genes at different time intervals.

suggested the reduced level of ethylene resulting in better root elongation and plant architecture as evident by Camehl et al.[62]

In conclusion we can say that MTCC5279 mediated PGP in *Arabidopsis thaliana* is governed by many reported and new up and downregulated genes identified in this study. Further experimentation and manipulation of the identified candidate genes and processes could lead to generate important information on plant-microbial interactions, especially on plant growth promotion and their role in the interaction of bacteria with plants.

Materials and Methods

Plant growth promotion using *Arabidopsis thaliana* as a host plant. Bacterial culture *Pseudomonas putida* MTCC5279 was isolated from hot deserts of Rajasthan, India. The strain was characterized for plant growth promotional trait following standard protocols like P-solubilization, auxin production, abiotic stress tolerance.[63–65] Bacterial culture was grown in Nutrient broth at 30°C for 48 h in a rotary shaker at 180 rpm. Bacterial cells were pelleted after 48 h at 6000 rpm for 5 min and washed pellets was used for inoculating the *Arabidopsis thaliana* plants after resuspending in sterile 0.85% saline, as described earlier.[8]

Plant growth conditions. The plant growth promotary effect of MTCC5279 on *Arabidopsis thaliana* was assessed in sterile soilrite. Seeds of *Arabidopsis thaliana* ecotype Columbia (*Col-0)* were surface-sterilized by 0.4% NaClO and 70% ethanol followed by repeated washing with distilled water. Surface sterilized seeds of *Arabidopsis thaliana* were sown (~10 seeds) on sterile soilrite and pots were transferred to temperature controlled culture room (set at 22°C) in continuous light conditions after 3 d of cold treatment. After 7 d of germination, a 48 h grown culture of *Pseudomonas putida* MTCC 5279 resuspended in 0.85% saline (final density of 10^9 CFU ml^{-1}), was used for inoculating the plants to maintain the moisture up to 40%. The control set were inoculated with same amount of sterile saline water. Plants were irrigated weekly with the nutrient solution and the plants were grown for 6 weeks. Plant growth promotional effect of the *Arabidopsis thaliana* plants grown as control and treated sets were evaluated by taking the root length, shoot length, number of branches, number of siliques and dry weight of the four plants from each replicates and each treatment has six replicates. Rhizosphere and phyllosphere colonization of MTCC5279 was evaluated as described earlier.[65]

RNA extraction and microarray analysis. Rosette leaves of 6 week old *Arabidopsis thaliana* plants were stored in RNA *later*™ (Qiagen) and stored at -80°C. Preserved leaf material of control and treated samples was used for the isolation of total RNA using RNeasy mini plant kit (Qiagen) as per manufacturer's instructions. Quality of total RNA was assessed spectrophotometrically (NanoDrop) and integrity was checked using agilent bioanalyzer (Agilent Technologies).

Ten micrograms of the total RNA was converted to cRNA using ExpressArt aminoallyl mRNA amplification Kit (AmpTec GmbH) as per the manufacturer's instruction. Amplified aminoallyl cRNA was purified and 20 µg was coupled with fluorescent Cy3 and Cy5 dye for a dual channel experiment with dye swap. Fifteen µg of labeled control and treated samples were mixed in 100 µl of the hybridization buffer and was hybridized to *Arabidopsis thaliana 25 K OciChip*™ (Ocimum Biosolutions Ltd.) on the automated Hyb station (Tecan HS4800 Pro; Tecan Groups Ltd).

Data analyses. The 24652 probes on *Arabidopsis thaliana 25 K OciChip*™ were used to compare across samples and MAD scaling was performed on each sample.[66] Differentially expressed genes were identified by outlier detection of a contaminated bivariate distribution.[67] To determine the differentially expressed candidate genes from these outliers, the fold-change value were calculated providing up and downregulated genes across the samples in respect to control. The biological significance and functional classification of differentially expressed genes was performed using Gene Ontology.

Semi-quantitative RT-PCR analysis. Validation of the transcriptome profiling experiments was performed by semiquantitative RT-PCR on selected candidate genes identified from the microarray experiments and some of the selected genes from others report.[20] Total RNA for RT-PCR verification was extracted from the liquid nitrogen frozen plant materials (control and treated) from the same set of experimental plant leaves. Total RNA was extracted with the RNeasy plant mini kit (Qiagen Sciences), according to the manufacturer's instructions using on-column DNaseI digestion. RNA preparations were first reverse transcribed for first-strand cDNA synthesis using oligo-dT primer using revertaid H^{minus} first strand cDNA synthesis kit (Fermentas). Subsequently, cDNA made were used for PCRs with the gene of interest by adding the corresponding gene specific primer combinations. The genes were amplified for 28 cycles in a PCR program, 95°C for 3 min followed by 94°C for 30 sec, 30 sec at the annealing temps (52°C-63°C), and 30 sec

for 72°C followed by the final extension of 72°C for 10 min (**Table 3**).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplementary Material

Supplementary material may be found here:
http://www.landesbioscience.com/journals/psb/article/18957

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