Pranav *et al. Annals of Microbiology* (2024) 74:40
https://doi.org/10.1186/s13213-024-01784-5

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE Open Access

Genome-wide identification of root colonization fitness genes in plant growth promoting *Pseudomonas asiatica* employing transposon-insertion sequencing

Parameswaran Sree Pranav[1], Ramamoorthy Sivakumar[1], Vemparthan Suvekbala[2] and Jeyaprakash Rajendhran[1*]

Abstract

Background *Pseudomonas* spp. are well-studied plant growth promoters, particularly in the context of root colonization. However, the specific genetic factors that determine its fitness in the rhizosphere remain largely unexplored. This study breaks new ground by employing transposon insertion sequencing (Tn-Seq) to identify the genetic factors in *Pseudomonas asiatica* JR11 that are crucial for colonizing corn roots.

Results We created a transposon mutant library of *P. asiatica* JR11 with 91,884 insertion sites and subjected it to three consecutive enrichment cycles within the corn root system. A total of 79 genes were identified as essential for root colonization (negatively-selected), while 22 genes were found to counteract root colonization efficiency (positively-selected), with both sets being commonly present across all three cycles. These genes involve amino acid metabolism, cell wall biosynthesis, and protein functions. Additionally, we found four negatively-selected and four positively-selected hypothetical proteins that consistently influenced root colonization fitness.

Conclusions The identification of these molecular determinants opens up exciting possibilities for further research. Understanding these pathways could lead to the development of novel strategies for enhancing the fitness of *P. asiatica* JR11 during corn root colonization, with potential implications for plant growth promotion and agricultural practices.

Keywords Plant-growth promoting rhizobacteria, Pseudomonas, Root colonization, INSeq, Tn-Seq, Transposon mutagenesis, Next generation sequencing

*Correspondence:
Jeyaprakash Rajendhran
jrajendhran@gmail.com; rajendhran.biological@mkuniversity.ac.in
[1]Department of Genetics, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu 625021, India
[2]Kerala State Council for Science, Technology & Environment, An Autonomous Society Under the S&T Department, Thiruvananthapuram, Kerala 695004, India

Background

The evaluation of genetic factors responsible for the rhizocompetence of the diverse community of phytobeneficial *Pseudomonas* species has been extensively studied. The employment of transposon mutagenesis and high-throughput sequencing to analyze molecular traits involved explicitly in plant root colonization would provide knowledge on the contribution of such conditionally essential genes and their associated fitness required for survival in the rhizospheric environment. The plant rhizosphere consists of a wide range of nutritional sources and chemoattraction signals that directly influence the rhizomicrobiome profile (Lareen et al. 2016). Root colonization by any bacterium in a rhizospheric environment is a complex process impacted by several biotic and abiotic factors. The primary step in successful colonization is detecting the root and subsequent movement towards it to access plant-derived nutrients. Such chemotactic response towards plant roots, followed by various signaling responses, facilitates successful colonization of *Pseudomonas* spp. (Pliego et al. 2008; Feng et al. 2019). Similarly, the direct correlation of the bacterial population in the rhizosphere with several genes that influence the initial adhesion to the root surface is well-documented (Espinosa-Urgel and Ramos 2004). The potential reports of PGPR (Plant growth promoting rhizobacterium) *Pseudomonas* spp. in the field of agriculture are steadily increasing due to its sustainability when compared with chemical fertilizers.

Pseudomonas asiatica is a member of the *P. putida* group, first isolated from clinical specimens of patients at a university hospital in Okinawa, Japan, in July 2013 (Tohya et al. 2019). A study on the genomic analysis of the strain *P. asiatica* JP233, isolated from cucumber rhizosphere soil, revealed the presence of numerous genes associated with plant growth promotion, including those involved in IAA biosynthesis, ethylene catabolism, siderophore production, abiotic stress tolerance, and phosphate solubilization (Wang et al. 2022). In addition, several studies have also reported on other members of the *P. putida* group, including *P. taiwanensis*, *P. guariconensis*, *P. entomophila*, *P. fulva*, and *P. donghuensis*; inhabiting the rhizosphere region of various plant hosts and facilitating plant growth (Cheng et al. 2021; Toro et al. 2013; Ansari et al. 2018; Pokojska-Burdziej et al. 2004; Krzyżanowska et al. 2023). However, the role of genetic factors in the cascade of biological processes that influence plant root colonization has not yet been fully understood (Hartmann et al. 2009). Therefore, this study focuses on the molecular determinants of the fitness of *P. asiatica* JR11 for root colonization.

Genome sequencing and computational analysis have opened various possibilities for analyzing such factors. Transposon Insertion Sequencing has emerged as a powerful functional tool in providing a genome-wide scale of detection of genetic factors that are attributed to a particular biological function (Goodman et al. 2009; Van Opijnen et al. 2009). Tn-Seq is an approach that combines transposon mutagenesis with high-throughput sequencing to assess an organism's fitness in a specific condition.

Previously, several studies have reported the conditionally essential genes of *Pseudomonas* spp. involved in plant root colonization using transposon insertion sequencing (Cole et al. 2017; Liu et al. 2018; Sivakumar et al. 2019). A total of 115 genes in *P. simiae* WCS417r and 269 genes in *Pseudomonas* sp. WCS365 were identified as essential for colonizing the *Arabidopsis thaliana* root system (Cole et al. 2017; Liu et al. 2018). In both of these studies, the majority of negatively-selected genes that influence the colonization of *Arabidopsis thaliana* root system are found to be functionally involved in amino acid metabolism and transport, energy production and conversion, cell motility, cell wall/membrane/envelope biogenesis, transcription and a significant number of genes lacks functional annotation. A study on *P. aeruginosa* PGPR2 evaluated the fitness determinants required for corn root colonization and revealed 108 essential genes. Similar to the previous findings, most genes are involved in amino acid metabolism and transport, energy production and conversion, cell wall/membrane/envelope biogenesis, and several genes with unknown functions (Sivakumar et al. 2019).

P. asiatica JR11, a potential plant growth-promoting bacterium isolated from the sugarcane rhizosphere, contains 31 genes associated with pyoverdine biosynthesis, transport, and regulation (Unpublished data; Genome Accession No. JBFDAD000000000). In this study, we utilized transposon insertion sequencing to unravel the fitness determinants of *P. asiatica* JR11 required for corn root colonization. The genome-wide Tn-Seq screening was conducted for three consecutive cycles. We identified key colonization-depleted (negatively-selected genes) and colonization-enriched genes (positively-selected genes) that could influence corn root colonization.

Methods

Bacterial strains and growth conditions

Pseudomonas asiatica JR11 and *Escherichia coli* were grown at 28 and 37 °C, respectively, and routinely subcultured in Luria Bertani (LB) medium. When required, the medium was solidified using 2% agar. Antibiotics were added at the following concentration (unless otherwise specified): ampicillin, 100 μg ml^{-1}; gentamicin, 40 μg ml^{-1}; irgasan, 25 μg ml^{-1}.

Construction of pSAM-BT20 vector

The transposon delivery vector, pSAM-BT20, was derived from pSAM-BT (Goodman et al. 2009; Sivakumar et al. 2019).

Construction of transposon insertion mutant library

The transposon insertion mutant library was constructed as previously described (Sivakumar et al. 2019). Briefly, the transposon library was constructed by conjugating *P. asiatica* JR11 with donor strain *E. coli* S17 λ-*pir*, harboring pSAM-BT20 plasmid. The donor and recipient strains were grown separately overnight. The donor and recipient cells were mixed at a 1:3 ratio and pelleted, then washed with 1 ml of fresh LB medium, resuspended in 100 µl of the medium, and spotted on LB agar plates without antibiotics. The plates were incubated at 28 °C for 8 h, and the cells were scraped off from the surface and resuspended in 2 ml of fresh LB medium. The suspension was inoculated on LB medium supplemented with gentamicin (30 µg ml^{-1}) and irgasan (25 µg ml^{-1}) for counter-selection against the donor *E. coli* strain, and the plates were incubated at 28 °C for 24 h. We have generated a library of ~1,00,000 mutants in which the integration of transposon into the genome was confirmed by PCR amplification from the genomic DNA of 10 random mutants, which indicated the presence of the gentamicin resistance cassette but not the transposase gene (Fig. S1). The colonies were pooled using sterile phosphate-buffered saline containing 15% glycerol, and one ml of mutant suspension was aliquoted to vials and stored at -80 °C until further use. Later, one vial was retrieved from the stock and enumerated by standard plate counting and used for further experiments.

Plant model for INSeq experiment

The corn seedlings were surface-sterilized and germinated on moist filter paper for three days, and the germinated seedlings were transferred to the gnotobiotic hydroponic plant nutrient medium (Hoagland and Arnon 1950). An aliquot of the mutant library was thawed, pelleted, and washed three times with sterile 10 mM MgSO4. The suspension was diluted to ~4×10^6 CFU ml^{-1} and inoculated onto germinated seedlings. The experiment was performed in triplicate ($n=3$). A portion of the suspension was used for genomic DNA isolation (input pool). The corn plants were maintained at greenhouse conditions for 16 h of light and 8 h of dark period. After seven days post-inoculation, the roots were aseptically excised, washed gently to eliminate weakly adhered bacterial cells, and transferred to 50-ml tubes containing 0.85% saline and ten glass beads (3 mm in diameter). The tubes were vortexed briefly to detach bacteria from the root surface (output pool of 1st cycle). Then, the output population from the 1st cycle was pooled and inoculated into 2nd set of plantlets in triplicate and maintained in above mentioned conditions. Repeatedly, the forthcoming output pool from 2nd enrichment cycle was introduced as input for 3rd enrichment cycle. Genomic DNA was isolated from all the input and output populations in 3 enrichment cycles. A detailed scheme of the workflow is shown in Fig. 1.

Tn-Seq library preparation and sequencing

Total DNA was isolated from the input and output populations using the QIAGEN DNeasy blood and tissue kit according to the manufacturer's instructions. The extracted DNA was used as a template to amplify transposon-insertion junctions with appropriate barcodes, as previously described (Goodman et al. 2011). The amplicons obtained were ligated with Ion Torrent adapters, and samples were pooled in equimolar concentration and sequenced using Ion Torrent PGM on a 318 chip.

Data analysis

Initially, the trailing transposon sequences in the 5' and 3' end reads were trimmed off from the raw reads using Fastx-Clipper v0.0.14. The adapter sequences were then trimmed using cutadapt v4.0 (Martin 2011). Fastx-Barcode splitter v0.0.14 was used to group the reads based on the barcodes of each input and output library. Once again, the cutadapt v4.0 was used to trim off the barcode sequences in the reads, and they were pooled manually based on the condition. The pre-processed 16 bp FASTA sequences were analyzed in TRANSIT software v3.2.7 (https://www.bv-brc.org/app/Tnseq) in the PATRIC Database. To identify the conditionally essential genetic regions, the conditionally essential resampling mode and *mme1* parameter were used to compare read count sums between Input vs. Cycle 1-Output, Input vs. Cycle 1-Output, Input vs. Cycle 3-Output-3 (as control vs. treatment) and mapped to the annotated genome sequence of *P. asiatica* JR11 as reference. The statistical analysis was performed within the TRANSIT software, employing a permutation test-based method to assess the differences in read counts across genomic regions between each condition and to calculate fitness scores. As a selective parameter, the genes were considered negatively-selected when they exhibited a log2FC < -2 and positively-selected with a log2FC>2, both with a statistically significant p-value<0.05, calculated using Benjamini and Hochberg procedure (DeJesus et al. 2015). The COG functional annotation was assigned to the genome sequences of *P. asiatica* JR11, *P. aeruginosa* PGPR2, *P. simiae* WCS417r, and *Pseudomonas* sp. WCS365 using eggNOG-mapper v2.1.9 (Huerta-Cepas et al. 2017). The volcano plots were generated using ggplot2 (Wickham 2011) and the circular heatmap was generated using SRplot (Tang et al. 2023).

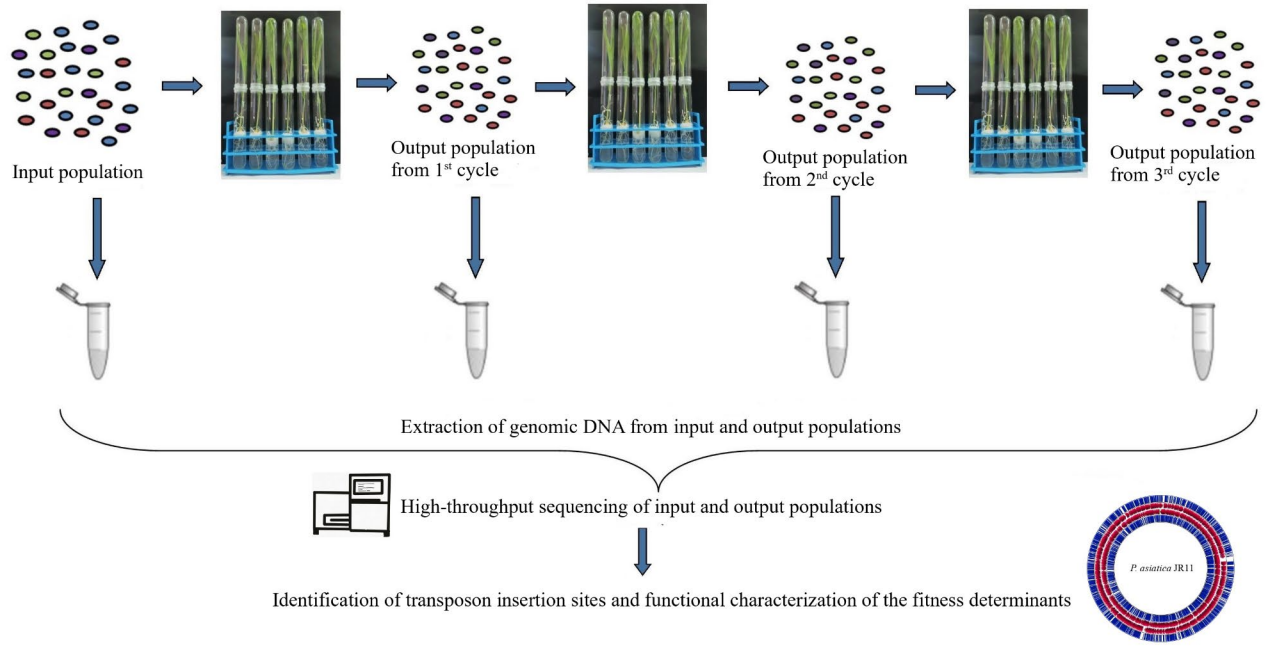

Fig. 1 A schematic representation of the overall experimental workflow for identifying fitness determinants in *P. asiatica* JR11 during corn root colonization using transposon insertion sequencing. The figure illustrates the novel approach of this study, which involves serially propagating the input population through three enrichment cycles. Following this, DNA extraction and high-throughput sequencing of both input and output populations are performed using the Ion Torrent platform to identify and functionally characterize the conditionally essential genes

Root colonization assay

The surface sterilized and germinated corn seeds were transferred to a gnotobiotic hydroponic medium (Hoagland and Arnon 1950) (50 ml) and kept for 7 days with a 16:8 light: dark cycle. After 24 h, each corn plantlet grown in a hydroponic system was inoculated with ~4×10^6 CFU (OD600=0.2) of *P. asiatica* JR11. The inoculum was prepared by diluting the overnight culture of the strains to 0.2 OD600 in 10 mM magnesium sulfate. The roots of 7-day-old plants were recovered, and their fresh weight was recorded. The bacterial cells colonized on the root surface were vortexed with glass beads and 20 ml of 0.85% saline. The 100ul suspension containing the colonized bacteria was serially diluted and platted on LB agar, and incubated at 28° C for 48 h. The normalization of bacterial cell count was carried out based on the root weight, and the colony-forming units (CFU) were expressed as logs (CFU/g of fresh root weight).

Results

Tn-Seq identified colonization fitness determinants in *P. asiatica* JR11

The *P. asiatica* JR11 mutant library was generated to investigate the fitness determinants responsible for corn root colonization from approximately ~ 1,00,000 mutants with a sum of 91,884 insertion sites found among 5,533 protein-coding genes, having an approximate average of 16 insertions per gene. A total of 1,106,858 raw reads were obtained from Ion Torrent 318 Chip sequencing. Of these, 1,104,775 reads (99.8%) contained adapter sequences and were retained for further analysis. Based on the barcodes, the reads were binned into four groups (Input, Output-1, Output-2, and Output-3) and analyzed using the TRANSIT tool, as described in the methods. The genome-wide map representing the random transposon insertion across the *P. asiatica* JR11 genome is depicted in Fig S2. Each corn seedlings in the gnotobiotic system were inoculated with ~4×10^6 CFU of the transposon insertion mutant library. The genes essentially contributing to the colonization fitness were evaluated by the comparison of the number of reads with insertion sites in the output population to the number of reads in the input population, with a minimum threshold of $\log_2$fc < -2 for negatively-selected genes and $\log_2$fc > 2 for positively-selected genes with p-value < 0.05 (Fig. 2). The genome-wide Tn-Seq screen of all three cycles led to the identification of an overall of 977 colonization-depleted genes or negatively-selected genes (17.7% of protein-coding genes in *P. asiatica* JR11) upon the insertion of the transposon resulting in reduced root colonization. Further, 59 colonization-enriched genes or positively-selected genes (1% of protein-coding genes in *P. asiatica* JR11) (Table S2) exhibited enhanced colonization ability upon transposon insertion. A total of 79 colonization-depleted genes and 22 colonization-enriched genes were consistently found across all three cycles (Table S1), i.e.,

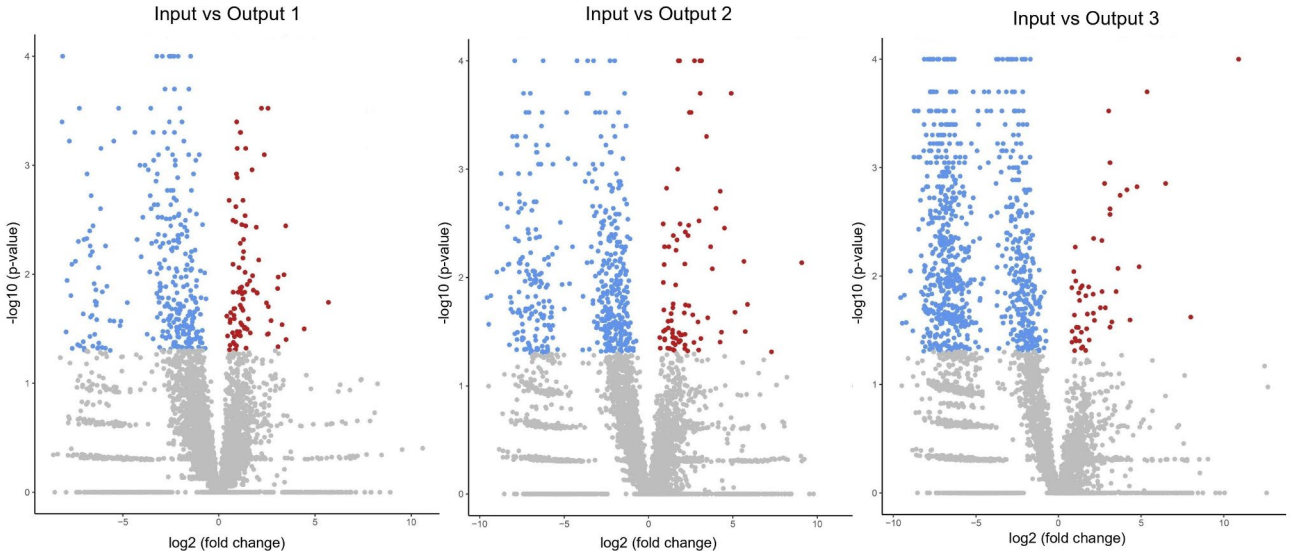

Fig. 2 Volcano plot representing the fitness of *P. asiatica* JR11 mutants during corn root colonization is presented. The plot displays the log2 fold-change on the x-axis and statistical significance on the y-axis for the negatively-selected (blue) and positively-selected (red) genes across three conditions

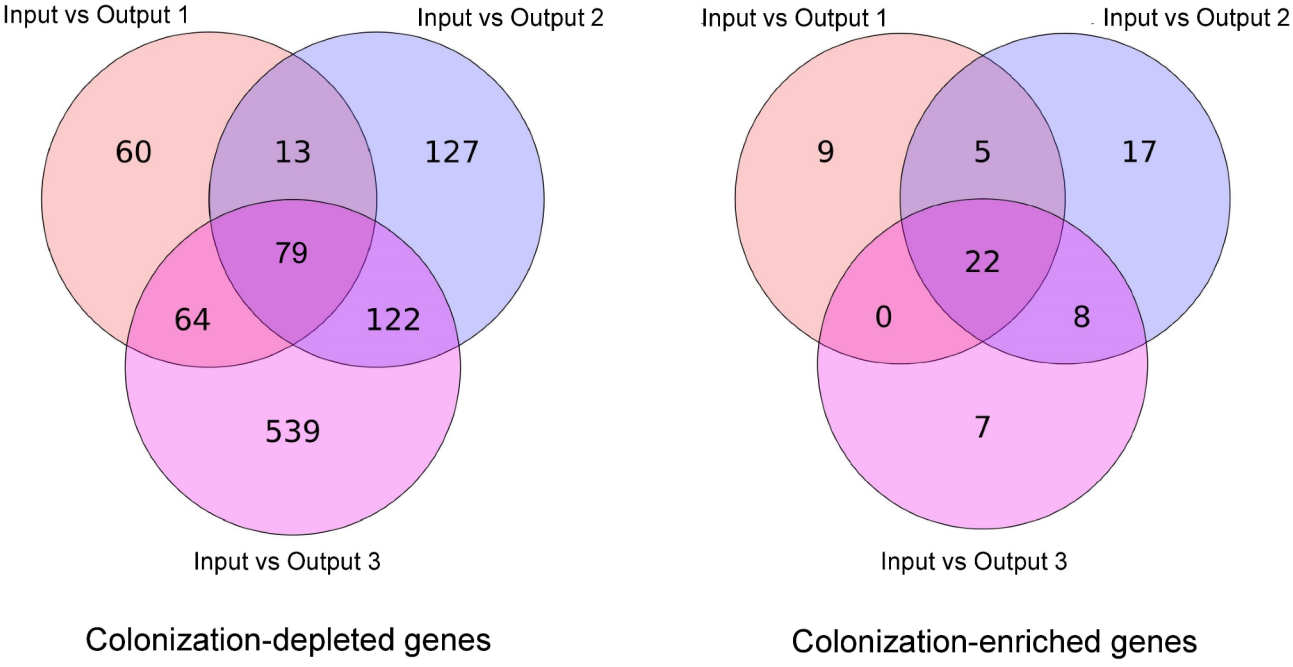

Fig. 3 A Venn diagram depicting the total number of unique and shared genes that negatively (colonization-depleted) or positively (colonization-enriched) regulate the fitness of *P. asiatica* JR11 during corn root colonization across three experimental cycles (Input vs. Output-1, Input vs. Output-2, and Input vs. Output-3) was generated using the Multiple List Comparator (www.molbiotools.com/listcompare.html)

Input vs. Output-1, Input vs. Output-2, and Input vs. Output-3) as shown in Fig. 3. These genes were then functionally classified based on Cluster of Orthologous Groups (COG) annotation system. The functional categorization of all negatively- and positively-selected genes identified in this study in each cycle is provided in Fig. S3.

Root colonization-depleted genes in *P. asiatica* JR11

The functional categorization of the commonly present 79 significantly underrepresented fitness genes (Supplementary Table S1) (Fig. 4) revealed that the majority of the negatively-selected essential genes are involved in amino acid transport and metabolism, class of function unknown, transcription, energy production, and conservation. Several other genes responsible for maize root

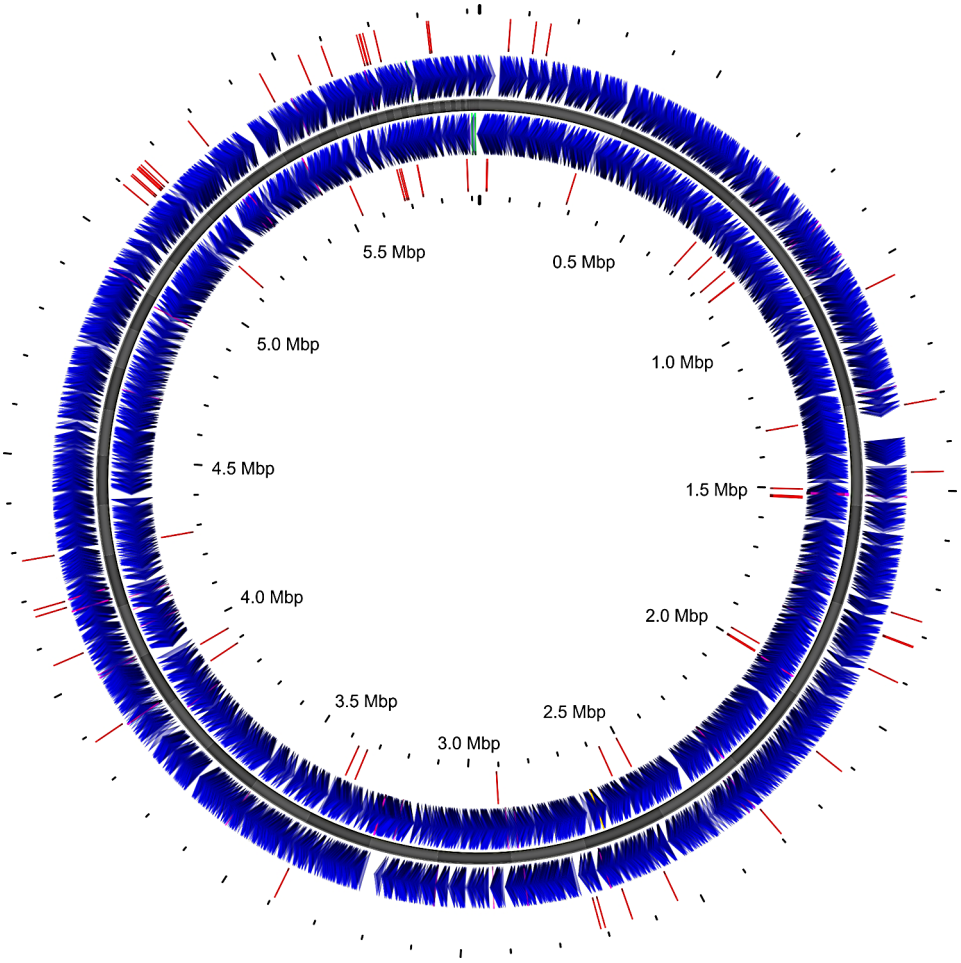

Fig. 4 The genome-wide map showing the distribution of negatively-selected conditionally essential genes commonly found in all three cycles in *P. asiatica* JR11. The red bars in the inner and outer circles represent the transposon insertion sites. The outer and inner circles (blue) represent the forward and reverse strands, respectively

colonization linked with other functional categories are represented in Fig. 5.

Metabolism

An overall 17% of genes were classified under the category metabolism. Among them, 16 genes are involved in amino acid transport and metabolism, which is of crucial importance to corn root colonization. Mutants with the transposon insertions in the genes *ilvD*, *ilvE*, *ilvH*, and *leuB* required for the biosynthesis of valine, leucine, and isoleucine were underrepresented in all three cycles of root colonization with the fold-change ranging from −7.78 to -2.19. Similarly, mutants with transposon insertions in two genes involved in tryptophan biosynthesis were significantly underrepresented after corn root colonization. We have also identified that the transposon insertion in the genes *astB*, *astD*, *alaA*, *and ansB*, which are involved in the arginine succinyltransferase pathway, has shown a significantly reduced fitness with fold-change in the range of -2.09 to -6.24 in all three cycles (Table S1). The transposon insertion in the genes involved in cysteine and methionine metabolism (*metX*, *metZ*) were underrepresented in all 3 cycles of the output population with the fold change ranging from from −2.33 to -8.13. The disruption of the gene *soxG*, playing a vital role in glycine, serine, and threonine metabolism, has also displayed reduced fitness (Table S1). The transposon insertion in the gene *gltD* involved in glutamate synthesis is significantly underrepresented in all three cycles. Furthermore, three genes responsible for histidine biosynthesis were considerably underrepresented in the output populations.

The genes responsible for lipid metabolism coding for glycerol-3-phosphate dehydrogenase, acyltransferase PA1621, and oxidoreductase were identified to be essential, and mutants of these genes were significantly underrepresented with the fold-change ranging from −2.08 to -7.65. Mutants of gene coding for L-sorbosone dehydrogenase categorized under carbohydrate metabolism were significantly underrepresented. Several genes involved

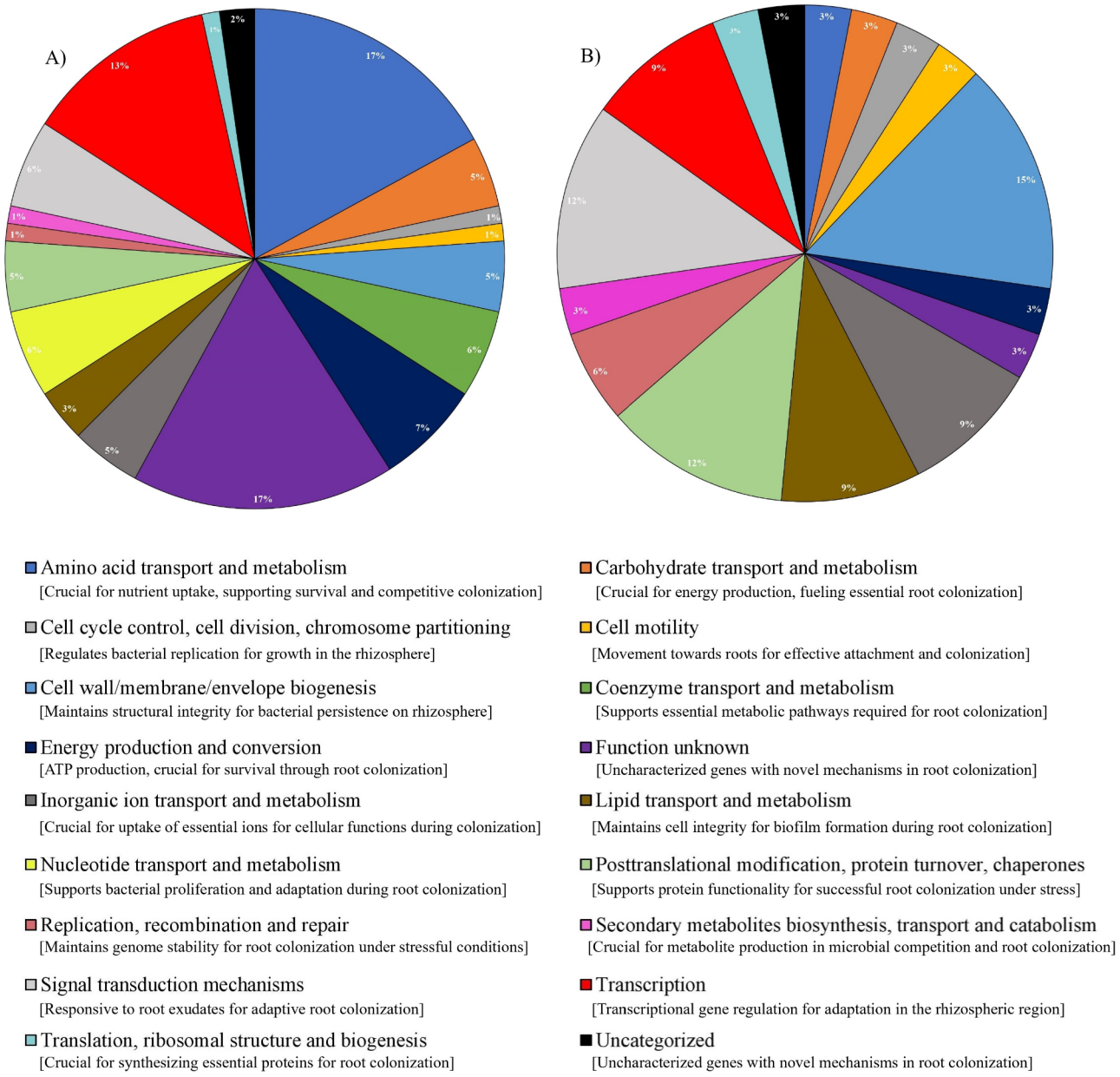

Fig. 5 The functional categorization and distribution of the COG categories of colonization-depleted genes (**A**) and colonization-enriched genes (**B**), consistently found among all three enrichment cycles in *P. asiatica* JR11

in purine metabolism were also found to be essential for root colonization. Transposon insertion in the gene *surE* of 5'-nucleotidase and *purN* coding for phosphoribosylglycinamide formyltransferase, involved in purine biosynthesis, were significantly underrepresented in the output population. The transposon insertion mutants of genes coding for transaldolase and succinyl-CoA ligase were underrepresented with the fold-change ranging from −2.09 to -7.73. The transposon insertion in the gene *dkgB* encoding 2,5-didehydrogluconate reductase involved in ketogluconate metabolism has resulted in reduced fitness in corn root colonization with fold change ranging from −4.26 to -6.66 (Table S1).

Also, mutants *nuoC* and *petB* genes, which play a vital role in oxidative phosphorylation were underrepresented with fold-change ranging from −2.21 to -7.8. The genes *cysI* and *nirB*, involved in the assimilation of sulfate and nitrite, respectively, were significantly underrepresented upon transposon insertion. Additionally, the transposon insertion in the genes *bioC*, *pabA* and *cobC* coding for vitamin B7, B9 and B12 respectively, has resulted in depleted colonization fitness with the fold-change ranging from −2.31 to -7.92.

Motility and extracellular structures

Transposon insertion in *cheR*, a crucial gene involved in the catalysis of the methylation of chemotaxis receptors in the cytoplasmic domain, has resulted in a significant reduction in the colonization fitness with the fold-change ranging from −2.39 to -3.31. Mutants with transposon insertion in *nlpD* gene responsible for activating peptidoglycan amidases were significantly underrepresented. Besides, the transposon insertion in the genes *rlpA*, *prc* and a putative glycosyl transferase identified under the category of cell wall biosynthesis and processing has led to an observable decrease of fitness in the output populations of JR11 in all three cycles (Table S1).

Stress response and detoxification

The disruption of genes *clpX* and *clpP*, coding for ATP-dependent Clp proteases involved in the degradation of misfolded and damaged proteins, showed a significant reduction in fitness fold change ranging from −2.53 to -5.96. Transposon insertion in the gene *ahpD* encoding alkylhydroperoxidase, responsible for detoxifying peroxides during oxidative stress, has reduced fitness. Also, the insertion in the gene *lon* has decreased fitness, with the fold-change ranging from −2.1 to -5.7 among all three cycles. This gene plays a crucial role in maintaining cellular integrity by degrading short-lived regulatory proteins and damaged proteins.

Signal transduction

Transposon insertion in *colS* two-component system (TCS) sensor histidine kinase was significantly underrepresented with the reduction in the colonization fitness (Table S1). The disruption of a gene encoding for an osmosensitive K+channel histidine kinase (KdpD), which is responsible for stimulus perception and signaling with a response regulator KdpE as a part of TCS, has shown a decrease in fitness with the fold change ranging from −2.26 to -3.96. Further, transposon insertion in the gene coding for RNA polymerase-binding transcription factor has reduced fitness, with the fold-change ranging from −3.07 to -7.7.

Transcriptional regulators

A total of nine transcriptional regulators were identified as essential fitness factors required for corn root colonization by *P. asiatica* JR11. Mutants with the transposon insertions in the genes coding AraC, AcrR, LysR, RpiR, LacI transcriptional regulator were significantly underrepresented with fold-change ranging from −2.12 to -8.35. Further, the insertion in *cysB*, which acts as a positive regulator required for cysteine biosynthesis, has been significantly underrepresented. The disruption of two-component transcriptional response regulator of OmpR family exhibited decreased fitness. Furthermore, the mutants with the disruption in gene *pdhR* coding for a lactate-responsive regulator and a DNA-binding transcriptional regulator of the MocR family have been significantly underrepresented (Table S1).

Transporters

The transposon insertion in two ABC transporters significantly reduced the fitness. Mutants with disruption of *kdpA* gene involved in ATP-driven potassium transport were significantly underrepresented in the output population. The gene *yffB* coding for a glutathione-dependent thiol reductase was significantly underrepresented in all three cycles (Table S1). The gene *perM* coding for putative permease responsible for the mediation of transport of autoinducer-2 involved in quorum sensing has decreased fitness with fold-change ranging from −2.18 to -5.26. Besides, disruption of an uncharacterized MFS (major facilitator superfamily)-type transporter has displayed reduced fitness.

Miscellaneous genes

Four hypothetical proteins of unknown function were predicted to be significantly underrepresented, with fold-change ranging from −2.14 to -7.44 fold. The gene *ruvB* responsible for Holliday junction migration during homologous recombination has reduced fitness with the fold change of -6.39 in all three cycles (Table S1). Similarly, an uncharacterized membrane protein and pirin were found to be essential for corn root colonization with reduced fitness fold-change ranging from −2.34 to -7.69. The insertion in gene *yeiH* has reduced colonization fitness, with the fold-change ranging from −2.83 to -6.65. Mutants with transposon insertion in a gene involved in the deo xylulose pathway of isoprenoid biosynthesis were significantly underrepresented. The disruption of gene *yhdP* coding for a protein of unknown function has decreased the colonization fitness (Table S1).

Root colonization-enriched genes in *P. asiatica* JR11

An overall of 22 conditionally-enriched genes were identified among all three cycles. Transposon mutation of these genes enhanced corn root colonization fitness. The majority of these genes belong to the classes cell wall/membrane/envelop biogenesis, transcription, signal transduction, inorganic ion transport, and metabolism (Fig. 3). Mutants of *barA* signal transduction histidine-protein kinase gene and its response regulator *uvrY* were significantly overrepresented, with the fold-change ranging from 5.24 to 10.89. These genes are well-known to be involved with adaptive response regulation in stressful environmental conditions, cell division, carbon metabolism, iron metabolism and pili formation. Disruption of RNA polymerase sigma factor RpoS coding gene, a well-known transcriptional regulator of several genes during

oxidative stress response and carbon-deprived stress conditions, has enhanced the colonization fitness, with the fold-change ranging from 7.8 to 9.06. Mutants with transposon insertions in genes coding for two hypothetical proteins were significantly overrepresented, with the fold-change ranging from 2.98 to 6.93. The disruption of genes *yejA*, *yejB yejE*, and *yejF* coding for ABC transporters increased the root colonization fitness (Table S1). The transposon insertion on *bifA* and *pedS1* coding for c-di-GMP phosphodiesterase and sensory box histidine kinase/response regulator enhanced the root colonization fitness. These genes mediate cell motility and biofilm formation. The disruption of a gene involved in the degradation of carnitine, which acts as the sole source of both carbon and nitrogen, has resulted in an increased fitness with the fold-change ranging from 3.09 to 8. Transposon insertion in the genes *wblG*, *wecB*, and *galE* coding for glycotransferases involved in the utilization of uracil-diphosphate glucose has increased the colonization fitness fold ranging from 2.43 to 4.75. Mutants of RNA helicase SrmB involved in ribosome assembly were significantly overrepresented in output populations. The insertion in gene *wzx* coding for o-antigen flippase has increased fitness with the fold-change ranging from 2.56 to 4.02. Further, the transposon insertion in a hypothetical protein of unknown function and a hypothetical protein belonging under the categories cell motility and signal transduction have also displayed increased fitness fold change ranging from 2.22 to 5.06 (Table S1).

Comparison of essential genes with previously reported studies

The 79 negatively-selected genes predicted to be present in all three cycles, and other essential genes associated with colonization fitness potential (Table S2), were compared with previously published studies on *P. simiae* WCS417r and *Pseudomonas* sp. WCS365, which were examined *Arabidopsis thaliana* roots (Cole et al. 2017; Liu et al. 2018)d *aeruginosa* PGPR2, investigated in the corn roots (Sivakumar et al. 2019). This comparison of genes with annotation information provides an overview of the root colonization potential of *Pseudomonas* species, assessed through transposon insertion sequencing. Interestingly, the strain *P. asiatica* JR11 consists of a total of 511 unique negatively-selected gene sets required for plant root colonization which were not previously described in the mentioned studies (Fig. 6A). This comparison has revealed the intersection of conditionally essential genes *gltB*, *gltD*, *leuB*, *metF*, *metZ*, *trpD* involved in amino acid metabolism and the outer membrane porin *oprF*, found in *P. asiatica* JR11, *P. aeruginosa* PGPR2, and *Pseudomonas* sp. WCS365. Biotin synthase *bioB* and the gene *cbrA* work along with *cbrB* as a two-component regulatory system involved in the utilization of carbon and nitrogen sources and were found in *P. asiatica* JR11, *P. aeruginosa* PGPR2, *P. simiae* WCS417r. Additionally, the corn root colonizer *P. aeruginosa* PGPR2 shares 17 genes with *P. asiatica* JR11 (*aceA*, *aruF*, *clpX*, *flgD*, *glcB*, *glnD*, *leuC*, *mdoG*, *mdoH*, *metX*, *nuoB*, *nuoG*, *prc*, *purF*, *ruvB*, *thiG* and *trpE*) involved in amino acid metabolism, nucleotide metabolism, coenzyme metabolism, replication and repair, cell wall/membrane/envelop biogenesis, cell motility, post-translational modification, energy production and conversion, protein turnover, chaperone functions, inorganic ion transport and metabolism. The biocontrol bacterium *P. simiae* WCS417r involved in the colonization of the *Arabidopsis thaliana* root system shares 9 underrepresented colonization fitness genes (*actP*, *algR*, *fleQ*, *flgB*, *flgG*, *flhA*, *fliA*, *hutC* and *tig*) required for cell motility, transcription, cell cycle control and mitosis, inorganic ion transport and metabolism. A total of 34 genes (*acoC*, *acrA*, *apbE*, *aroE*, *arsC*, *bdhA*, *cmoM*, *comM*, *cysG*, *cysW*, *gtsB*, *hisB*, *ilvC*, *ilvD*, *ilvI*, *lon*, *motB*, *nuoF*, *petA*, *pitA*, *pykA*, *resA*, *rlmM*, *sdaA*, *serA*, *spuC*, *tal*, *tolC*, *trpA*, *ttcA*, *ugpQ*, *yheS*, *znuA* and *znuC*) previously described to be exhibiting decreased fitness by *Pseudomonas* sp. WCS365 in the rhizosphere of *Arabidopsis thaliana*, has also been predicted to be displaying reduced colonization fitness in *P. asiatica* JR11, essentially for corn root colonization (Fig. 6A). Interestingly, the comparative analysis revealed that none of the genes were commonly present across all four Tn-Seq studies investigating the root colonization potential of *Pseudomonas* spp. This could be attributed to variations in sequencing strategies, differences in plant hosts examined, or the diverse experimental conditions employed in each study. Further, we conducted a functional-level comparison of the conditionally essential genes encoded by these *Pseudomonas* spp., categorizing them based on COG functional annotation (Fig. 6B) to highlight the similarities and differences among their functional classes. The overall distribution and functional annotation of each gene identified across the *Pseudomonas* spp. are listed in Supplementary Table 3. Ultimately, the circular heatmap (Fig. 6B) summarizes the genes associated with the functional classes of amino acid metabolism and transport, carbohydrate metabolism and transport, cell cycle control and mitosis, cell motility, and cell wall/membrane/envelope biogenesis are consistently present across all four strains, highlighting their importance in plant root colonization.

Discussion

Understanding the genetic factors responsible for the rhizocompetence of phytobeneficial bacteria is crucial for developing bioinoculants. *P. asiatica* JR11 is an effective root colonizer of corn plant roots isolated from the sugarcane rhizosphere (Unpublished data). In this study,

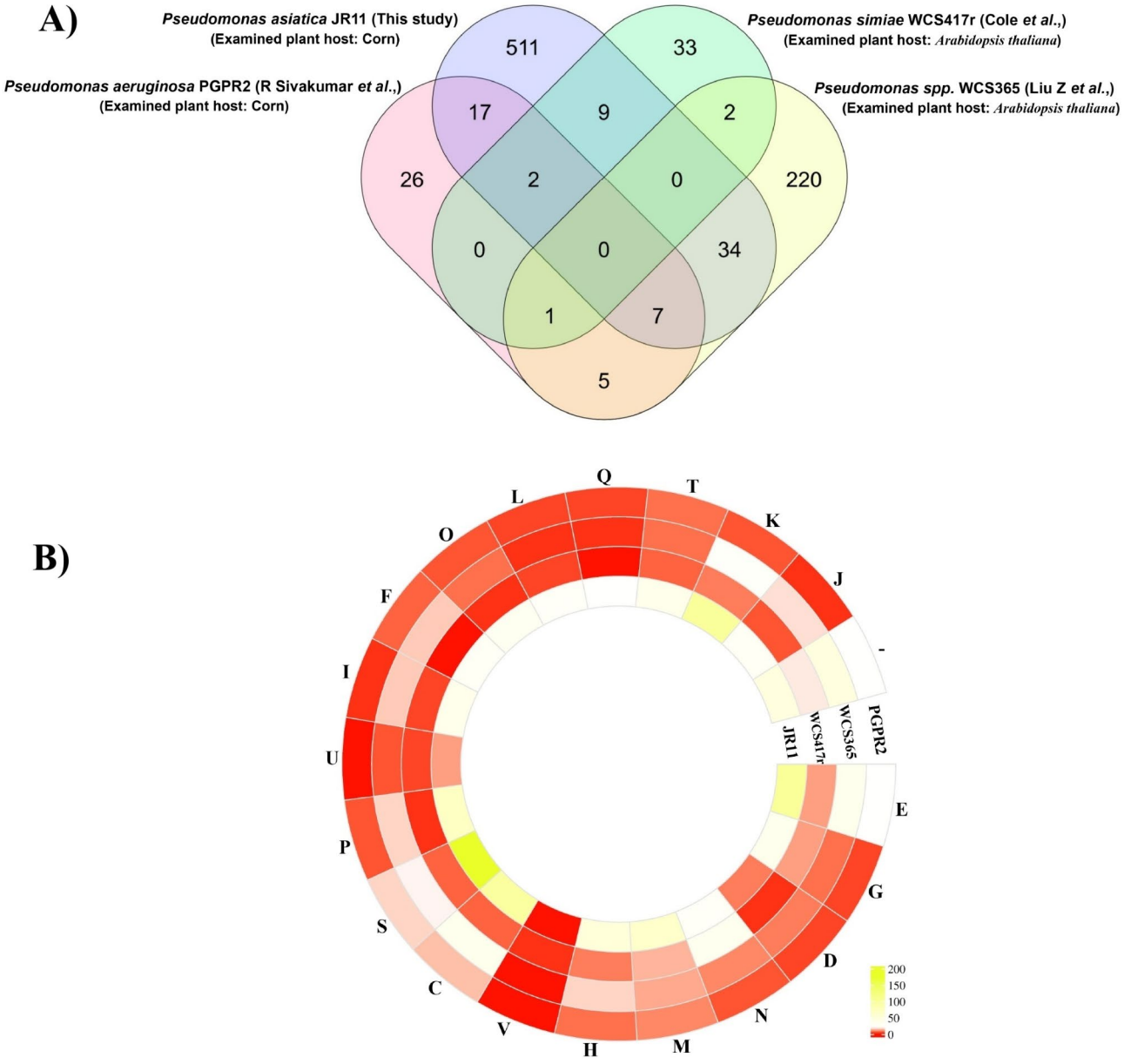

Fig. 6 **A**) Comparison of negatively-selected conditionally essential genes in *P. asiatica* JR11 with previously reported conditionally essential genes of *P. aeruginosa* PGPR2, *P. simiae* WCS417r, and *Pseudomonas* sp. WCS365, providing insights into common and unique colonization fitness determinants identified in *Arabidopsis thaliana* and corn root systems. The Venn diagram was generated using the Multiple List Comparator (www.molbiotools.com/listcompare.html). **B**) Circular heatmap illustrating the distribution of colonization-depleted genes essential for plant root colonization in identified *Pseudomonas* spp. The heatmap displays the following functional categories: E - Amino Acid metabolism and transport, G - Carbohydrate metabolism and transport, D - Cell cycle control and mitosis, N - Cell motility, M - Cell wall/membrane/envelop biogenesis, H - Coenzyme metabolism, V - Defence mechanisms, C - Energy production and conversion, S - Function Unknown, P - Inorganic ion transport and metabolism, U - Intracellular trafficking and secretion, I - Lipid metabolism, F - Nucleotide metabolism and transport, O - Post-translational modification, protein turnover, chaperone functions, L - Replication and repair, Q- Secondary Structure, T- Signal transduction, K -Transcription, J - Translation and "–" indicating no functional COG annotation for the conditionally essential genes

transposon insertion sequencing (Tn-Seq) has unraveled the genes that are responsible for the colonization under rhizospheric conditions, which resulted in the identification of an overall of 977 negatively-selected genes and 59 positively-selected genes with unique insertion sites across the genome sequence of *P. asiatica* JR11. Figure 7 illustrates a model that summarizes the key findings of this study, highlighting the colonization-depleted and enriched genes identified in *P. asiatica* JR11, which are essential for corn root colonization. In this study, the genes that confer fitness potential in the rhizospheric environment are predicted to intersect with all three

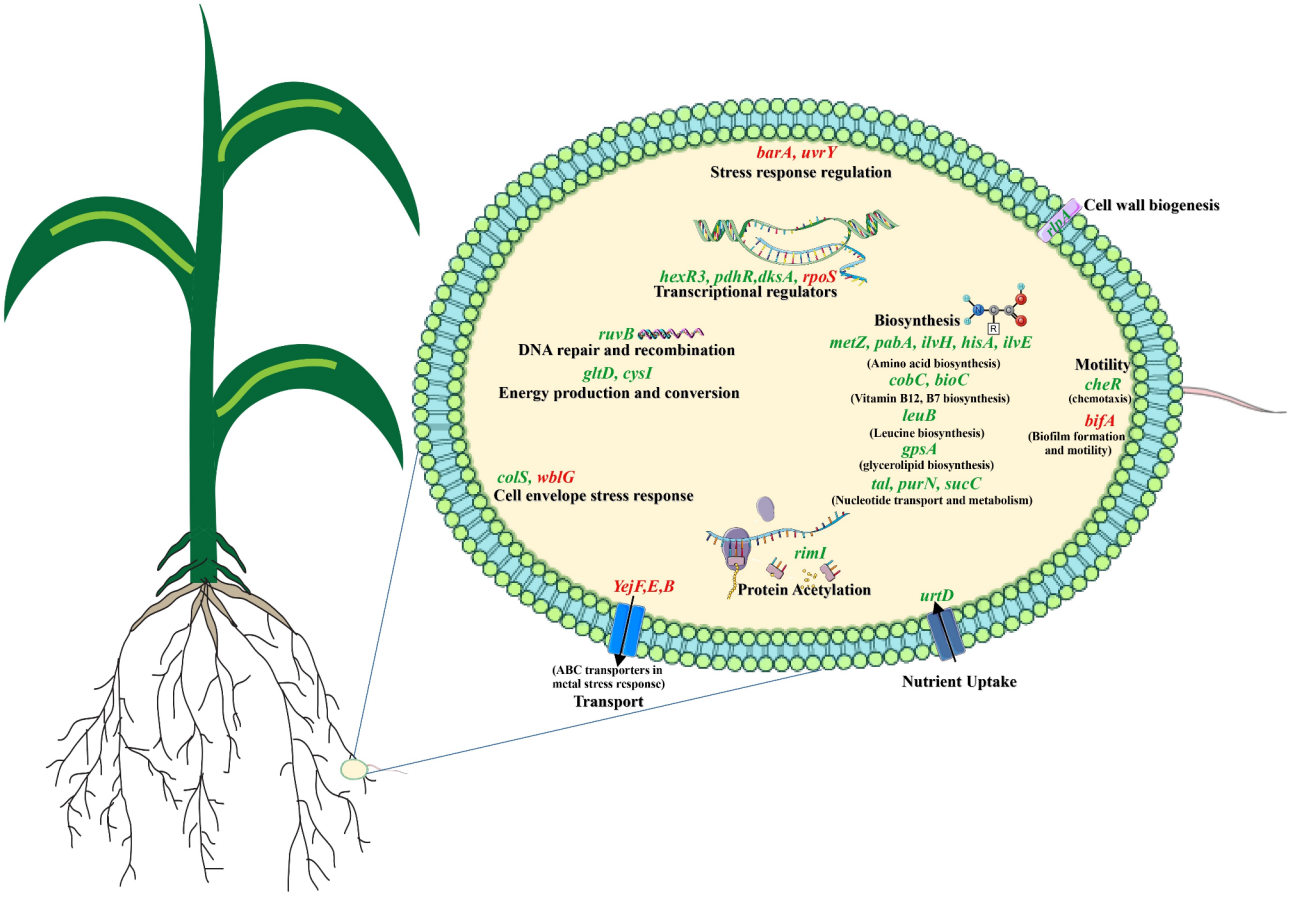

Fig. 7 A model depicting roles of colonization-depleted and colonization-enriched genes of *P. asiatica* JR11 during corn root colonization. Colonization-depleted (green) are essential for root colonization and are primarily involved in amino acid biosynthesis, nucleotide biosynthesis, glycerolipid biosynthesis, transcriptional regulation and vitamin biosynthetic pathways. Colonization-enriched (red) genes counteracting the root colonization and are mainly associated with the cyclic-di-GMP signalling pathway, ABC transporter pathways, and a two-component regulatory system. This figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license (https://creativecommons.org/licenses/by/3.0/). The root illustration is adapted from Illustrations P (2017) Root illustrations (https://doi.org/10.6084/m9.figshare.c.3701038.v13)

cycles and were functionally categorized. In this study, the genes that confer fitness potential in the rhizospheric environment are predicted to intersect with all three cycles and were functionally categorized. Most of the genes identified in this study participate in amino acid metabolism (102 negatively-selected genes out of 977 genes found across the three cycles). In general, amino acids typically make up the majority of the carbon and nitrogen rhizodeposits found in the rhizosphere. Numerous studies have revealed that amino acids play a vital role, resulting in decreased root colonization fitness of the auxotrophic mutants (Moe 2013). In this investigation, the genes *leuB*, *ilvH*, *ilvE*, *ilvD*, *hisA*, *hisH*, *mazG*, *trpD*, *trpE*, and *argD* exhibited diminished fitness fold, involved in the biosynthesis of leucine, isoleucine, valine, histidine, tryptophan, and arginine. In one study, auxotrophic mutants of the aforementioned amino acids in *P. fluorescens* WCS365 have resulted in poor root colonization ability in tomato and potato plant roots (Simons et al. 1997). Similarly, the genes responsible for the biosynthesis of those amino acids are also indicated in *P. aeruginosa* PGPR2, *P. simiae* WCS417r, and *Pseudomonas* sp. WCS365, as their auxotrophic mutants, likewise contributed to enhanced root colonization. The cysteine and methionine metabolism genes *metX* and *metZ* in *P. asiatica* JR11 have similarly been found to be essential for root colonization in *P. aeruginosa* PGPR2 and *Pseudomonas* sp. WCS365. The genes *bioC* and *cobC* involved in the biosynthesis of biotin and cobalamin, respectively, have resulted in reduced fitness during root colonization. The biotin and cobalamin (vitamin B7 and B12) auxotrophs of *P. fluorescens* strain 267.1 showed a decrease in colonized bacterial population on the surface of clover roots compared with the wild-type strain (Marek-Kozaczukv and Skorupska 2001). The glycerol-3-phosphate dehydrogenase involved in glycerophospholipid metabolism, identified in this study as a negatively-selected fitness determinant, has also hampered the root colonization

ability of *Pantoea stewartia* in corn xylem (Royet et al. 2019). The purine metabolism gene *purN* was found to be underrepresented in *P. asiatica* JR11 and was also mentioned in *P. simiae* WCS417r to show reduced fitness during root colonization. Additionally, the genes *purF* and *purL* mutants in purine metabolism were found to be associated with decreased colonization fitness in *P. aeruginosa* PGPR2, *P. simiae* WCS417r, and *P. stewartia* (Royet et al. 2019. Transaldolase, a key component in the pentose phosphate pathway, necessarily known for maintaining cellular redox homeostasis and serving as a precursor to cellular biosynthetic metabolism, has been found to exhibit reduced fitness in *P. asiatica* JR11 during corn root colonization (Stincone et al. 2015). In common, ABC transporters are transmembrane proteins known to be involved in the transport of carbohydrates, lipids, and the extrusion of cytotoxic compounds with a direct impact on plant growth promotion (Do et al. 2018). A total of 30 different negatively-selected and two positively-selected ABC transporters have been identified to be having decreased colonization fitness in *P. asiatica* JR11 found across the three cycles. In *P. asiatica* JR11, the gene *nuoC* has been found to be crucial for corn root colonization. This gene is involved in oxidative phosphorylation, which produces the ATP required for several cellular processes with direct implications on plant root colonization. Previously, the transposon insertion in *nuo* operon reported in *P. fluorescens* WCS365 (Carvajal et al. 2002) and in *P. aeruginosa* PGPR2 was found to have an impact on diminished root colonization. Further, *P. asiatica* JR11 comprises a total of 25 positively-selected and four negatively-selected genes involved in chemosensory metabolic pathways and chemotaxis towards plant roots. The *clpP* gene previously reported to be required for the initiation of biofilm formation in *P. fluorescens* WCS365 mutant was also identified in *P. asiatica* JR11 with reduced colonization fitness (O'Toole and Kolter 1998). The gene *nirB* contributes to nitrate assimilation has resulted in reduced colonization fitness. Similarly, the impact of nitrogen assimilation in the host root colonizing plant pathogen *R. solanacearum* was reported earlier (Dalsing and Allen 2014). Apart from the genes with known functions, 131 negatively-selected and 17 positively selected hypothetical proteins with no annotation information were identified. Of these, four negatively-selected and four positively-selected hypothetical proteins were commonly found among all three cycles. Besides the genes consistently identified across all three conditions, several sets of negatively-selected and positively-selected genes were detected in specific conditions not identified in the other two conditions. From the 215 genes identified in the first enrichment cycle, 91 were carried over to the second. In the second enrichment cycle, out of 340 negatively-selected genes, 200 were carried into the third, which originally included a total of 803 negatively selected genes. This progression highlights both the retention and absence of specific genes across cycles. For instance, the negatively selected genes *serA*, *ccoP*, *queG*, *sucA*, and *dgcB* (involved in energy production), *hisC*, *ybiB*, *gltB*, and *metF* (amino acid transport), and *apbE*, *metH*, *PA1766*, *pdxB*, and *ilvI* (coenzyme metabolism) were identified with significant fitness scores in the first cycle but were absent in the second. This pattern highlights the need to conduct multiple enrichment cycles to fully identify and functionally characterize conditionally essential genes in *P. asiatica* JR11. Each cycle reveals distinct essential genes vital for corn root colonization, reinforcing the importance of this approach. Further details on the functional categorization of the genes identified across all three cycles are presented in Fig. S3. A minor limitation of this study is that all in-planta enrichment experiments were conducted in a sterile hydroponic system, which contrasts with the dynamic natural rhizospheric environment that presents various challenges to successful plant root colonization. In a study investigating the expression of genes during the root colonization of *P. putida* KT2440 in the rhizosphere of *Pinus halepensis*, *Quercus ilex*, *Cupressus sempervirens*, and *Rosemarinus officinalis*, researchers identified the activation of various metabolic genes essential for its successful colonization (Fernández et al. 2013). Interestingly, the genes *putA* (proline dehydrogenase, involved in energy production and conservation), *trpF* (involved in amino acid transport and metabolism), and *clpA* (involved in stress response) found in *P. putida* KT2440 also exhibiting reduced fitness during corn root colonization in *P. asiatica* JR11 (Supplementary Table 2), validating its survival and successful colonization in the rhizospheric environment. Similarly, a study using in vivo expression technology to investigate the colonization potential of the same organism in the maize rhizosphere revealed the presence of *aceA* (isocitrate lyase) and *aceE* (pyruvate dehydrogenase), both involved in energy production and conservation. Additionally, transcriptional regulators *asnC*, *araC*, and *colS* (integral membrane sensor histidine kinase) were identified (Ramos-González et al. 2005). All of these negatively-selected genes are essential for maize root colonization by *P. asiatica* JR11 (Supplementary Table 2), further validating its colonization potential in both sterile hydroponic and rhizospheric soil environments.

Conclusions

Through the Tn-Seq approach, we identified fitness genes associated with root colonization in *P. asiatica*. Similarly, certain genes counteracting fitness were also identified. Further investigation and molecular characterization of these genes would help us understand the pathways

contributing to their fitness during corn root colonization. Eventually, better root colonizing strains can be generated through gene editing tools.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13213-024-01784-5.

Supplementary Material 1: Fig. S1. Confirmation of transposon integration in P. asiatica JR11 mutant library. The Lane M indicates 1 kb molecular weight DNA marker (Thermo Fisher Scientific, USA); "-" indicates negative control, "+" indicates positive control (pSAM_BT plasmid DNA), and the following numbered lanes indicate the number of samples.

Supplementary Material 2: Fig. S2 The genome-wide map of P. asiatica JR11 showing the overall distribution of transposon insertion sites throughout the genome. The outer and inner circles (red) represent the forward and reverse strands, respectively. The blue bars in both the outer and inner circle represents the transposon insertion sites present in the forward and reverse strands, respectively

Supplementary Material 3: Fig. S3 The functional categorization and distribution of the COG categories of colonization-depleted genes and colonization-enriched genes, identified in all three enrichment cycles in P. asiatica JR11.

Supplementary Material 4: Table S1. Details on negatively- and positively-selected genes, which regulate the fitness of P. asiatica JR11, commonly identified among all three cycles during corn root colonization. The table includes read counts, gene annotation information, fitness scores (log2 fold change values), p-values, and COG functional categories for both negatively- and positively-selected genes.

Supplementary Material 5: Table S2. Details on negatively and positively-selected genes that regulate the fitness of P. asiatica JR11, identified across all three cycles during corn root colonization. The table provides read counts, gene annotations, fitness scores (log2 fold change) and p-values of all the colonization-related genes identified in this study.

Supplementary Material 6: Table S3. A comprehensive comparison of colonization-depleted genes identified Pseudomonas asiatica JR11, Pseudomonas aeruginosa PGPR2, Pseudomonas simiae WCS417r, and Pseudomonas sp. WCS365, with their COG functional categories.

Acknowledgements
The authors acknowledge the Science and Engineering Research Board (SERB), New Delhi, for providing financial support under the SERB-Core Research Grant (CRG) Scheme (File No. CRG/2020/003287). The authors also gratefully acknowledge the MKU-RUSA, UGC-NRCBS, DST-PURSE, and DST-FIST programs of Madurai Kamaraj University.

Author contributions
PSP, RS, VS, and JR designed the experiments; PSP, RS, and VS did experiments; PSP and JR wrote the manuscript; RS, VS, and JR edited the manuscript; all authors have read and approved the manuscript.

Funding
This research was funded by the Science and Engineering Research Board (SERB), New Delhi, under the SERB – Core Research Grant (CRG) Scheme (File No. CRG/2020/003287).

Data availability
The whole genome sequence of *P. asiatica* JR11 is available at DDBJ/ENA/GenBank under the accession number JBFDAD000000000. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not Applicable: No animal or human samples were used in this study.

Consent for publication
Not Applicable: No personal data are used in this manuscript.

Competing interests
The authors declare that they have no competing interests.

Received: 19 August 2024 / Accepted: 14 November 2024
Published online: 27 November 2024

References

Ansari FA, Ahmad I (2018) Biofilm development, plant growth promoting traits and rhizosphere colonization by *Pseudomonas Entomophila* FAP1: a promising PGPR. Adv Microbiol 8:235–251. https://doi.org/10.4236/aim.2018.83016

Carvajal MMC, Wijfjes AH, Mulders IH, Lugtenberg BJ, Bloemberg GV (2002) Characterization of NADH dehydrogenases of *Pseudomonas fluorescens* WCS365 and their role in competitive root colonization. Mol Plant Microbe Interact 15:662–671. https://doi.org/10.1094/MPMI.2002.15.7.662

Cheng C, Yu Y, Zhang X, He S, Wang Y, Guo Q, Wang J, Sun X (2021) Wheat-associated *Pseudomonas taiwanensis* WRS8 reduces cadmium uptake by increasing root surface cadmium adsorption and decreasing cadmium uptake and transport related gene expression in wheat. Environ Pollut 268:115850. https://doi.org/10.1016/j.envpol.2020.115850

Cole BJ, Feltcher ME, Waters RJ, Wetmore KM, Mucyn TS, Ryan EM, Visel A (2017) Genome-wide identification of bacterial plant colonization genes. PLoS Biol 15:e2002860. https://doi.org/10.1371/journal.pbio.2002860

Dalsing BL, Allen C (2014) Nitrate assimilation contributes to *Ralstonia solanacearum* root attachment, stem colonization, and virulence. J Bacteriol 196:949–960. https://doi.org/10.1128/JB.01378-13

DeJesus MA, Anaya-Castro A, Ranjitkar S, Schein CH, Gakhar L et al (2015) TRANSIT-a software tool for Himar1 TnSeq analysis. PLoS Comput Biol 11:e1004401. https://doi.org/10.1371/journal.pcbi.10

Do THT, Martinoia E, Lee Y (2018) Functions of ABC transporters in plant growth and development. Curr Opin Plant Biol 41:32–38. https://doi.org/10.1016/j.pbi.2017.08.003

Espinosa-Urgel M, Ramos JL (2004) Cell density-dependent gene contributes to efficient seed colonization by Pseudomonas putida KT2440. Appl Environ Microbiol 70:5190–5198. https://doi.org/10.1128/AEM.70.9.5190-5198.2004

Feng H, Zhang N, Fu R, Liu Y, Krell T, Du W, Shao J, Shen Q, Zhang R (2019) Recognition of dominant attractants by key chemoreceptors mediates recruitment of plant growth-promoting rhizobacteria. Environ Microbiol 21:402–415. https://doi.org/10.1111/1462-2920.14472

Fernández M, Duque E, Molina L, Ramos J-L (2013) In vivo gene expression of Pseudomonas putida KT 2440 in the rhizosphere of different plants. Microb Biotechnol 6:307–313. https://doi.org/10.1111/1751-7915.12037

Goodman AL, McNulty NP, Zhao Y, Leip D, Mitra RD, Lozupone CA, Knight R, Gordon JI (2009) Identifying genetic determinants needed to establish a human gut symbiont in its habitat. Cell Host Microbe 6:279–289. https://doi.org/10.1016/j.chom.2009.08.003

Goodman AL, Wu M, Gordon JI (2011) Identifying microbial fitness determinants by insertion sequencing using genome-wide transposon mutant libraries. Nat Protoc 6:1969–1980. https://doi.org/10.1038/nprot.2011.417

Hartmann A, Schmid M, Tuinen DV, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257. https://doi.org/10.1007/s11104-008-9814-y

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. Circular. California agricultural experiment station, 347 (2nd Edition)

Huerta-Cepas J, Forslund K, Coelho LP, Szklarczyk D, Jensen LJ, Von Mering C, Bork P (2017) Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. Mol Biol Evol 34:2115–2122. https://doi.org/10.1093/molbev/msx148

Krzyżanowska DM, Szafranski J, Koczyk G, Czarnecki O, Szulc A, Gawroński P, Mieczkowski A, Wasilewski K (2023) Host-adaptive traits in the plant-colonizing Pseudomonas donghuensis P482 revealed by transcriptomic responses to exudates of tomato and maize. Sci Rep 13:9445. https://doi.org/10.1038/s41598-023-36494-6

Lareen A, Burton F, Schäfer P (2016) 0Plant root-microbe communication in shaping root microbiomes. Plant Mol Biol 90:575–587. https://doi.org/10.1007/s11103-015-0417-8

Liu Z, Beskrovnaya P, Melnyk RA, Hossain SS, Khorasani S, O'Sullivan LR, Wiesmann CL, Bush J, Richard JD, Haney CH (2018) A genome-wide screen identifies genes in rhizosphere-associated *Pseudomonas* required to evade plant defenses. MBio 9:e00433–e00418. https://doi.org/10.1128/mBio.00433-18

Marek-Kozaczuk M, Skorupska A (2001) Production of B-group vitamins by plant growth-promoting *Pseudomonas fluorescens* strain 267 and the importance of vitamins in the colonization and nodulation of red clover. Biol Fertil Soils 33:146–151. https://doi.org/10.1007/s003740000304

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17:10–12. https://doi.org/10.14806/ej.17.1.200

Moe LA (2013) Amino acids in the rhizosphere: from plants to microbes. Am J Bot 100:1692–1705. https://doi.org/10.3732/ajb.1300033

O'Toole GA, Kolter R (1998) Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Mol Microbiol 28:449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x

Pliego C, De Weert S, Lamers G, De Vicente A, Bloemberg G, Cazorla FM, Ramos C (2008) Two similar enhanced root-colonizing *Pseudomonas* strains differ largely in their colonization strategies of avocado roots and *Rosellinia necatrix* hyphae. Environ Microbiol 10:3295–3304. https://doi.org/10.1111/j.1462-2920.2008.01721.x

Pokojska-Burdziej A et al (2004) Effect of endophytic bacterium Pseudomonas fulva on growth of pine seedlings (Pinus sylvestris), formation of mycorrhizae and protection against pathogens. Phytopathol Pol 32:33–47

Ramos-González MI, Campos MJ, Ramos JL (2005) Analysis of Pseudomonas putida KT2440 gene expression in the maize rhizosphere: in vitro expression technology capture and identification of root-activated promoters. J Bacteriol 187:4033–4041. https://doi.org/10.1128/jb.187.12.4033-4041.2005

Royet K, Parisot N, Rodrigue A, Gueguen E, Condemine G (2019) Identification by Tn-seq of *Dickeya dadantii* genes required for survival in chicory plants. Mol Plant Pathol 20:287–306. https://doi.org/10.1111/mpp.12754

Simons M, Permentier HP, de Weger LA, Wijffelman CA, Lugtenberg BJ (1997) Amino acid synthesis is necessary for tomato root colonization by *Pseudomonas fluorescens* strain WCS365. Mol Plant Microbe Interact 10:102–106. https://doi.org/10.1094/MPMI.1997.10.1.102

Sivakumar R, Ranjani J, Vishnu US, Jayashree S, Lozano GL, Miles J, Handelsman J, Rajendhran J (2019) Evaluation of InSeq to identify genes essential for *Pseudomonas aeruginosa* PGPR2 corn root colonization. G3 (Bethesda) 9:651–661. https://doi.org/10.1534/g3.118.200928

Stincone A, Prigione A, Cramer T, Wamelink MM, Campbell K, Cheung E, Olin-Sandoval V, Grüning NM, Krüger A, Tauqeer Alam M, Keller MA, Breitenbach M, Brindle KM, Rabinowitz JD, Ralser M (2015) The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. Biol Rev Camb Philos Soc 90:927–963. https://doi.org/10.1111/brv.12140

Tang D, Wang C, Wang J, Yu X, Zhao J, Cheng D, Tang J (2023) SRplot: a free online platform for data visualization and graphing. PLoS ONE 18. https://doi.org/10.1371/journal.pone.0294236

Tohya M, Watanabe S, Teramoto K, Uechi K, Tada T, Kuwahara-Arai K, Kirikae T (2019) Pseudomonas asiatica sp. nov., isolated from hospitalized patients in Japan and Myanmar. Int J Syst Evol Microbiol 69:1361–1368. https://doi.org/10.1099/ijsem.0.003316

Toro M, Ramirez-Bahena MH, Cuesta MJ, Velazquez E, Peix A (2013) Pseudomonas guariconensis sp. nov., isolated from rhizospheric soil. Int J Syst Evol Microbiol 63:4413–4420. https://doi.org/10.1099/ijs.0.051193-0

van Opijnen T, Bodi KL, Camilli A (2009) Tn-seq: high-throughput parallel sequencing for fitness and genetic interaction studies in microorganisms. Nat Methods 6:767–772. https://doi.org/10.1038/nmeth.1377

Wang L, Li H, Chen C, Li H, Yu Y, Chen F, Zhang R (2022) Genomic analysis of Pseudomonas Asiatica JP233: an efficient phosphate-solubilizing bacterium. Genes 13:2290. https://doi.org/10.3390/genes13122290

Wickham H (2011) ggplot2. Wiley Interdiscip Rev Comput Stat 3:180–185. https://doi.org/10.1002/wics.147

Publisher's note