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Short Communication

Draft genome sequence of a halotolerant plant growth-promoting bacterium *Pseudarthrobacter oxydans* NCCP-2145 isolated from rhizospheric soil of mangrove plant *Avicennia marina*

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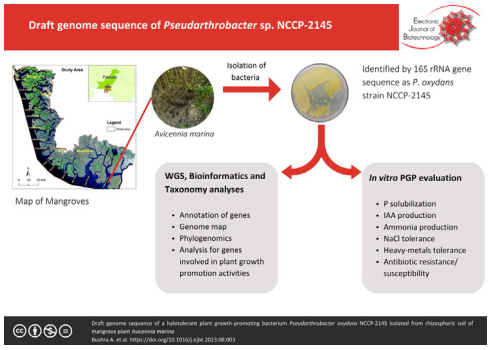
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Limited knowledge exists regarding the diversity of mangrove plant-associated *Pseudarthrobacter* species. This study presents the first draft genome and phylogenetic analysis of a *Pseudarthrobacter oxydans* NCCP-2145, isolated from the rhizospheric soil of *Aviciana marina*, a native mangrove plant found in Miani Hor, Lasbela-Baluchistan, Pakistan.
Results: The genome of *P. oxydans* NCCP-2145 comprises 4,495,869 base pairs, with a G+C content of 65.9% and 4,207 coding sequences. Genome annotation revealed the presence of multiple biosynthesis pathways. The analysis also identified genes responsible for plant growth-promoting traits, such as the synthesis of indole acetic acid, nitrogen fixation, and phosphorus solubilization. Experimental evaluations confirmed strain NCCP-2145 positive reactions for phosphorus solubilization, indole-3-acetic acid production, and ammonia production. Furthermore, strain NCCP-2145 exhibited tolerance to heavy metals (nickel, copper, and cadmium) and salinity levels up to 10% NaCl. Antibiotic susceptibility testing indicated resistance only to ceftazidime and the combination of amoxicillin/clavulanic acid. For phylogenomic analysis, strain NCCP-2145 was analyzed and compared to the closely related validly published type species *P. oxydans* DSM 20119T, revealing a similarity score of 98.64% based on 16S rRNA gene sequences and 89.1% based on DNA-DNA hybridization, confirming its classification as a member of species, *P. oxydans*.

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Conclusions: This study significantly contributes to our understanding of the genomic characteristics, functional capabilities, and potential plant growth-promoting attributes of *P. oxydans* NCCP-2145. Future research should focus on unraveling the precise mechanisms underlying its plant growth-promoting abilities and exploring practical applications in sustainable agriculture and environmental restoration.

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1. Introduction

Pseudarthrobacter species are a group of Gram-positive endophytic bacteria that belong to the family *Micrococcaceae*. These bacteria are well known for their diverse metabolic capabilities and are found in various environments, including soil, water, and extreme habitats [1]. *Pseudarthrobacter* species have been the subject of many studies due to their potential in plant growth promotion, bioremediation, production of antibiotics, enzymes, and biocatalysts [2]. To date, there are only 14 type strains of the genus *Pseudarthrobacter* (https://www.bacterio.net/, accessed on June 15th 2023). Due to the potential of utilizing inorganic and organic compounds as a metabolic substrate, these bacteria act as biofertilizers in agriculture. Moreover, many soil-dwelling *Pseudarthrobacter* species have been reported as biofertilizer because of their ability to exhibit plant growth-promoting features comprising indole-3-acetic acid (IAA) synthesis, fixation of nitrogen, and solubilization of potassium and phosphate [3,4]. In a study, *Pseudarthrobacter chlorophenolicus* BF2P4-5 showed the plant growth-promoting properties for tomato plants, suggesting that the strain BF2P4-5 could be recognized as an environmentally friendly biofertilizer for tomatoes and promote plant growth in other vital plants [5]. The flavonoid content of *Geum aleppicum* and plant growth are controlled by *Pseudarthrobacter* sp. NIBR-BAC000502770. Production of high levels of IAA by NIBR-BAC000502770 is reported in *G. aleppicum* plants treated with this PGPR strain showing improved root and shoot growth, distinguishing it as an environment friendly biofertilizer with significant benefits on small farms as well as in large-scale agriculture [2]. Here, the study presents the first draft genome of a halotolerant as well as heavy metal tolerant plant growth-promoting bacterium *Pseudarthrobacter oxydans* NCCP-2145, isolated from rhizospheric soil of mangrove plant *Avicennia marina*, a natural mangrove plant distributed in Miani Hor, Lasbela- Balochistan region of Pakistan.

2. Experiment

2.1. Isolation of bacteria

A soil sample from the rhizosphere of the mangrove plant *A. marina* was collected from Miani Hor, Lasbela-Baluchistan, Pakistan (66°31′55.88119″E, 25°27′56.07014″N). The sample was carefully stored in a sterilized plastic bag and transported to the Bioresource Conservation Institute (BCI), NARC, Islamabad inside a light-restricted container. To inoculate the rhizospheric soil, 10% NaCl was added to nutrient agar (NA) medium, and one hundred microliters of serially diluted soil from *A. marina* were utilized. The plates were subsequently kept in an incubator set at a temperature of 30°C for a duration of 24 h [6]. The isolate was accessioned in the National Culture Collection of Pakistan (NCCP) with the code NCCP-2145.

2.2. In vitro analysis of plant growth promotion characteristics

The phosphorus solubilization capability of NCCP-2145 was qualitatively assessed by cultivating the strain on Pikoyskaya agar plates. The plates were then placed in an incubator at 30°C for 7 d. As a response to bacterial growth, a distinct halo zone was observed on the Pikoyskaya medium. The solubilization efficiency and solubilization index were determined based on previous studies [7,8].

To evaluate the production of IAA, tryptone broth (1%) was used for inoculation and subjected to agitation in a shaking incubator at 30 ± 2°C for 48 h. After 48 h of culture growth, 1 mL of Kovac's reagent (consisting of p-dimethylamino benzaldehyde (DMAB) – 5 g and isoamyl alcohol – 75 mL), along with concentrated HCl – 25 mL, was added according to previous studies [9].

The catalase test was conducted on the isolate NCCP-2145 using the method according to previous studies [10]. In brief, a clean autoclaved glass slide was labeled in the center. A small amount of hydrogen peroxide solution (3%) was placed onto the slide, and a few colonies of bacteria (24 h old) were mixed with the help of an autoclaved toothpick. The presence of effervescence (gas bubbles) indicated a positive result.

A qualitative test was performed to assess the ammonia production ability of *P. oxydans* NCCP-2145, following the method outlined by Agbodjato et al. [11]. The strain was cultivated in a 10 mL peptone broth and placed in an incubator at a temperature of 36 ± 2°C for a period of 48 to 72 h. Following incubation, a 0.5 mL portion of Nessler's reagent was added to the bacterial suspension. The appearance of a brown-to-yellow color signified the production of ammonia.

The tolerance of strain NCCP-2145 to heavy metals was also evaluated using TSA medium supplemented with varying concentrations of $CdCl_2$, $CuSO_4$, and $NiCl.6H_2O$ (ranging from 0 ppm to 500 ppm). The isolate was streaked on TSA medium containing different concentrations of Cd, Cu, and Ni, and then incubated at 28°C for 24 h. Positive results were determined by visible growth of the bacteria at specific concentrations.

2.3. Antibiotic susceptibility analysis

The susceptibility of antibiotics was assessed utilizing the disc diffusion method on Muller-Hinton agar plates. Various antibiotic discs were employed, including ciprofloxacin (CIP 5 μg), amikacin (AK 30 μg), tetracycline (TE 30 μg), amoxicillin / clavulanic acid (AMC 30 μg), ceftazidime (CAZ 30 μg), levofloxacin (LEV 5 μg), septran (SXT 25 μg), rifampin (RA 5 μg) and cefpodoxime (CPD 30 μg) obtained from Oxoid, UK. Initially, the isolates were enriched in tryptic soy broth at 28°C for 48 h and standardized to a concentration of 5 × 108 cells mL^{-1}, using a 0.5 McFarland scale. Subsequently, the cultures were streaked onto sterile Muller Hinton agar (MHA) plates employing sterile cotton swab. Following that, the antibiotic discs were placed on the plates with sufficient

spacing to prevent overlap of inhibition zones. Subsequently, the diameter of inhibition zones was measured and recorded in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations [12] and recorded measurements were then categorized as resistant, intermediate, or sensitive.

2.4. Genomic DNA isolation, PCR amplification and whole-genome sequencing

The isolation of genomic DNA was performed using the PureLink Genomic DNA, Mini Kit (Thermo Fisher, USA) following the protocol for Gram-positive strains. The 16S rRNA gene was sequenced following the method described in a previous study [13]. The strain NCCP-2145 underwent whole-genome sequencing (WGS) analysis utilizing the Illumina NovaSeq PE150 platform (Illumina Inc.) at Macrogen (South Korea). To ensure data quality, the sequence reads from each dataset underwent filtration [14], only high-quality paired-end reads were used for the de novo assembly process. The assembly was performed using SPAdes v.3.13 https://cab.spbu.ru/software/spades/ [15]. To assess the genome's completeness and contamination, CheckM [16] was employed.

2.5. Bioinformatic and phylogenetic analyses

The identification of the 16S rRNA gene was conducted using EzBioCloud Server [17]. Gene annotation was predicted by utilizing several online databases, including the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg), Clusters of Orthologous Groups (COG) (https://www.ncbi.nlm.nih.gov/research/cog-project), Rapid Annotation using Subsystems Technology (RAST) (https://rast.nmpdr.org/rast.cgi), Carbohydrate-Active enZymes (CAZy) (https://www.cazy.org), and the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca). To identify clustered regularly interspaced short palindromic repeat (CRISPR) regions, CRISPRFinder (https://crispr-cas.i2bc.paris-saclay.fr) was utilized. Moreover, an evaluation of virulence and pathogenicity concerning human health was performed using PathogenFinder v.1.1, (https://cge.cbs.dtu.dk/services/PathogenFinder/).

The Genome-to-Genome Distance Calculation (GGDC 2.0, https://ggdc.dsmz.de) [18] and the orthoANI calculator on EzTaxon-e server were utilized to determine the digital DNA-DNA hybridization (digi-DDH) and average nucleotide identity (ANI) values of strain NCCP-2145, in comparison to closely related validly named type strains. Finally, a circular map of the genome of strain NCCP-2145 was generated utilizing CGView (https://cgview.ca), highlighting protein-coding and plant-growth promoting genes (Fig. 1).

3. Results and discussion

The genus *Pseudarthrobacter* has undergone reclassification from the genus *Arthrobacter* due to variations in its phylogenetic position and chemotaxonomic traits, such as polar lipid, quinones, and peptidoglycan profiles [19]. Current information available in the literature and microbial databases related to the genus *Pseudarthrobacter*, such as LPSN (https://lpsn.dsmz.de/genus/pseudarthrobacter), is limited to thirteen deposited genomes (https://www.ncbi.nlm.nih.gov/genome/?term=Pseudarthrobacter). However, until now to the best of our knowledge, there have not been any reported genome sequences for *Pseudarthrobacter* species specifically related to mangrove soil ecology. Therefore, it is essential to characterize and report a *Pseudarthrobacter* species isolated

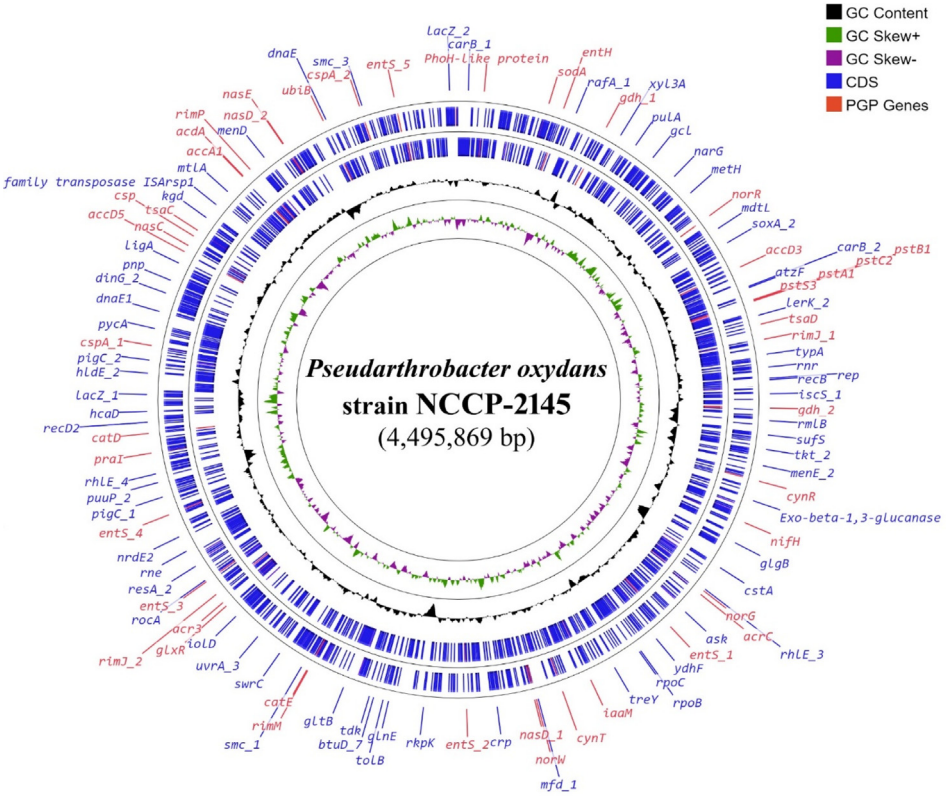

Fig. 1. A graphical circular map of *P. oxydans* NCCP-2145. From outside to center, Rings 1 and 2 are annotated protein-coding and plant growth-promoting genes, respectively. Ring 3 is representing the GC content while GC skew pattern is represented by the inner most ring where purple indicates negative values and green color indicating positive values.

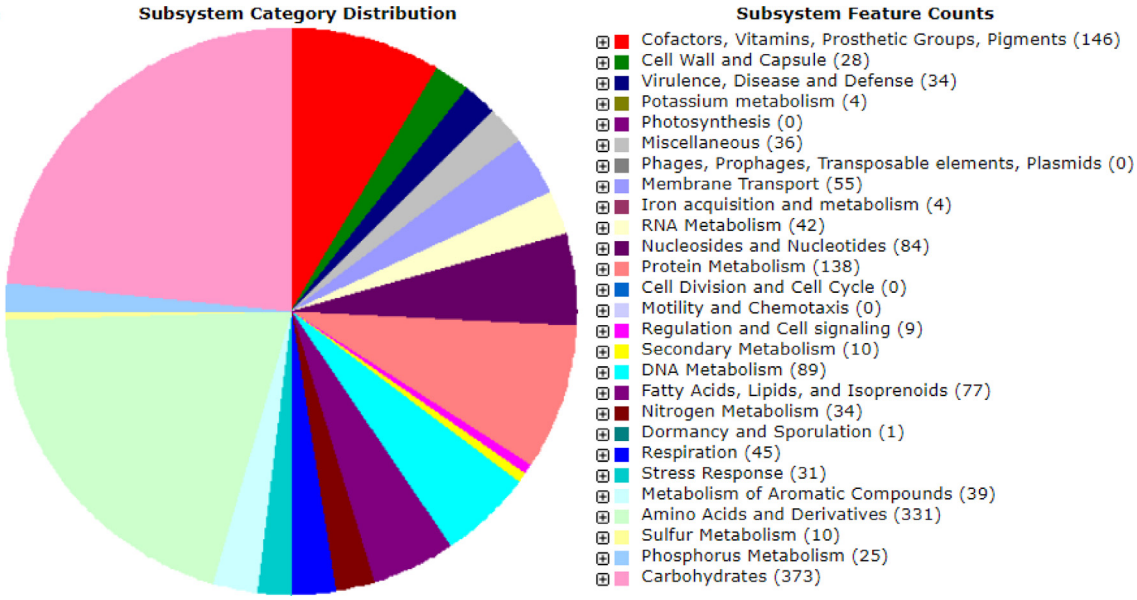

Fig. 2. An overview of functional classification of annotated genes using subsystem categories predicted in the genome of the *P. oxydans* strain NCCP-2145 based on RAST Server.

from the rhizospheric soil of the *A. marina*, a natural mangrove plant, which exhibits tolerance to high salt levels, resistance to multiple heavy metals, and having plant growth-promoting capabilities.

3.1. Genome sequence attributes and taxonomic study of P. oxydans NCCP-2145

The draft genome of *P. oxydans* NCCP-2145 has 48 contigs, a size of 4,495,869 bp and of 65.9% GC content. The sequencing yielded a total read count of 13,859,369 bp, with raw read and trimmed read statistics of, respectively, 3,952 million (M) and 4,922 M. The L50 count was 10, indicating that 10 contigs consist half of the assembled genome, while the N50 count was 161,478 bp, signifying that the contig with a size of 161,478 bp signifies the median length of the contigs. From a total of 4,259 genes, 3,952 genes were identified as protein-coding sequences (CDSs). The genome of strain NCCP-2145 also contains 53 transfer RNA (tRNA) genes, 3 ribosomal RNA (rRNA) genes and has a coverage of 140-fold. To determine the functional classification of genes in the NCCP-2145 genome, we employed the Rapid Annotation using Subsystem Technology (RAST) (https://rast.nmpdr.org/rast.cgi). The analysis revealed that a majority of the genes are engaged in carbohydrate metabolism, aligning with the bacteria lifestyle which necessitates the acquisition and mobilization of essential nutrients like phosphate, nitrogen, and iron to facilitate the symbiotic interaction between the bacteria and plants (Fig. 2). Additionally, several composite sequence search databases predicted the industrially important genes through CAZy (168), SwissProt (1315), COG (3175), MetaCyc (561), PHI (142), Pfam (3381). The KEGG database is also a valuable resource that provides a wealth of information about the functions as well as interactions of genes and genomes. Out of all the genes analyzed, 3920 were linked to KEGG pathways. These genes were classified into 23 functional categories using KEGG orthology (Fig. 3).

The strain NCCP-2145 is identified as a bacterium of the *P. oxydans* by 16S rRNA gene sequence and phylogenomic studies. 16S rRNA gene sequence (accession number: LC708055) analysis indicated that NCCP-2145 is closely related to *P. oxydans* DSM 20119^T (98.85% similarity). Moreover, the genomic diversity and taxonomic position of the species could both be elucidated by the genome-level phylogenetic analysis [20]. To comprehend the phylogenetic linkage using Type Strain Genome Server [18], the genome-based phylogeny showed that the strain NCCP-2145 is closely associated with the *P. oxydans* DSM 20119^T (Fig. 4) which has garnered significant scientific interest due to their remarkable ecological and biotechnological importance. Additionally, strain NCCP-2145 demonstrated the calculated values for digi-DDH and ANI, respectively, 89.1% and 98.64% to the closest *P. oxydans* DSM 20119^T (Table 1) that was above the cut-off values of 70% and 96% for species delineation and showed the strain NCCP-2145 to be a member of the *P. oxydans*.

3.2. Antibiotic susceptibility testing

Findings of the antibiotic susceptibility analysis demonstrated that strain NCCP-2145 was susceptible to fluoroquinolones (CIP, LEV), aminoglycosides (AK), tetracyclines (TE), third-generation cephalosporins (CPD), trimethoprim/sulfonamide (SXT), and ansamycin (RA) antibiotics. These findings are in line with previous studies [21,22,23]. However, the strain NCCP-2145 demonstrated intermediate resistance to β-lactams (AMC) and resistance to third-generation cephalosporins (CAZ), consistent with the earlier reports [24,25].

The strain NCCP-2145 was subjected to genome mining to identify antibiotic-resistant genes. The analysis, conducted with the help of the CARD database, revealed a total of 36 antibiotic resistance genes (Fig. 5). These annotated resistant genes were associated with various classes of antibiotics, such as aminoglycoside, tetracyclines, fluoroquinolone, rifamycin, and others. However, a substantial number of identified virulence factor genes (138 in total) and antibiotic resistance genes displayed less than 60% similarity, suggesting a weak homology with the genes present in the database [20]. Moreover, the strain NCCP-2145 is predicted as a non-human pathogen and no CRISPR arrays were found in its genome. The lack of phenotypic resistance to commonly employed antibiotics, along with the intrinsic nature of the resistance to specific antibiotics, strongly encourage its prospective application

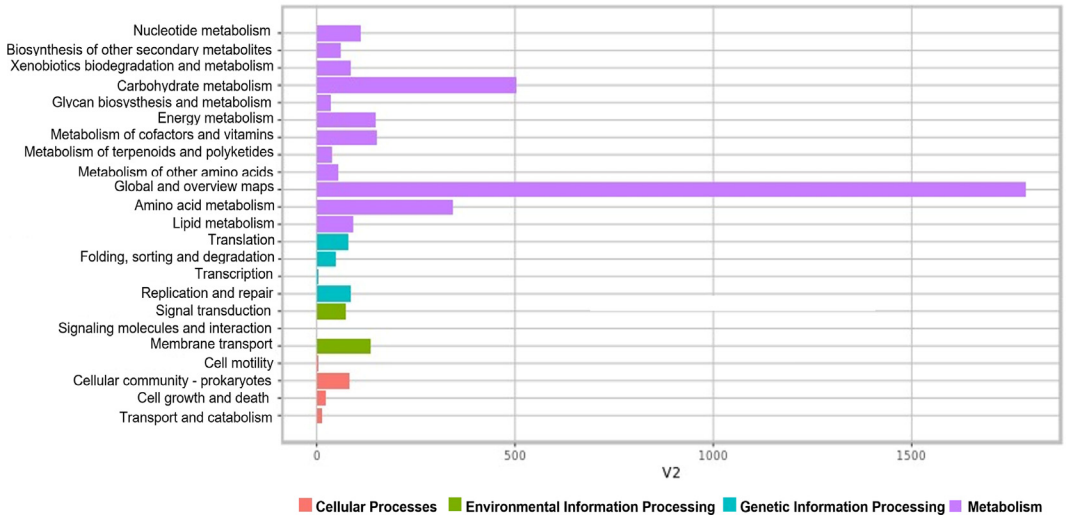

Fig. 3. The Kyoto Encyclopedia of Genes and Genomes (KEGG) function annotation of *P. oxydans* strain NCCP-2145, showcasing the genetic features and metabolic pathways.

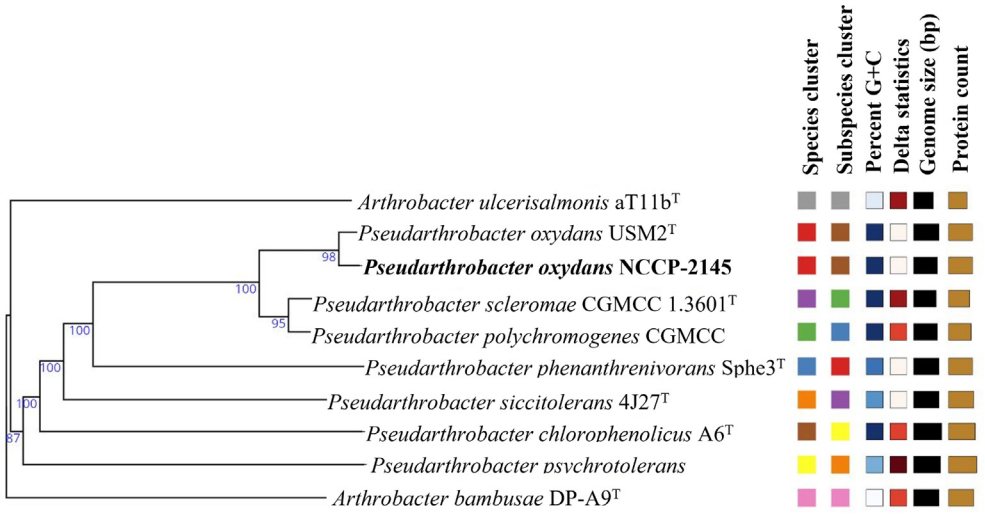

Fig. 4. A phylogenomic tree was constructed on the basis of whole genome sequences of *P. oxydans* NCCP-2145 (GCA_023708005.1) and related members of the genus *Pseudarthrobacter* inferred with FastME 2.1.6.1 Server from Genome BLAST Distance Phylogeny (GBDP) distances. The GBDP distance formula d5 is used to scale the branch lengths. The values of bootstrap support (from 100 replications) > 60% are given at nodes. The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a bioinformatics platform available at https://tygs.dsmz.de for a whole genome-based taxonomic analysis.

Table 1
Genome sequence characteristics, 16S rRNA gene sequence similarity, average nucleotide identity (ANI) and digital DNA–DNA hybridization (digi-DDH) of strain NCCP-2145 relative to the closely related type species in the genus *Pseudarthrobacter*.

Species	WGS ID	Genome Size (bp)	G + C Content (%)	Contigs	16S similarity (%)	ANI (%)	Digi-DDH (%)
P. oxydans NCCP-2145	GCA_023708005.1	4,495,869	65.92	48	–	–	–
P. oxydans DSM 20119^T	NZ_JABTYH000000000.1	4,689,826	65.7	90	98.85	98.64	89.1
P. polychromogenes DSM 20136^T	NZ_BMKU00000000	4,349,807	65.8	33	98.77	93.54	52.5
P. scleromae YH-2001^T	NZ_BMKV00000000	4,199,943	66	19	98.77	94.44	57.2
P. psychrotolerans YJ56^T	NZ_CP047898	5,170,670	64.7	3	98.11	80.01	23.3
P. phenanthrenivorans Sphe3^T	GCA_000189535.1	4,535,320	65.3	56	97.98	84.57	28.3
P. siccitolerans 4J27^T	NZ_CAQI00000000	4,765,168	65.1	64	97.91	82.73	25.8

for onsite implementation. However, further *in-situ* investigations are required to explore additional features and potential which may not only increase the yield but also may provide various health advantages to the consumers.

3.3. In vitro evaluation of plant growth-promoting features

Phosphate-solubilizing rhizobacteria (PSRB) constitute a significant subset of Plant Growth-Promoting Rhizobacteria (PGPR)

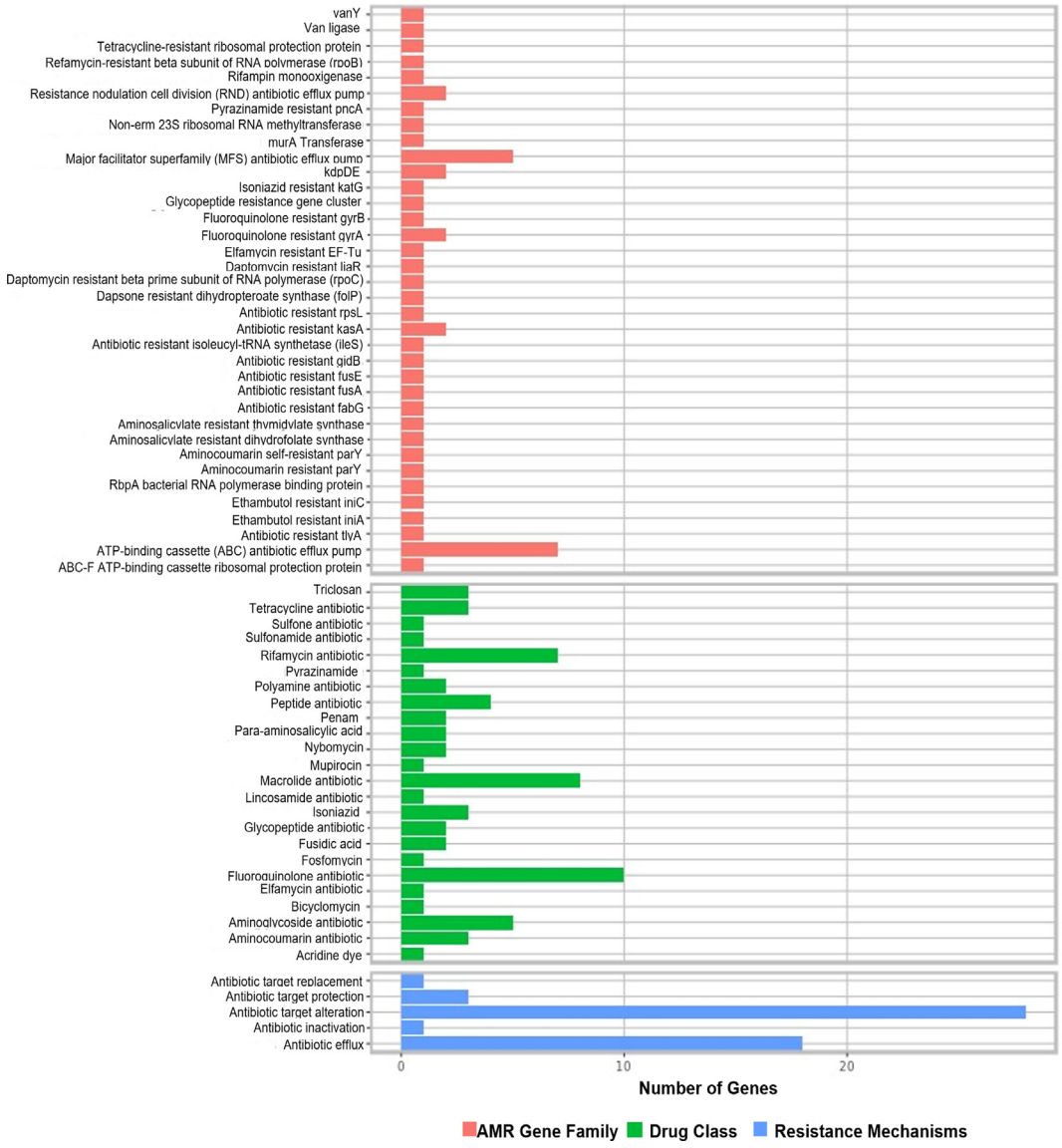

Fig. 5. The Comprehensive Antibiotic Resistance Database (CARD) assisted ontology to predict antibiotic resistance genes in the genome of *P. oxydans* strain NCCP-2145.

renowned for their capacity to enhance plant growth through the facilitation of phosphorus and nitrogen uptake [26]. The utilization of PSRB as biofertilizers has become widespread as a means to improve soil health. PGPR perform a pivotal role in promoting diverse plant developmental processes by producing phytohormones like auxins (IAA) and cytokinins, as well as making 1-amino cyclopropane-1-carboxylate (ACC) deaminase. These mechanisms ultimately improve plant resistance and mitigate environmental stresses [27]. Ammonia, a chemical compound, has multiple beneficial effects on plant health, primarily by inhibiting the growth of phytopathogens whereas catalase, an enzyme responsible for catalyzing the breakdown of hydrogen peroxide into hydrogen and oxygen, plays a crucial role in protecting cells from oxidative damage [28]. The isolate NCCP-2145 exhibited positive results in tests for phosphorous solubilization, IAA production, ammonia production, and catalase activity. However, it tested negative for ZnO and hydrogen cyanide. IAA production was confirmed by the appearance of a cherry red ring upon the addition of Kovac's reagent [29]. The formation of a clear halo zone around the bacterial growth on Pikovskaya agar medium demonstrated the strain's ability to solubilize phosphorus [30]. Strain NCCP-2145 also displayed the highest capability for ammonia production, which may contribute to improved nitrogen absorption by plants. Additionally, strain NCCP-2145 exhibited moderate to high tolerance to several heavy metals, including 250 ppm of Ni, 100 ppm of Cu, and 30 ppm of Cd as the minimum inhibiting concentrations. These findings align with previous work [31,32].

3.4. The biotechnological potential of Pseudarthrobacter oxydans NCCP-2145 as a plant bio-stimulant

The genome of strain NCCP-2145 has been found to contain several important biosynthesis pathways that are industrially significant. These pathways include auxin, biotin, thiamin, menaquinone and phylloquinone, pyridoxine (Vitamin B6), NAD and NADP cofactors, folate, phenylpropanoids, and trehalose (Fig. 2). Additionally, multiple open reading frames responsible for plant growth promotion traits, secondary metabolism, and various cellular functions have been annotated in the genome of strain NCCP-2145 (Fig. 1). Phosphorus is the most essential nutrient for optimal plant growth and yield [33]. The Pst phosphate transport system, which consists of *PstA*, *PstB*, and *PstS* genes [34], has been identified in the

Table 2
Plant-growth promotion traits found in *P. oxydans* NCCP-2145.

Plant-growth Promotion traits	Protein name (gene name)	Protein code (identity; UniProt accession)
Phosphate solubilization	Phosphate-binding protein (*PstS*)	NCCP2145_08270 (92.5%, GKV71446)
	Phosphate transport system permease protein (*PstA*)	NCCP2145_08290 (95.1%, GKV71448)
	Phosphate import ATP-binding protein (*PstB*)	NCCP2145_08300 (99.2%, GKV71449)
	Phosphate signaling complex protein (*PhoU*)	NCCP2145_13830 (95%, GKV72002)
	Alkaline phosphatase synthesis transcriptional regulatory protein (*PhoP*)	-
	Phosphate regulon sensor protein (*PhoH*)	-
	Phosphate regulon sensor protein (*PhoR*)	-
	Phosphate regulon transcriptional regulatory protein (*PhoB*)	-
Indole acetic acid	indole-3-glycerol phosphate synthase IGPS (*TrpC*)	NCCP2145_23270 (93.8%,GKV72946)
Nitrogen fixation	GAF domain-containing protein (*nifH*)	NCCP2145-17950 (80.7%, GKV72942)
	Glutamate synthase protein (*glxC*)	NCCP2145_03420 (88.2%, GKV70961)

NCCP-2145 strain, indicating its capability for phosphorus solubilization (Table 2). Both plants and soil bacteria are capable of synthesizing indole-3-acetic acid (IAA), which is an auxin hormone that plays a crucial role in plant growth and development. The production of IAA is influenced by factors such as the availability of tryptophan and other abiotic factors [35,36]. Soil bacteria employ multiple pathways, including indole-3-acetonitrile (IAN), indole-3-pyruvate (IPyA), and indole-3-acetamide (IAM) pathways [36,37] to produce IAA from L-tryptophan. The *ipdC* gene encodes indole-3-pyruvate decarboxylase, an essential enzyme for IAA biosynthesis via the IPyA pathway. The *nifH* gene plays a key role in N_2 fixation, thus contributing to the function of plant growth-promoting rhizobacteria [38]. The *nifH* gene sequence provides valuable insights into the genetic diversity of N_2-fixing bacteria [39]. All of these genes associated with plant growth promotion have also been identified and annotated in the genome of strain NCCP-2145.

4. Conclusions

This study sheds light on the genomic characteristics, functional capabilities, and potential plant growth-promoting attributes of *P. oxydans* NCCP-2145, offering insights into microbial diversity in mangrove ecosystems of Pakistan. The genome of this bacterium showcases promising traits as a bio-fertilizer, such as indole-3-acetic acid (IAA) biosynthesis, nitrogen fixation, and phosphate solubilization. *P. oxydans* NCCP-2145 exhibits non-pathogenic in nature and a low rate of antibiotic resistance, minimizing environmental risks. However, further research is needed to explore its precise mechanisms of plant growth promotion, practical applications in sustainable agriculture, and real-field evaluations.

Author contribution

Study conception and design: I Ahmed; B Uzair
Data collection: R Bushra; S Manzoor
Genome sequencing and data analysis: R Bushra; A Ali; S Abbas; I Ahmed
Analysis and interpretation of results: R Bushra; S Abbas; I Ahmed
Draft manuscript preparation: R Bushra; A Ali; I Ahmed
Revision of the results and approved the final version of the manuscript: R Bushra; B Uzair; A Ali; S Manzoor; S Abbas; I Ahmed

Conflict of interest

None.

Availability of data

The draft genome sequence of *Pseudarthrobacter oxydans* NCCP-2145 has been deposited to the DDBJ database and is available under the accession number BRCG01000001. The corresponding bio-sample, bio-project, and 16S rRNA gene sequence have been assigned the accession numbers SAMD00492352, PRJDB13574, and LC708055, respectively.

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