Draft genome sequence of a cold-adapted phosphorous-solubilizing *Pseudomonas koreensis* P2 isolated from Sela Lake, India

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Abstract

The draft genome sequence of a cold-adapted phosphorus-solubilizing strain *Pseudomonas koreensis* P2 isolated from the Sela Lake contains 6,436,246 bp with G + C content of 59.8%. The genome sequence includes 5743 protein coding genes, 68 non-protein coding genes, 1007 putative proteins, 5 rRNA genes, 64 tRNAs and two prophage regions in 40 contigs. Besides these, genes involved in phosphate solubilization, siderophore production, iron uptake, heat shock and cold shock tolerance, multidrug resistance and glycine-betaine production were also identified.

Keywords Phosphorus (P) · *Pseudomonas koreensis* · Genome · Phosphate solubilization · PGPR

Introduction

Phosphorus (P) is a key macronutrient and very essential for the development and growth of plants. At present, P is available in a very limited amount in agricultural soil due to its low solubility and fixation (Kwak et al. 2016). Several reports indicated that *Pseudomonas* sp. is one of the most powerful phosphate solubilizers (Hernandez-Salmeron et al. 2016; Lafi et al. 2016). Several strains from this genus have been documented to ameliorate crop growth, nutrient mobilization and yield, in addition to the biological control of plant pathogen (Sharma et al. 2018; Solanki et al. 2014).

Pseudomonas is one of the widely distributed genera which is present in versatile environmental conditions.

Pseudomonas koreensis is a non-spore forming, gram-negative, rod-shaped motile bacteria and was reported for the first time from farming soil in Korea (Kwon et al. 2003). A number of species have been documented from different environments, having excellent plant growth-promoting (PGP) attributes. The majority of these rhizobacteria exhibit multifarious PGP traits and include solubilization of phosphate, siderophore formation, 1 amino cyclopropane-1-carboxylate deaminase (ACC) and hydrogen cyanide (HCN) production etc. (Lugtenberg and Kamilova 2009). Besides these traits, rhizobacteria also acquire some environment-specific PGP attributes, for instance, heavy metal resistance, cold tolerance and antibiotic resistance. Because of these properties, they are of interest to use as inoculants for plant growth promotion (Lugtenberg and Kamilova 2009). In spite of the pervasive literature related to plant growth-promoting rhizobacteria (PGPR) and their modes of action, the molecular significance of these PGPR is still to be identified and difficult to understand, because the PGPR status is not always well defined. There are varieties of microorganisms having genes directly endure the plant beneficiary activity, for example *nif*, *phl* and *pqq* (Ahmad et al. 2008). There are a number of PGPR which still have to be identified. Nowadays, the next-generation sequencing technologies have numerous applications to study genomes which have been further employed to study PGPR present in several species such as *Pseudomonas* sp., *Bacillus* sp., and *Klebsiella*

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sp. (Hernandez-Salmeron et al. 2016). In the present work, draft genome sequence of *Pseudomonas koreensis* P2 isolated from the cold environment of the Sela Lake in the soil having inorganic phosphate-solubilizing plant growth-promoting traits with environmental tolerance was reported. The P2 strain solubilized phosphorous, synthesized siderophores, indole acetic acid (IAA) and HCN. This strain also possessed some means and skills to survive and sustain in a cold environment. The genome sequencing analysis will disclose the basics of the functioning of the organism and will provide accurate and deep understanding into evolutionary changes and future studies in *Pseudomonas* sp. Furthermore, the genome analysis and understanding highlight the additional factors which may contribute to the plant growth.

Materials and methods

PGPR strain and characterization of plant growth-promoting (PGP) attributes

P2 is a recently identified gram-negative, short-rod with yellowish white pigmentation and motile rhizobacteria isolated from the Sela lake (Latitude: 27°30'37.27"N, Longitude: 92°6'9.30"E). The full-length 16S rRNA gene sequencing was done and has been deposited to NCBI GenBank under the accession number KJ580528. The 16S rRNA sequences of different sub-species of the *P. koreensis* was obtained from the NCBI GenBank database and compared using MEGAX32. The 16S rRNA sequence of P2 elucidated approximately 100% sequence similarity to *Pseudomonas koreensis* (Fig S1). Details of the PGP properties (phosphorous solubilization, siderophore and HCN production) of P2 are shown in Table 1. Due to the advantageous characteristics of P2, it has been chosen to characterize at the molecular and genomic levels. Phosphate solubilization was quantified using three different sources of phosphate (Udaipur rock phosphate, tri-calcium phosphate and di-calcium phosphate) at a concentration of 5 g L⁻¹ in NBRIP broth at three different temperatures (4 °C, 15 °C and 35 °C). Phosphorus was estimated in the culture supernatant by the method as described elsewhere (Fiske and Subbarow 1925). Siderophore release was estimated at three different temperatures

(4 °C, 15 °C and 35 °C) on the Chrome azurol S agar plate (Sigma-Aldrich Ltd.) as described by Schwyn and Neilands (1987). Similarly, production of IAA was quantified using Salkowski reagent as described by Bric et al. (1991) and the formation of HCN was quantified using protocol mentioned by Lorck (1948) at three different temperatures (4 °C, 15 °C and 35 °C). The plant growth-promoting efficiency of P2 strain was validated in wheat, maize and chickpea plants in pot experiments (ICAR-NBAIM Annual Report 2014–2015; Kashyap et al. 2015).

Genomic DNA manipulations and draft genome sequencing

For total genomic DNA isolation, P2 was grown on LB agar (Luria Bertani) plate and a single colony was inoculated in LB broth (10 ml) and grown overnight in shaking incubation at 37 °C and 150 rpm. For draft genome sequencing of *Pseudomonas koreensis* strain P2, total genomic DNA was isolated according to Sharma et al. (2018) and quality of the DNA was checked on agarose gel under transilluminator. The genome sequence of P2 was determined by the Illumina HiSeq platform in paired-end module. Primary genome assembly was done using Velvet version 1.2.10. (Zerbino and Birney 2008). Bowtie version 2 (Langmead and Salzberg 2012) was employed for *de novo* genome validation and quality check. tRNA and rRNA were identified by employing ARAGORN version 1.2.36 (Laslett and Canback 2004) and RNAmmer version 1.2 tools (Lagesen et al. 2007), respectively. Webcutter 2.0 (<https://rna.lundberg.gu.se/cutter2/>) and plasmid finder version 1.3 (Carattoli et al. 2014) were used to perform plasmid contamination search and plasmid sequences determination. Prophage regions were identified by employing the PHAST server (Zhou et al. 2011). CONTIGuator version 2.7 (Galardini et al. 2011) was used to finish the draft genome with *Pseudomonas koreensis* as a reference genome.

Genome annotation and bioinformatic analysis

Annotation of P2 genome was done by Rapid Annotations using Subsystems Technology “(RAST, Version 2.0)” web service (Aziz et al. 2008). Ring Image Generator (BRIG)

Table 1 Plant growth-promoting attributes of P2 strain at different temperature regimes

Temperature (°C)	IAA (mg L ⁻¹)	Siderophore	HCN	P-solubilization (mg L ⁻¹)		
				Rock phosphate	Tri-calcium phosphate	Di-calcium phosphate
4	–	+	+	–	–	–
15	14.9	+	+	14.00 ± 1.03	216.44 ± 4.27	667.55 ± 10.12
35	14.10	+	+	120 ± 3.78	532.03 ± 5.98	860.40 ± 21.57

version 0.95 was used for genome comparison (Alikhan et al. 2011). The circular genomic map was constructed with BLAST+, with standard default parameters. *Pseudomonas koreensis* D24 was taken as a reference genome. Next, the identified genes from P2 were functionally annotated with the help of BLASTKOALA (Kanehisa et al. 2016). This server has a modified version of the internally used KOALA algorithm for KO assignment. The result file was used further for pathway mapping and comparative pathway analysis. Phylogenetic tree construction was done using MEGAX32 version. Sequences were aligned by MUSCLE and phylogenetic tree was made by the neighbor-joining method (Tamura et al. 2013).

Results and discussion

The draft genome sequencing of the strain P2 generated a total of 77.43 million reads with a total sequence data of 7820.5 Mb. High-quality reads (> 95%) with an average read length of 101 bp were considered for downstream analysis. Primary genome assembly generated 40 contigs with 59.8% GC content and a scaffold N50 value of 438,962 bp. Similar reports of genome assembly of *P. koreensis* strains from soybean and rice rhizosphere have been published earlier (Lozano et al. 2019; Lin et al. 2016). In this study, genome validation and quality control using Bowtie software revealed 29 million reads mapped concordantly and 6 million reads mapped discordantly with an overall alignment rate of 97.82%. Two prophage regions were present in the genome while plasmid sequences were missing. Both the prophage regions were intact and complete with 36.9 kb and 43.2 kb. *Denovo* genome identification and draft genome preparation were done using *P. koreensis* genome sequence as a reference (Table 2). Draft genome annotations and functional characterization by RAST allowed for the identification of 5743 protein coding genes, 68 non-protein coding genes, 1007 putative proteins, 5 rRNA genes and 64 tRNAs

(Fig. 1; Table 2). RAST is a fully automated web-based tool and has been extensively employed in genome sequence annotation of several microbes including *P. koreensis* and *P. moraviensis* (Lin et al. 2016; Lujan et al. 2017; Srivastava et al. 2019). The intra-species sequence similarity was checked by calculating ANI value using reference of *P. koreensis*, *P. agarici*, *P. moraviensis*, and *P. rhizosphaerae* (Table 3). The ANI between the query genome and the reference genome was calculated as the mean identity of all the BLASTN matches. The ANI was calculated based on the BLAST algorithm, ANIb. The ANIb score between different subspecies of *Pseudomonas* is between 77 and 87%. The ANI score with references to *P. koreensis* was ~86.53. The result show more closeness to the *P. koreensis*.

Comparative analysis of the draft genome of different sub-species *Pseudomonas koreensis* (P2, D26, 57B-090624, BS3658, CI12, CRS05-R5, IMBL1, LB-090714, P19E3) using RAST (Table 2) identified around 5000–6600 coding sequences where P19E3 was identified with the highest number of coding sequences and 57B-090624 was identified with lowest coding sequences. Further, comparative study also indicated that the draft genome of different *P. koreensis* strains differed in the number of sub-system (234–242) and GC content (59.2–60.6%) (Table 2). Highest number of RNA (92) was identified with BS3658 and CRS05-R5, while lowest number was in 57B-090624. Further comparison of *P. koreensis* genome along with the *P. agarici*, *P. moraviensis* and *P. fluorescens* 113 using BRIG analysis (Fig. 2) indicated gap in between the sequences which represent the dissimilarity between the genome of different species of *Pseudomonas*. Even, there are some differences in between the *P. koreensis* subspecies because they have been isolated from the different geographical locations. These differences have led to the adaptation of the organism to particular climatic condition. Besides this, analysis revealed that P2 has significantly higher gene abundance of phosphorous metabolism, siderophore and HCN production and many more PGPR traits (Table S3) which allow this bacterium to be considered

Table 2 Comparison of genome assembly statics of different species of *Pseudomonas koreensis*

Genome	P2	D26	57B-090624	BS3658	CI12	CRS05-R5	IMBL1	LB-090714	P19E3
Size	6,436,246	6,301,761	5,983,798	6,123,913	6,622,028	5,991,225	6,100,607	6,070,527	7,498,194
GC content	59.8	59.6	60	60.5	59.2	60.6	59.9	59.9	59.2
N50	438,962	NA	234,574	NA	608,098	NA	324,256	299,940	6,444,290
L50	5	1	8	1	4	1	7	7	1
Number of contigs (PEGs)	40	1	38	1	16	1	36	47	5
Number of subsystems	239	240	242	238	241	234	240	239	236
Number of coding sequences	5743	5638	5292	5471	5798	5345	5392	5332	6605
Number of RNAs	69	84	67	92	85	92	86	74	90

Names are written in abbreviated form. P2, D26, 57B-090624, BS3658, CI12, CRS05-R5, IMBL1, LB-090714, P19E3 represent the subspecies of *Pseudomonas koreensis*

Fig. 1 RAST annotation of *Pseudomonas koreensis* P2: The subsystem category distribution shows the percentage distribution of the genes in different pathways, which is labeled with different colours and the number of genes in one feature is mentioned in the bracket

Table 3 ANI analysis of *Pseudomonas koreensis* P2

Organism name	Regression_value	ANIb value
<i>Pseudomonas_koreensis</i>	0.98867	86.53
<i>Pseudomonas_moraviensis</i>	0.98771	86.55
<i>Pseudomonas_agarici</i>	0.9259	79.81
<i>Pseudomonas_rhizosphaerae</i>	0.91148	77.72

as a potential genomic resource for developing efficient biological fertilizer for agricultural usage.

Solubilization of insoluble phosphate by bacteria is another way to enhance plant growth promotion. Gluconic acid (GA) is recognized as one of the major organic acids liable for efficient solubilization of mineral phosphates (Oteino et al. 2015). It is a well-known fact that the synthesis of GA is catalyzed by glucose dehydrogenase (GDH). P2 exhibits both UDP-dependent (UDP-GDH) and pqq-dependent glucose dehydrogenase (PQQ-GDH) and genome mining revealed the presence of pqqFABDE operon in between 156,040 and 158,264 bp in P2 genome (Fig. 3, Table S3), and still require detailed investigation to confirm their functioning in GA synthesis. However, conserved nature of phosphate regulon transcriptional regulatory proteins PhoB and sensor protein PhoR in *Pseudomonas* (Monds et al. 2006) strongly advocate the possibility that inorganic phosphate uptake in P2 strain is promoted by phosphate regulon transcriptional regulatory proteins PhoB and sensor protein PhoR with involvement of two other high-affinity phosphate transport systems, PstBACS and PhnE2E1. P2 also

possesses sodium-dependent phosphate transporter NhaA and NhaD. Studies on in vitro mineral phosphate solubilization showed that temperature has substantial effects on TCP solubilization as P release at higher temperature (35 °C) was more than at lower temperature (15 °C and 4 °C). A significant solubilization of rock phosphate (14 and 120 mg L⁻¹), tri-calcium (216.44 and 532.03 mg L⁻¹) and di-calcium phosphate (667.55 and 860.40 mg L⁻¹) by P2 at 15 °C and 35 °C temperature was observed (Table 1). Similarly, Selvakumar et al. (2011) also reported that P release by *Pseudomonas lurida* M2RH3 was affected by temperature and the maximum P release was observed at 30 °C as compared to 4 °C and 15 °C. They also observed that tri-calcium phosphate (TCP)-solubilizing ability of *Pseudomonas* sp. strain PGERs17 reduced to 42.3 mg L⁻¹ at 4 °C from 74.1 mg L⁻¹ at 28 °C. At 4 °C, P solubilization was absent for all the three insoluble P sources by P2 strain in the present study.

Siderophore-producing PGPR perform a key function in iron (Fe) nutrition of plants and act as a vital factor for improving crop health and yield (Solanki et al. 2014). P2 strain was found siderogenic at 35 °C and able to retain its siderogenic activity at a temperature of 4 °C too (Fig S2). *Pseudomonas* sp. has multiple systems to sense or asunder iron in its environment. There are various positive and negative regulatory factors in the *Pseudomonas* which are able to regulate cellular iron acquisition and storage. The genes associated with the siderophore production were identified in the P2 genome as well (Table S3). For example, the gene *Fur* (ferric uptake regulator) controls the transcription of *PvdS*, which is further needed for the transcription of several

Fig. 2 BRIG output image of different sub-species of *Pseudomonas koreensis* along with *Pseudomonas agarici*, *Pseudomonas moraveinsis*, *Pseudomonas fluorescens* 113 genome. *Pseudomonas koreensis*

D24 was taken as reference genome. GC content and species name are labelled in the figure with different colour

Fig. 3 Operon structure of *pqqFABDE* (*pqqF*, *pqqA*, *pqqB*, *pqqD*, *pqqE*) in *Pseudomonas koreensis* P2

genes responsible for PVD biosynthesis and all these genes responsible for PVD biosynthesis are present in P2 genome. In addition, the P2 genome also possesses TonB- and TonR-dependent transport system along with the ABC transporters (Table S3).

IAA production by cold-tolerant bacteria like P2 strain has received little attention in the past. Recently, accumulative reports suggested that IAA production is superior in the mesophilic range relative to extremely low and chilling temperature. Selvakumar et al. (2008) recorded high levels of IAA production by *Pantoea dispersa* at 30 °C in contrast to 4 °C temperature. Although similar trend was observed in the present study, the IAA production in *P. koreensis* P2 was not much affected at 15 °C (Table 1). Similarly, cyanogenesis by PGPR is considered as another main factor to control pathogenic fungi. In the current study, *P. koreensis* P2 showed HCN production in all the three temperature regimes (Fig S2). In the genome analysis, association of *HcnABC* gene with HCN synthesis has been observed.

P2 possesses various cold shock proteins as it was identified from the cold environment. In the present study, three paralogs of CspA are determined in the P2 genome. Other cold shock proteins [CspCDG (CspC, CspD, CspG)] required for the cellular function at lower temperature were also identified (Table S2). The cold shock proteins and the heat shock proteins were identified from the subspecies of *P. koreensis* and some other species of *Pseudomonas* like *P. agarici*, *P. moraviensis* and *P. fluorescens* 113. In the present study, three different copies of the *cspA* in all the cases except *P. fluorescens* 113 (4 copies of *cspA*) were identified. The phylogenetic analysis of *cspA* and the GroEL subunit of heat shock protein (Hsp60) indicates a very minor differences in the data coverage (Fig S3). Furthermore, multiple sequence alignment revealed significant variation in the nucleotide as well as protein sequences of cold shock protein (Fig S4A and S4B) as compared to heat shock protein (Fig S5A and S5B). Phylogenetic analysis performed by Awasthi et al. (2019) indicated that the CspA was highly diverse in Pseudomonads (35–100%) with apparent gene transfer among different subgroups of *Pseudomonas*. Additionally, amino acid composition of the CspA from cold-adapted *P. koreensis* was different from mesophilic *Pseudomonas* and even a small amino acid change between CspA plays a significant role in determining cold adaptive and mesophilic species. Similarly, the findings of present study indicate that the variation of the CSP sequence may be contributing to the

adaptation in a cold environment in case of *P. koreensis* P2, while there is not much variation in the protein as well as nucleotide sequence of heat shock protein.

There is a two-component response regulator in P2, i.e., *CbrA* and *CbrB* genes which encode a sensory box histidine kinase and a response regulator, respectively. Similar orthologs have already been identified in *P. aeruginosa* and moreover, a paramount role in central metabolism and the knockout of orthologs with adverse effect on growth at the lower temperature have been documented (Reva et al. 2006).

Overall, the present study was able to annotate 51% of the P2 genome. Besides these, genes for phosphate solubilization, siderophore production, iron uptake, heat shock and cold shock tolerance, multi-drug resistance and glycine-betaine production were also identified. Besides these PGPR traits, we also identified many genes that are well known to be responsible for the production of antimicrobial compounds such as 4-hydroxybenzoate, phenazine and GABA (Table S3). These compounds are mainly responsible for the suppression of the pathogen and enhance the plant growth. The P2 genome sequencing confirmed the presence of 1-aminocyclopropane-1-carboxylase (ACC) deaminase (*acdS*) as well.

Conclusion

The current study elucidates the plant growth-promoting properties and genetic makeup of *Pseudomonas koreensis* P2. The draft genome sequencing and analysis of the genome support its role as a plant growth-promoting bacterium and has led to the confirmation of the presence of the several plant growth-promoting traits like phosphate solubilization, siderophore, HCN and IAA production, which can improve the growth of the associated plant. A total of 5743 protein coding genes, 68 non-protein coding genes, 4398 characterized protein and 1330 proteins with pathway annotation were identified in 40 contigs. The presence of the glucose dehydrogenase and *pqq* genes makes the inorganic phosphorus present in the soil bioavailable to the plant. Similarly, the presence of cold shock proteins confirms the adaptability to the cold environment. So, this genomic resource could be exploited in future for the further research and to develop a potential plant growth promoter with best suited characters for the survival under diverse environment.

Genome sequence accession number

This strain has been submitted in the NAIMCC culture collection under the accession number NAIMCC-B-01747. The whole-genome shotgun project has been deposited at NCBI GenBank under the accession number GCA_002177125.1 (BioProject ID: PRJNA328580).

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Author contributions AKS conceived the idea. AKS, PS, HC, MK and PLK are associated with the wet lab experiments and sequencing. JY, Anjney Sharma, and Anchal K. Srivastava contributed in sequencing and analysis. APB, RS and AKS analyzed the genome data. Anil K. Saxena gave critical inputs. APB and AKS wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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