



Complete genome sequence of the abscisic acid-utilizing strain *Novosphingobium* sp. P6W

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Abstract

The phytohormone abscisic acid (ABA) plays multiple roles in plant survival and fitness. Significant quantities of ABA are constantly introduced into soil via root exudation, root turnover and incorporation of abscised shoot tissues. In addition, some phytopathogenic fungi synthesize ABA in the course of plant–microbe interactions. The accumulation of soil ABA can inhibit seed germination and root growth but despite this observation, the biochemical pathways of ABA conversion by microorganisms and genetic determinants of the process remain unknown. Here we report on the complete genome sequence of strain P6W, an ABA-utilizing isolate of the genus *Novosphingobium*. Strain P6W was isolated from the rhizosphere of rice (*Oryza sativa* L.) seedlings using a selective ABA-supplemented medium. The genome of strain P6W consists of 6,606,532 bp, which includes two chromosomes and two plasmids. It comprises of 5663 protein-coding genes and 80 RNA genes. ANI values calculated based on the analysis of nine previously sequenced genomes of members of the genus *Novosphingobium* ranged from 77 to 92%, which suggests that strain P6W is potentially a new species of the genus *Novosphingobium*. Functional annotation of genes in the genome of strain P6W revealed a number genes that could be potentially responsible for ABA degradation.

Keywords Abscisic acid · *Novosphingobium* · Complete genome sequence · Rhizosphere · Plant–microbe interactions.

Abbreviations

ABA Abscisic acid
ANI Average nucleotide identity
PGPR Plant growth-promoting rhizobacteria

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Introduction

The plant growth-promoting rhizobacteria (PGPR) can stimulate plant growth by producing various phytohormones, such as auxins, gibberellins, and cytokinins (Dobbelaere et al. 2003; Dodd et al. 2010). The ability to synthesize ABA was found in different PGPR species (Naz et al. 2009), such as *Azospirillum lipoferum* (Cohen et al. 2009) and *Arthrobacter koreensis* (Piccoli et al. 2011). Several ABA-producing endophytic bacteria isolated from plants grown under extreme salinity were assigned to *Achromobacter xylosoxidans*, *Bacillus licheniformis*, *Bacillus pumilus*, and *Brevibacterium halotolerans* (Sgroy et al. 2009).

Bacteria can not only produce but also metabolize and utilise phytohormones as nutrients. However, the role of microbial metabolism of phytohormones in plant–PGPR interactions received relatively little attention (Dodd et al. 2010). It has been shown that many rhizobacteria metabolize the indole-3-acetic acid by several biochemical pathways (Leveau and Gerards 2008) and a precursor of the phytohormone ethylene 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC deaminase (Glick et al. 2009).

However, little is known about microbial utilization of other phytohormones. The bacterium *Serratia proteamaculans* strain B1 metabolized the synthetic cytokinin N6-benzyladenine as a carbon source in vitro via the enzyme xanthine dehydrogenase (Taylor et al. 2006). Biodegradation of gibberellins by *Azospirillum lipoferum* was also described (Piccoli et al. 1997).

Plants are presumed not to regulate ABA concentrations in the soil solution, because ABA transporter genes (if located in epidermal roots cells) may efflux ABA and its concentration may gradually increase during the vegetative period (Hartung et al. 1996). This can lead to inhibition of seed germination (Gaciarrubio et al. 1997). As has been reported earlier, ABA accumulation during water stress may function in maintaining root growth in drying soil (Sharp and LeNoble 2002). Conversely, exogenous ABA may act as an inhibitor of root growth in well-watered plants (Sharp and LeNoble 2002). Moreover, the ability to synthesize and secrete ABA may be related to virulence of fungal pathogens (Schmidt et al. 2008; Hartung 2010). In this regard, information on the ABA-utilizing PGPR is of particular interest.

Recently, using a selective ABA-supplemented medium, two bacterial strains were isolated and assigned to *Rhodococcus* sp. P1Y and *Novosphingobium* sp. P6W. Both strains showed the ability to decrease ABA concentrations in rice and tomato seedlings and stimulate plant growth (Belimov et al. 2014). Since *Novosphingobium* sp. P6W had greater effects on ABA concentrations in plants, this strain has undergone further study in the presented work.

Materials and methods

Growth conditions and genomic DNA preparation

A culture of *Novosphingobium* sp. strain P6W was grown aerobically in M9 medium with glucose as carbon source at 28 °C. Genomic DNA was isolated using phenol–chloroform extraction method (Sambrook et al. 1989).

Genome sequencing, assembly, and annotation

Illumina sequencing reads were generated from three libraries: Nextera XT 2 × 150 (average insert size 249 bp), Nextera XT 2 × 250 (insert size 508 bp, 1.8 mln reads) and Nextera Mate pair 2 × 300 (average insert size 3331 bp, 8.1 mln reads). Filtering and trimming of Illumina reads were performed with Prinseq lite v. 0.20.4 (Schmieder and Edwards 2011) for standard paired end libraries and with NxTrim v0.4.2 (O'Connell et al. 2015) for the mate pair library. Genome was also sequenced on a MK1B MinION

device using R9 version flow cells. The library was prepared using the ONT SQK-LSK108 Ligation sequencing kit 1D without optional shearing steps to select for long reads. The run duration was 48 h. Total 37,045 reads were obtained (average 5647, max 175,097 bp, 32× coverage). All reads were assembled into two contigs and one large scaffold with a single gap by SPAdes v3.12.0. However, the gap in this scaffold could not be closed using available data. Therefore, nanopore-only assembly was performed with canu v1.7, which resulted in a very similar assembly with the only significant distinction: the large scaffold split into two contigs. Since all four contigs had no gaps, were properly circularised and had the expected GC skew patterns, the nanopore-only assembly was considered as the correct one. The absence of rearrangements in this assembly was additionally verified with NxRepair (Murphy et al. 2015) using mate-pair data. Errors remained in the assembly were fixed by four rounds of error correction by pilon v1.22 (Walker et al. 2014) using Illumina data mapped onto the assembled contigs by BWA-mem (Li 2013).

Genome annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline v4.5 (Tatusova et al. 2016). The Phobius web server (Käll et al. 2007) was used for the identification of proteins with transmembrane helices and SignalP v4.1 (Petersen et al. 2011)—for identification of signal peptides. The Pfam database (Finn et al. 2016) was searched for conserved domains with hmmscan v3.1 from the HMMER package (Eddy 2011). Genome statistics was collected with Sigmoid v2.0 (Nikolaichik and Damienikan 2016). BPGA (Chaudhari et al. 2016) was used for COG assignment.

Multisubstrate analysis

The GEN III MicroPlate™ test panel and Microbial Identification Systems software OmniLog® Data Collection (Biolog, Inc., Hayward, USA) was used to obtain a phenotypic pattern for additional identification of the bacterium.

Results and discussion

Classification and features

Novosphingobium sp. P6W was isolated from the rhizosphere of rice (*Oryza sativa* L.) seedlings cultivated in sod-podzolic soil collected in St-Petersburg region (59.740112, 30.428968) (Belimov et al. 2014). It was deposited in the Russian Collection of Agricultural Microorganisms (RCAM, WDCM 966) and Belgian Coordinated Collections of Microorganism (BCCM/LMG, LMG 30848). This strain

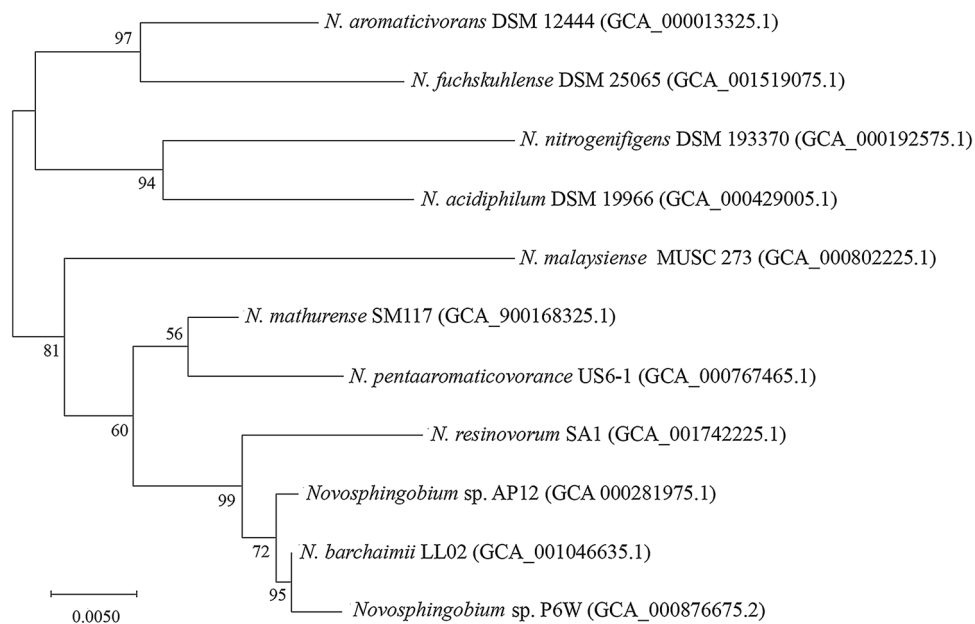


Fig. 1 Phylogenetic relationships in *Novosphingobium* genus. The phylogenetic tree was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the

evolutionary distances used to infer the phylogenetic tree. The evolutionary distances have been computed using the Jukes–Cantor method (Jukes and Cantor 1969) and are in the units of the number of base substitutions per site. The analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

produces auxins and has ACC deaminase activity (Belimov et al. 2014). Phylogenetic analysis based on the 16S rRNA gene sequences showed that *Novosphingobium* sp. P6W formed a clade with other members within the genus *Novosphingobium* (Takeuchi et al. 2001) (Fig. 1).

According to the Biolog GEN III MicroPlate assay P6W utilizes as carbon source: Dextrin, D-Maltose, D-Trehalose, Gentiobiose, β -Methyl-D-Glucoside, D-Salicin, α -D-Glucose, D-Fructose, D-Galactose, L-Fucose, L-Rhamnose, D-Glucose-6-PO₄, Gelatin, Glycyl-L-Proline, L-Aspartic Acid, L-Glutamic Acid, D-Glucuronic Acid, Glucuronamide, Citric Acid, L-Malic Acid, Bromo-Succinic Acid, Tween 40, β -Hydroxy-D,L-Butyric Acid, Acetoacetic Acid, Acetic Acid. This strain is sensitive to Troleandomycin, Minocycline, Guanidine Hcl, Niaproof 4, Vancomycin, Tetrazolium Blue, Lithium Chloride, Sodium Butyrate, Sodium Bromate, D-serine and resistant to Rifamycin SV, Lincomycin, Aztreonam.

Genome sequencing information

The genome of *Novosphingobium* sp. P6W was sequenced in 2014 using an Illumina MiSeq sequencing platform. The assembly and annotation of draft genome sequences were completed on 2015/02/17. GenBank Assembly Accession number is GCA_000876675.1. Illumina sequencing of mate-pair libraries was completed during 2016. In June 2018, the genome sequencing using Oxford Nanopore sequencing

technology was performed. The complete genome has been deposited in GenBank under accession numbers CP030352.1, CP030353.1, CP030354.1, and CP030355.1. A summary of the project information is presented in Table S1.

Table 1 Genome statistics

Attribute	Value	% of total
Genome size (bp)	6,606,532	100
DNA coding (bp)	5,715,607	86.51
DNA G + C (bp)	4,207,174	63.68
DNA scaffolds	4	100
Total genes	5964	100
Protein-coding genes	5663	94.95
RNA genes	80	1.34
Pseudo genes	221	3.7
Genes in internal clusters	Unknown	Unknown
Genes with function prediction	4653	82.16
Genes assigned to COGs	4990	88.12
Genes with Pfam domains	4732	83.56
Genes with signal peptides	693	12.24
Genes with transmembrane helices	997	17.61
CRISPR repeats	0	0

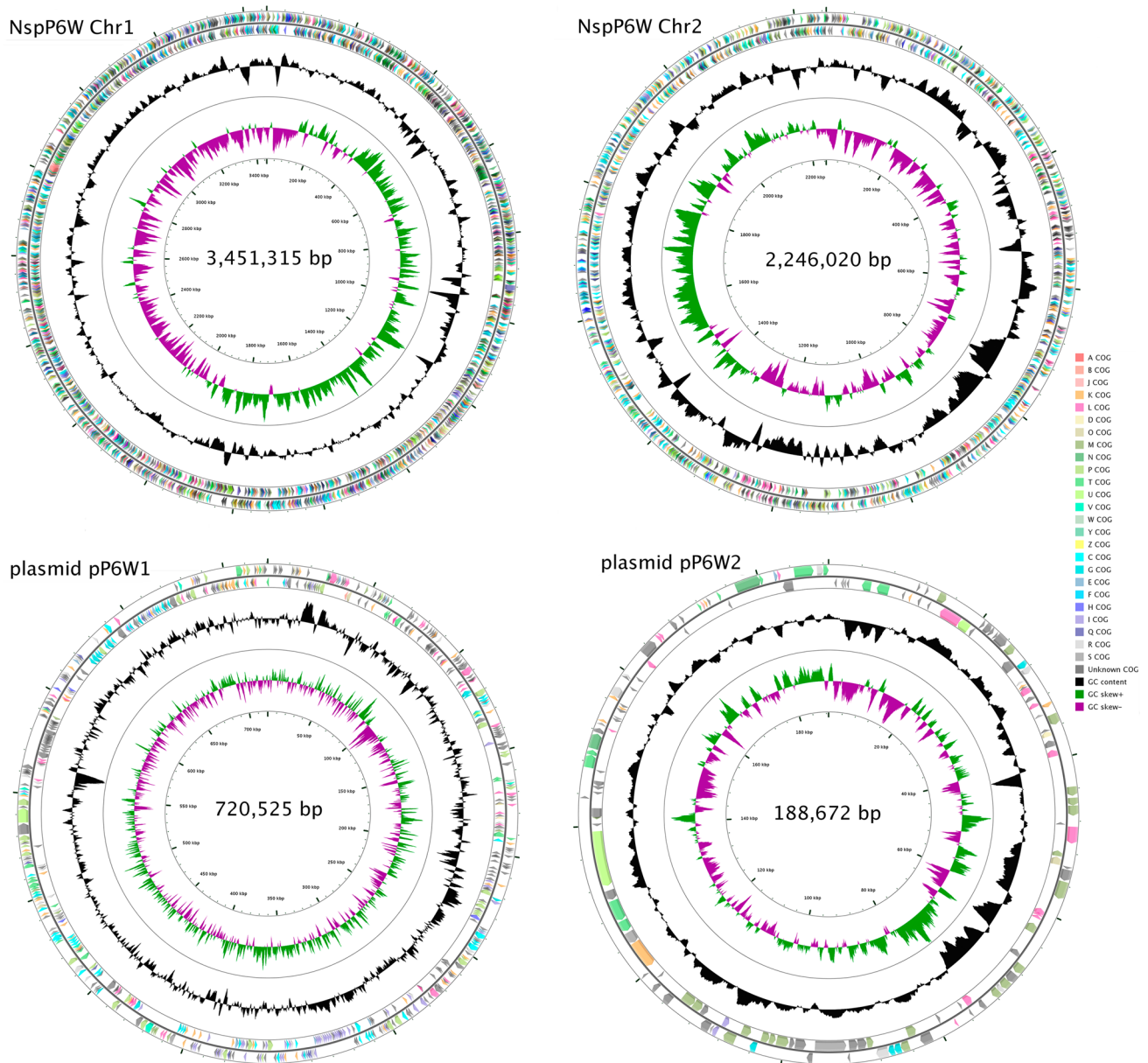


Fig. 2 Circular maps and genetic features of the chromosomes and plasmids of *Novosphingobium* sp. P6W displaying relevant genome features. From outside to center: genes located on forward

strand (colored by COG categories), genes located on reverse strand (colored by COG categories), GC content and GC skew

Genome properties

The total length of the complete genome sequence is 6,606,532 bp, which includes two chromosomes (3.45 and 2.25 Mb) and two plasmids pP6W1 (0.72 Mb), pP6W2 (0.19 Mb). The G/C content was determined to be 63.68%. There are 80 RNA genes which include 15 rRNAs, 62 tRNAs, and 3 ncRNAs. From the gene prediction results, 5663 CDSs were identified. The statistics of the genome are summarized in Table 1 and the distribution of genes

into COG functional categories is presented in Fig. 2 and Table 2.

Insights from the genome sequence

The ANI values were examined for nine genome-sequenced strains in the genus *Novosphingobium* (Fig. 3). The results show that the closest relative of *Novosphingobium* sp. P6W is *N. barchaimii* LLO2 (Niharika et al. 2013) with 92% similarity. This allows us to propose that strain

Table 2 Number of genes associated with general COG functional categories

Code	Value	% age	Description
J	172	3.45	Translation, ribosomal structure and biogenesis
A	0	0	RNA processing and modification
K	401	8.04	Transcription
L	246	4.93	Replication, recombination, and repair
B	2	0.04	Chromatin structure and dynamics
D	28	0.56	Cell cycle control, cell division, chromosome partitioning
V	51	1.02	Defense mechanisms
T	251	5.03	Signal transduction mechanisms
M	267	5.35	Cell wall/membrane biogenesis
N	65	1.30	Cell motility
U	124	2.49	Intracellular trafficking and secretion
O	145	2.91	Posttranslational modification, protein turnover, chaperones
C	329	6.59	Energy production and conversion
G	289	5.79	Carbohydrate transport and metabolism
E	360	7.22	Amino acid transport and metabolism
F	81	1.62	Nucleotide transport and metabolism
H	144	2.89	Coenzyme transport and metabolism
I	299	5.99	Lipid transport and metabolism
P	454	9.10	Inorganic ion transport and metabolism
Q	218	4.37	Secondary metabolites biosynthesis, transport and catabolism
R	715	14.33	General function prediction only
S	348	6.98	Function unknown
–	1480	26.30	Not in COGs

Fig. 3 The ANI values for strains in the genus *Novosphingobium*. The matrix was performed using the online resource <http://enve-omics.ce.gatech.edu/g-matrix/index>

	1	2	3	4	5	6	7	8	9	10	
100	89	81	80	80	80	79	79	78	77		1. <i>N. mathurense</i> SM117 (GCA_900168325.1)
89	100	82	81	80	81	80	79	79	78		2. <i>N. pentaaromaticovorance</i> US6-1 (GCA_000767465.1)
81	82	100	80	79	80	79	78	78	77		3. <i>N. malaysiense</i> MUSC 273 (GCA_000802225.1)
80	81	80	100	92	82	79	78	78	77		4. <i>N. barchaimii</i> LL02 (GCA_001046635.1)
80	80	79	92	100	82	78	78	78	77		5. <i>Novosphingobium</i> sp. P6W (GCA_000876675.2)
80	81	80	82	82	100	79	78	79	77		6. <i>N. resinovorum</i> SA1 (GCA_001742225.1)
79	80	79	79	78	79	100	80	80	78		7. <i>N. nitrogenifigens</i> DSM 193370 (GCA_000192575.1)
79	79	78	78	78	78	80	100	78	79		8. <i>N. aromaticivorans</i> DSM 12444 (GCA_000013325.1)
78	79	78	78	78	79	80	78	100	78		9. <i>N. fuchskuhlense</i> DSM 25065 (GCA_001519075.1)
77	78	77	77	77	77	78	79	78	100		10. <i>N. acidiphilum</i> DSM 19966 (GCA_000429005.1)

P6W represents a novel species of the genus *Novosphingobium* because ANI value is below the species-specific threshold set at 95% (Richter and Rosselló-Móra 2009).

At present, the process of ABA catabolism is studied exclusively in plants. It is known that the oxidative pathway is predominant in ABA catabolism in plants and it is triggered by hydroxylation at C-8' to produce 8'-hydroxy ABA. This reaction to be catalyzed by ABA 8'-hydroxylase, which is a cytochrome P450 belonging to CYP707A family (Kushiro et al. 2004). The 8'-hydroxy ABA is then spontaneously isomerized to form phaseic acid. It is possible that soil bacteria are also able to implement this mechanism. As shown

by Hartung et al. (1996), introducing the radioactive ABA into unsterile soil resulted in rapid degradation of this compound into phaseic acid and dehydrophaseic acid. According to our genome annotation the *Novosphingobium* sp. P6W has seven genes encoding various cytochrome P450 proteins (Table 3). However, the mechanisms of bacterial conversion of plant ABA may differ depending on traits of a particular strain. It was previously reported that *Corynebacterium* sp. converted ABA to dehydrovomifoliol in vitro and possessed vomifoliol dehydrogenase activity (Hasegawa et al. 1984).

Hydroxylation is one of the most common initial steps for catalytic degradation of many different compounds by bacteria

Table 3 List of potential ABA-modifying enzymes from *Novosphingobium* sp. 6W

Locus_tag	Protein ID	Annotation
TQ38_005800	AXB76083	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_006990	AXB76285	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_012410	AXB77196	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_013590	AXB78222	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_015200	AXB77682	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_017175	AXB78390	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_017220	AXB79839	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_017265	AXB78406	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_017270	AXB79840	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_019625	AXB78806	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_022045	AXB79199	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_022455	AXB79267	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_024365	AXB79586	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_024390	AXB79589	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_028770	AXB80532	Aromatic ring-hydroxylating dioxygenase subunit alpha
TQ38_022480	AXB79272	Aromatic ring-opening dioxygenase LigA
TQ38_024465	AXB79602	Cytochrome P450
TQ38_012650	AXB77235	Cytochrome P450
TQ38_020545	AXB78960	Cytochrome P450
TQ38_027480	AXB80322	Cytochrome P450
TQ38_022985	AXB79357	Cytochrome P450
TQ38_028530	AXB80494	Cytochrome P450
TQ38_005010	AXB75958	Cytochrome P450

in nature. In many cases, the hydroxylation is catalyzed by an oxygenase belonging to Rieske (di)oxygenases (RO) (Nojiri et al. 2014). Although ROs are known for their role in aromatic hydrocarbon biodegradation, these enzymes have a broad range of substrates, including terpenoids (Martin and Mohn 1999). According to the results of the annotation, *Novosphingobium* sp. P6W has 15 genes encoding subunit alpha of aromatic ring-hydroxylating dioxygenases (Table 3). Of these, 5 are located on chromosome 1, 9—on chromosome 2 and 1—on a plasmid P6W1.

Conclusions

Bacteria of the genus *Novosphingobium* possess specific biochemical features, including pollutant degradation (Niharika et al. 2013; Nojiri et al. 2014). In the present work we have carried out a genetic description of the strain, which is likely to be a new species of genus *Novosphingobium*. This strain utilizes ABA as a source of carbon and energy. Since ABA is relatively resistant to spontaneous hydrolysis, bioconversion by bacteria is probably important for maintaining low levels of this phytohormone in soil counteracting negative effects of high ABA concentrations on plant growth. The presented data on the complete genome of the *Novosphingobium* sp.

P6W will be useful for further studies of ABA catabolism by transcriptome analysis and reverse genetics.

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Author contributions NEG performed all types of the genomic sequencing and drafted the manuscript, YAN performed assembling, annotation and analysis of the genome, VYG performed the genome sequencing, TTI performed the phylogenetic analysis with additional genomic analysis, AAB performed the laboratory experiments and finalized the manuscript, VIS performed the laboratory experiments and phylogenetic analysis, YVG performed preparing DNA for nanopore sequencing, oversaw the project and finalized the manuscript. All authors read and approved the manuscript.

Compliance with ethical standards

Conflict of interest We declare no conflict of interest with respect to this paper.

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