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GENOME ANALYSIS OF *Pseudomonas brassicacearum* S-1 — AN ANTAGONIST OF CROP PATHOGENS

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Background. The strain *Pseudomonas brassicacearum* S-1 is the basis of the biopesticide "Ecogreen", which is used to control pathogens infecting vegetable and green spicy crops in small-scale hydroponics.

The aim of this work was to sequence and analyze the nucleotide sequence of the genome of strain *P. brassicacearum* S-1 (GenBank accession number CP045701).

Methods. Whole-genome sequencing was performed by both MiSeq (Illumina) and MinION (Oxford Nanopore). Analysis of the genome sequence was performed with a number of bioinformatics programs.

Results. The genome of the *P. brassicacearum* S-1 strain comprising a single circular 6 577 561-bp chromosome with GC content of 60.8 %. Genome analysis revealed genes that constitute valuable biotechnological potential of the S-1 strain and determine synthesis of a wide range of secondary metabolites. Moreover, mobile genetic elements, prophages and short repetitive sequences were identified in the S-1 genome.

Conclusions. Detected genetic determinants, which are responsible for the synthesis of practically valuable compounds, indicate a significant potential of the *P. brassicacearum* S-1 strain as a biocontrol agent.

Key words: genome, sequencing, *Pseudomonas brassicacearum*, secondary metabolites, biocontrol.

Numerous bacterial strains are now characterized physiologically and biochemically. Studied strains include those promising for further use (or already engaged) in biotechnology, particularly in the development of biological agents controlling plant diseases [1]. Elaboration of highly efficient next-generation biocontrol preparations based on bacteria with known genetic structure is one of the most prominent research directions in industrial biotechnology [2]. Molecular genetic and functional genome analysis allows one to directly enhance the activity of biotechnologically significant bacterial cultures without the introduction of extraneous genetic determinants, for example, by selecting the optimal cultivation conditions or removing negative regulatory genes. Such analysis contributes to comprehensive genetic characterization of strains essential for the design of biocontrol mixtures for agriculture.

Pseudomonas spp. are widely used as active agents in biocontrol preparations [3]. The biopesticide "Ecogreen" based on the strain *Pseudomonas brassicacearum* S-1 was developed at the Institute of Microbiology, National Academy of Sciences of Belarus and is now used to protect cucumber, tomato, and other crop plants from root rot diseases [4]. Hence, the aim of this work was to sequence and thoroughly analyze *P. brassicacearum* S-1 genome to facilitate research on plant growth-promoting, antifungal or antibacterial activities of this strain.

Materials and Methods

The genomic DNA was purified with the Bacteria DNA Preparation Kit (Jena Bioscience, PP-206S) from *P. brassicacearum* S-1 culture grown on LB medium until the late logarithmic growth phase.

The genome was sequenced using a MinION sequencer (Oxford Nanopore Technologies). The library for the MinION run was prepared using the Oxford Nanopore Technologies Ligation Sequencing Kit (SQK-LSK109). Additionally, the same DNA sample was sequenced on the Illumina MiSeq platform using MiSeq reagent kit v3. The paired-end library for the Illumina run was prepared using the MuSeek library preparation kit (Thermo Fisher Scientific, K1361).

The genome was assembled in a hybrid manner, combining Illumina and nanopore reads in the following way. 102 296 MinION reads (mean length 10 916.6 nt, mean quality 9.8) were assembled using Flye (v. 2.8 — https://github.com/fenderglass/Flye) into one contig. The nanopore assembly was polished with the Illumina reads. For this purpose, Bowtie2 v. 2.3.5.1 (https://github.com/BenLangmead/bowtie2) and Pilon v. 1.23 (https://github.com/broadinstitute/pilon) with default parameters were used. To correct errors in repeats and search for plasmids, 1 688 260 Illumina reads remaining after quality filtering were assembled into 613 contigs using SPAdes v. 3.14.1 (https://github.com/ablab/spades) with BayesHammer error correction. Combinator-FQ program (https://github.com/masikol/combinator-FQ) was used to detect adjacent contigs and estimate contig multiplicity. The SPAdes contigs were mapped to the draft genome for final corrections in the repeating regions.

The genome was annotated with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/). The genetic map of the chromosome was created using CGView Server (http://cgview.ca). MiGA (Microbial Genomes Atlas, http://microbial-genomes.org) and Mauve (http://darlinglab.org/mauve/mauve.html) were used for comparison of genome sequences and average nucleotide identity (ANI) calculation. For identifying secondary metabolites synthesis clusters, antiSMASH (bacterial version, 5.2.0) was used (https://antismash.secondarymetabolites.org). Prophage sequences and mobile genetic elements were detected using PHASTER (https://phaster.ca) and ISfinder (https://isfinder.biotoul.fr). Restriction–modification systems were predicted by REBASE (http://rebase.neb.com/rebase/rebase.html). BLAST software package (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and the GenBank database (https://www.ncbi.nlm.nih.gov/genbank) were used for comparing nucleotide sequences.

Results and Discussion

Whole-genome sequencing of strain *P. brassicacearum* S-1 was carried out by combining two technologies: Oxford Nanopore and Illumina. Combining reads generated by the two sequencers and thus having distinct, though complementary, properties is likely to improve assembly quality. Hybrid assembly produced a single circular 6 577 561-bp sequence with a GC-content of 60.8%. 5 726 genes were detected, 5 568 annotated as protein-coding, 73 — as pseudogenes, 65 — as tRNA genes, 4 — as ncRNA genes and 16 — as rRNA genes. Genes coding for ribosomal RNA (5S, 16S, 23S) grouped into five clusters. No extrachromosomal genetic elements were found. *P. brassicacearum* S-1 genome sequence was deposited in GenBank with accession number CP045701.

Genome analysis confirmed the taxonomic classification of the strain *P. brassicacearum* S-1 (Domain: *Bacteria*; Phylum: *Proteobacteria*; Class: *Gammaproteobacteria*; Order: *Pseudomonadales*; Family: *Pseudomonadaceae*; Genus: *Pseudomonas*; Species: *Pseudomonas brassicacearum*). Among the genomes present in GenBank, *P. brassicacearum* subsp. *brassicacearum* NFM421 (NC_015379) and *P. kilonensis* DSM 13647 (GCA_001269885) were the closest to that of *P. brassicacearum* S-1 with ANI values of 94.48% and 93.80%, respectively (Table 1).

Comparative analysis of the S-1 genome and related genomes was performed to detect genome rearrangements and unique regions. S-1 appeared to contain unique genome regions absent from the genomes of closely related strains (Fig. 1). Such loci comprise prophage areas (genome coordinates 1 333 519-1 370 191, 4 408 475-4 449 567, 4 841 053-4 877 530), non-ribosomal peptide synthetases (GFU70_14995 — GFU70_15065, GFU70_15270) and the gene cluster determining synthesis of flagellar proteins (GFU70_10800 — GFU70_11030). The chromosome of strain S-1 is the smallest compared to the ones of related strains. It lacks the 241 393 bp fragment which in the genome of strain *P. brassicacearum* subsp. *brassicacearum* NFM421 spans from 2 423 627 to 2 665 019 bp (PSEBR_a1987 — PSEBR_cmegm61). Massive gene reshuffling was also detected within studied bacterial genomes. The genome of strain *P. brassicacearum* subsp. *brassicacearum* NFM421 has the highest similarity in gene positioning compared to the genome of the strain S-1.

Many bacterial strains have specialized defense systems to protect themselves against the penetration and replication of phages or horizontal gene transfer. S-1 genome contains genes coding for DNA methyltransferases of type I (GFU70_25180) and type II (GFU70_09340, GFU70_09360, GFU70_18880, GFU70_20705). Additionally, genes encoding restriction endonucleases of type I (GFU70_25195) and type IV (GFU70_25205) were detected in the genome. Despite this, three complete prophage regions were found: locus tags GFU70_05685 — GFU70_05895 (similar to phage *Vibrio parahaemolyticus*: Vibrio_VP882 (NC_009016)), GFU70_20685 — GFU70_20930 (similar to Vibrio_VP58.5 (NC_027981)) and GFU70_18645 — GFU70_18930 (similar to *Escherichia coli* phage Entero_HK022 (NC_002166)) (Fig. 2). No CRISPR-Cas system was detected in the S-1 genome.

We were able to explore the genomic instability of the S-1 genome. The mobilome of the *P. brassicacearum* S-1 is quite diverse, as the strain was found to carry several putative transposable elements and short repetitive sequences.

Three different families of IS elements were identified by genome analysis:

IS66 (GFU70_08270 — GFU70_08275, GFU70_18595 — GFU70_18600, GFU70_21595 — GFU70_21600, GFU70_11650 — GFU70_11660, GFU70_13735 — GFU70_13745),
IS110 (GFU70_01205, GFU70_16560),
IS1182 (GFU70_01115, GFU70_02845, GFU70_05675, GFU70_19305, GFU70_22695, GFU70_25155, GFU70_28780, GFU70_26625).

Furthermore, six different group II introns (GFU70_04185, GFU70_04810, GFU70_05155, GFU70_23400,

Table 1. **Overview of related *Pseudomonas* genomes**

Strain (accession number)	Total length, bp	GC-content,%	ANI*,%
P. brassicacearum subsp. *brassicacearum* NFM421 (NC_015379)	6 843 248	60.8	94.48
P. kilonensis DSM 13647 (GCA_001269885)	6 385 813	60.9	93.80
P. thivervalensis DSM 13194 (GCA_001269655)	6 581 995	61.2	91.79
P. mediterranea CFBP 5447 (GCA_000774145)	6 319 692	61.2	87.79
P. corrugata NCPPB2445 (GCA_001411965)	6 083 940	60.6	87.30
P. fuscovaginae LMG 2158 (NZ_LT629972)	6 592 354	61.4	82.86

Note: * ANI between subject and *P. brassicacearum* S-1.

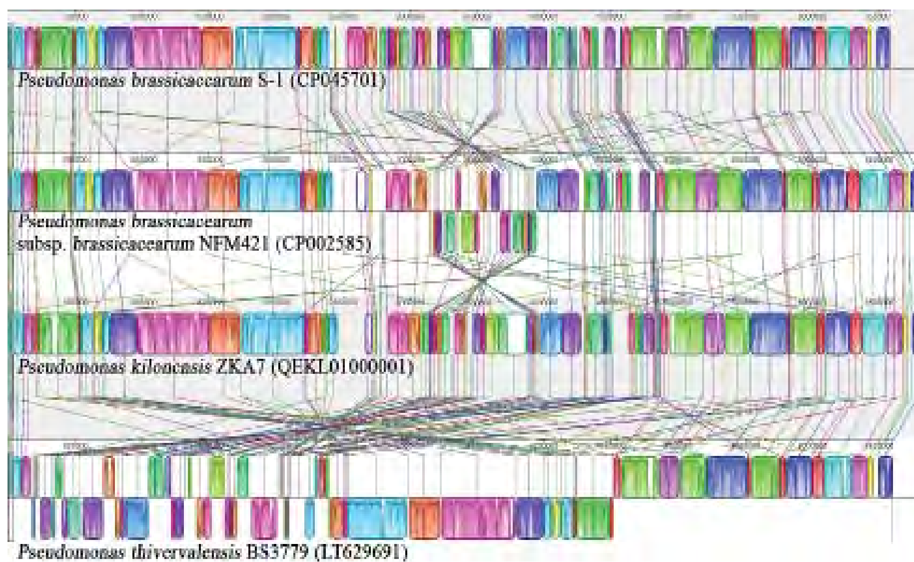

Fig. 1. **MAUVE progressive alignment of *Pseudomonas* sp. genomes**
Boxes of the same color indicate high similarity regions. Boxes below the horizontal strain lines indicate inverted regions. Rearrangements are shown by colored lines. The scale is in base pairs.

GFU70_25585, GFU70_26015) were found in the genome.

Two groups of repetitive extragenic palindromes (REPs) were identified: GTAG-type (784 occurrences) and GTGG-type (1254 occurrences). Four tyrosine transposases (RAYTs) that might be responsible for the transposition of REPs [5] are present in the genome: GFU70_00680, GFU70_07390, GFU70_16415, GFU70_22750.

Multiple mobile genetic elements can lead to the loss of important properties that determine the biotechnological potential of the strain, although these elements might also help *P. brassicacearum* S-1 to adapt to changing environmental conditions in the wild.

We could detect neither genes responsible for antibiotic resistance nor genes associated with virulence or phytotoxicity in the S-1 genome. The genes encoding the components of type III and type IV secretion systems were not found. On the other hand, the *tss* genes encoding the components of the type VI secretion system (GFU70_16125 — GFU70_16190, GFU70_27965 — GFU70_28045, GFU70_28090 — GFU70_28155) were identified. It is known that soil microorganisms use this system to fight against other bacteria [6].

Genome analysis highlighted many genetic loci that might determine the high biotechnological potential of *P. brassicacearum* S-1. The most important of these are

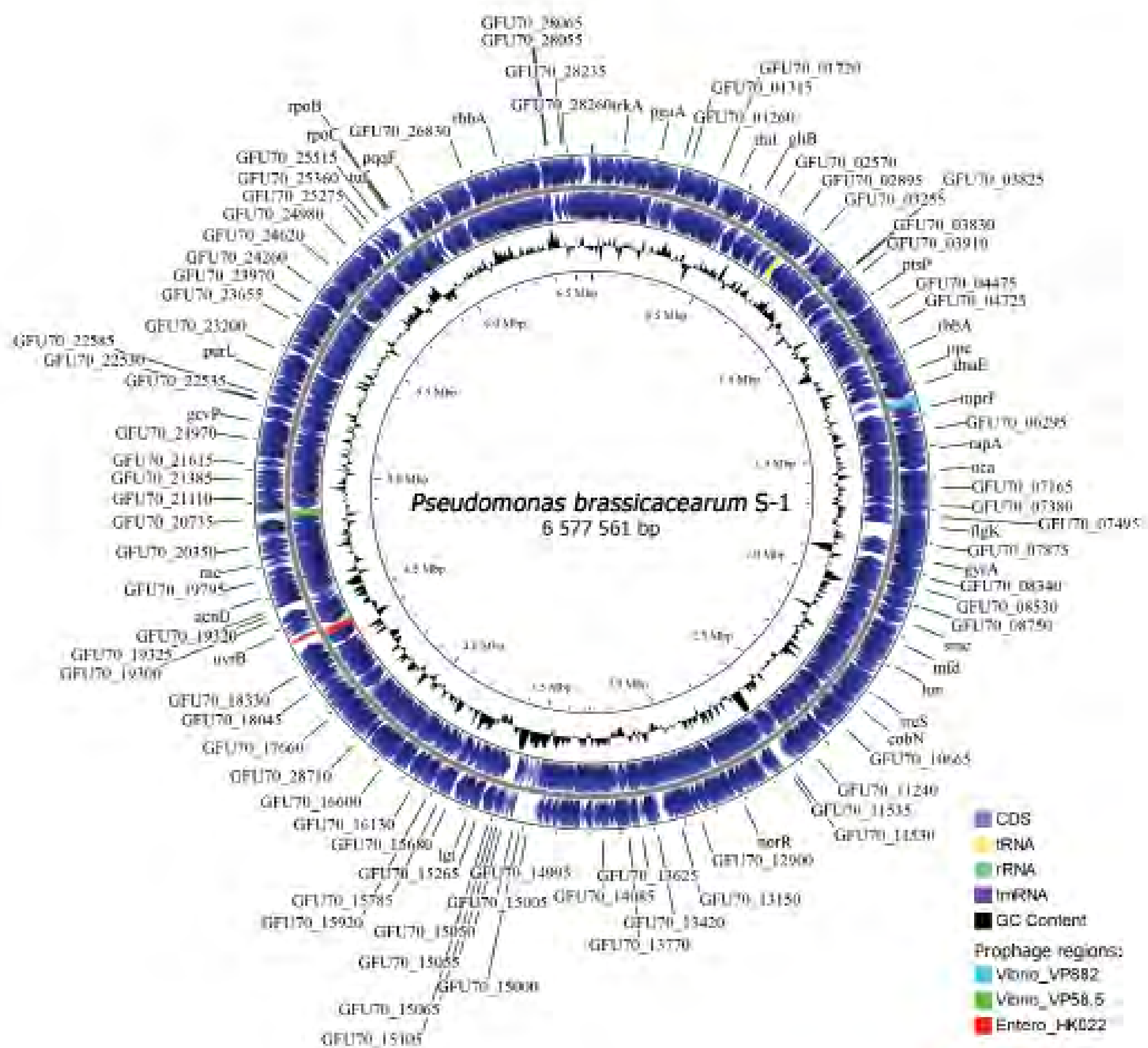

Fig. 2. **Circular map of the *P. brassicacearum* S-1 chromosome**

summarised in Table 2 and also described in more detail below.

Destruction of Lignin. Genes predicted to confer the ability to degrade lignin (GFU70_24415, *dyp*, *copA*, *vanB*, *pcaFHGDC*, *pcaQHG*) were identified in the S-1 genome. Cultures capable of lignin destruction are currently widely used for wood waste recycling [7].

Plant Growth Promotion. The *acdS* gene found in the S-1 genome encodes 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Various stress effects affecting plants provoke the production of phytohormone ethylene, which accelerates plant senescence, promotes leaf yellowing and fruit fall. The reaction of ACC conversion into ammonium and α-ketobutyrate catalyzed by AcdS contributes to decreasing ethylene concentration [8].

The ability of rhizosphere microorganisms to convert inorganic phosphorus-containing compounds into plant-accessible form is regarded as one of the key factors for plant nutrition. Phosphate solubilization by *P. brassicacearum* is associated with the synthesis and secretion of gluconic acid [9]. The biosynthesis of gluconic acid starts from the oxidation of glucose molecules, and the latter reaction is catalyzed by periplasmic

Table 2. **Biocontrol genetic determinants of *P. brassicacearum* S-1**

Locus tag (gene name)	Size, bp	Protein function or product	Strains with similar genes (accession number)	Identity, %
Destruction of Lignin				
GFU70_24415 (-)	726	polyphenol oxidase	*P. fluorescens* FW300-N2E2 (CP015225.1) *P. fluorescens* F113 (CP003150.1)	95.87 95.32
GFU70_10030 (*dyp*)	879	DyP-type peroxidase	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* F113 (CP003150.1)	95.68 95.34
GFU70_22265 (*copA*)	1 734	copper resistance system multicopper oxidase	*P. fluorescens* F113 (CP003150.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	94.80 94.72
GFU70_13435 (*vanB*)	951	vanillate O-demethylase	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* F113 (CP003150.1)	97.17 96.73
GFU70_06610 — GFU70_06640 (*pcaFHGDC*)	6 885	protocatechuate 3,4-cleavage pathway	*P. thivervalensis* PLM3 (CP022202.1) *P. thivervalensis* BS3779 (LT629691.1)	91.78 91.69
GFU70_26120 — GFU70_26130 (*pcaQHG*)	2 392	protocatechuate 3,4-cleavage pathway	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. brassicacearum* L13-6-12 (CP014693.1)	96.28 96.24
Exoprotease Activity				
GFU70_14220 (*aprA*)	1 458	M10 family metallo-peptidase	*P. fluorescens* F113 (CP003150.1) *P. fluorescens* FW300-N2E2 (CP015225.1)	94.37 94.30
GFU70_16545 (-)	1 062	M4 family peptidase	*P. fluorescens* F113 (CP003150.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	96.70 96.61
GFU70_18035 (-)	2 064	S8 family serine peptidase	unique	-
GFU70_28710 (-)	9 633	S8 family serine peptidase	*P. corrugata* BS3649 (LT629798.1) *P. cichorii* JBC1 (CP007039.1)	82.86 78.98
Plant Growth Promotion				
GFU70_26155 — GFU70_26180 (*pqqFABCDE*)	6 044	pyrroloquinoline quinone biosynthesis	*P. fluorescens* F113 (CP003150.1) *P. fluorescens* Pf275 (CP031648.1)	93.78 90.78

Table 2 (continuation)

Locus tag (gene name)	Size, bp	Protein function or product	Strains with similar genes (accession number)	Identity, %
Plant Growth Promotion				
GFU70_26155 — GFU70_26180 (*pqqFABCDE*)	6 044	pyrroloquinoline quinone biosynthesis	*P. fluorescens* F113 (CP003150.1) *P. fluorescens* Pf275 (CP031648.1)	93.78 90.78
GFU70_10675 (*acdS*)	1 017	1-aminocyclopropane-1-carboxylate deaminase	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* F113 (CP003150.1)	96.45 96.35
GFU70_25410 (*ribA*)	618	GTP cyclohydrolase II	*P. brassicacearum* BS3663 (LT629713.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	96.93 96.76
GFU70_25440 (*ribB*)	1 092	bifunctional 3,4-dihydroxy-2-butanone-4-phosphate synthase/GTP cyclohydrolase II	*P. fluorescens* FW300-N2E2 (CP015225.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	97.25 96.98
GFU70_25435 (*ribE*)	477	6,7-dimethyl-8-ribityllumazine synthase	*P. fluorescens* F113 (CP003150.1) *P. brassicacearum* BS3663 (LT629713.1)	99.37 98.95
GFU70_25450 (*ribD*)	1 134	bifunctional diaminohydroxyphosphoribosylaminopyrimidine deaminase/5-amino-6-(5-phosphoribosylamino)uracil reductase	*P. brassicacearum* L13-6-12 (CP014693.1) *P. brassicacearum* BS3663 (LT629713.1)	96.03 95.94
GFU70_00875 — GFU70_00880 (*trpAB*)	2 045	tryptophan synthase subunits	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* L13-6-12 (CP014693.1)	95.83 95.70
GFU70_25925 (*trpC*)	837	indole-3-glycerol phosphate synthase	*P. brassicacearum* BS3663 (LT629713.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	95.07 95.34
GFU70_25930 — GFU70_25945 (*trpDEG*)	4 090	anthranilate biosynthesis	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* L13-6-12 (CP014693.1)	96.12 96.03
Phosphate Solubilization				
GFU70_14025 (*phyC*)	1 926	phytase	*P. brassicacearum* L13-6-12 (CP014693.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	93.35 93.30
GFU70_25420 (*pgpA*)	504	phosphatidylglycerophosphatase A	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* FW300-N2E2 (CP015225.1)	96.83 96.63
GFU70_25950 (*gph*)	819	phosphoglycolate phosphatase	*P. brassicacearum* BS3663 (LT629713.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	95.85 95.73
GFU70_08095 (*mupP*)	672	N-acetylmuramic acid 6-phosphate phosphatase	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* F113 (CP003150.1)	97.02 96.58

Table 2 (end)

Locus tag (gene name)	Size, bp	Protein function or product	Strains with similar genes (accession number)	Identity, %
GFU70_08100 (*ubiG*)	699	bifunctional 2-polyprenyl-6-hydroxyphenol methylase/3-demethylubiquinol 3-O-methyltransferase	*P. brassicacearum* BS3663 (LT629713.1) *P. fluorescens* F113 (CP003150.1)	96.85 96.71
Exopolysaccharides Biosynthesis				
GFU70_23090 — GFU70_23145 (*algDKEGXLIJFA, alg8, alg44*)	16 567	alginate biosynthesis	*Pseudomonas* sp. MPDS (CP054128.1) *Pseudomonas* sp. A214 (LT707062.1)	92.60 92.57
GFU70_25220 (*sacB*)	1 275	glycoside hydrolase family 68 protein	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	97.65 97.33
Lipopolysaccharides (LPS) Biosynthesis				
GFU70_28450 (*alkP*)	2 211	lipoteichoic acid synthase	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* L13-6-12 (CP014693.1)	94.26 94.17
Secondary Metabolites With Antagonistic Activity				
GFU70_16380 — GFU70_16390 (*hcnABC*)	2 981	cyanide-forming glycine dehydrogenases subunits	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. fluorescens* F113 (CP003150.1)	94.77 94.53
GFU70_11155 — GFU70_11195 (*phlHGFACBDEI*)	9 063	2,4-diacylphloroglucinol biosynthesis	*P. brassicacearum* L13-6-12 (CP014693.1) *P. fluorescens* F113 (CP003150.1)	95.36 95.38
GFU70_08745 — GFU70_08750 (*pvdSL*)	13 608	pyoverdine biosynthesis and regulation	*P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1) *P. brassicacearum* LBUM300 (CP012680.1)	94.45 94.44
GFU70_19280 — GFU70_19330 (*pvdIJKDEONMP*)	46 331	pyoverdine biosynthesis and regulation	*P. brassicacearum* L13-6-12 (CP014693.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	95.18 95.14
GFU70_08785 (*pvdH*)	1 413	aspartate aminotransferase	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* L13-6-12 (CP014693.1)	94.83 94.76
GFU70_19235 (*pvdA*)	1 335	L-ornithine N5-oxygenase	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* subsp. *brassicacearum* NFM421 (CP002585.1)	95.06 94.83

membrane-bound glucose dehydrogenase. This enzyme forms a complex with pyrroloquinoline quinone (PQQ). The PQQ biosynthesis gene cluster (*pqqFABCDE*) is present in the S-1 genome. PQQ serves as the redox cofactor for diverse bacterial dehydrogenases, stimulates plant growth, features antioxidant properties [10] and can be directly involved in the synthesis of antimicrobial compounds [11].

The ability to synthesize indole-3-acetic (IAA) acid is a major property of plant growth-promoting rhizosphere bacteria. Tryptophan has been identified as the main precursor for IAA biosynthesis pathways in bacteria. The tryptophan and anthranilate biosynthesis genes (*trpAB*, *trpCDEG*) were identified in the S-1 genome [12].

P. brassicacearum S-1 encodes genes for the biosynthesis of proteins with predicted exoprotease activity (*aprA*, GFU70_16545, GFU70_18035, GFU70_28710).

Plant Colonization. Exopolysaccharides are known to be essential constituents of biofilms facilitating colonization of plant rhizosphere [13]. The S-1 strain harbours several genes (*algDKEGXLIJFA, alg8, alg44*) presumably directing the biosynthesis of exopolysaccharide alginate [14]. The strain might have the genetic potential to produce exopolysaccharide levan since it was predicted to harbour gene *sacB* (GFU70_25220) coding for glycosyl hydrolase 68 (GH68) family protein [15].

LPS components of bacterial cell walls may constitute a barrier blocking the transfer of many antibiotics. Additionally, changes in lipid composition maintain functions of the outer membrane in response to environmental changes. It is worth mentioning that *P. brassicacearum* S-1 encodes lipoteichoic acid synthase presumably involved in the biosynthesis of lipoteichoic acid. Teichoic acid is considered to be an essential component of biofilms formed by gram-positive bacteria [16] but is not typical for gram-negative bacteria. It might be surmised that the synthesis of lipoteichoic acid enhances the adaptation capacity and survival rate of the S-1 strain in plant rhizosphere.

Secondary Metabolites and Antibiotics. Clusters *pvd*, *phl* and *hcn* were identified in the S-1 genome. They are responsible for the production of pyoverdine, phloroglucinol and cyanide, respectively. These metabolites can suppress the growth of a wide range of crop pathogens [17–19].

A detailed search for the genes controlling the biosynthesis of secondary metabolites using antiSMASH revealed 9 loci carrying genes, which are supposedly responsible for the synthesis of secondary metabolites with antagonistic activity (Table 3).

Region No. 1 (*nrps_1*) shows similarity to the gene determining the biosynthesis of fragin in *Burkholderia cenocepacia* H111 (37 % identity). This genome region was not described for closely related strains, so we compared it to the genome region of *B. cenocepacia* H111. This gene encodes a putative metallophore responsible for metal chelation and, therefore, antifungal activity [20].

Region No. 2 shows 40 % identity to the gene cluster determining the biosynthesis of aryl polyene in *Aliivibrio fischeri* ES114. Aryl polyene is regarded as a reactive oxygen species protection agent [21].

Region No. 3 (*naggn*) probably determines the synthesis of N–acetylglutaminylglu tamine. This compound is involved in osmotic stress adaptation [22].

Region No. 4 demonstrates high similarity to gene cluster responsible for fengycin synthesis in *Bacillus velezensis* FZB42. This compound is known to suppress the growth of fungal pathogens [23].

Region No. 5 (*nrps_2*) is similar to the gene cluster determining the synthesis of cupriachelin in *Cupriavidus necator* H16. This siderophore is involved in the chelation of ferric ions by their uptake from extracellular space and transferring into bacterial cytoplasm via ABC transporters [24].

Regions No. 6 and No. 9 are probably involved in the synthesis of polyketide synthases. Polyketide synthases are responsible for the synthesis of a huge diversity of secondary metabolites with antimicrobial activities [25]. The *nrps_5* gene (region No. 9) is unique and not typical for closely related strains (homologue is found in the genome of the strain *P. mediterranea* DSM 16733 (locus tags SAMN05216476_3741 — SAMN05216476_3742)). These regions are attractive objects for exploring the antagonistic activity of this strain.

Region No. 7 is presumably engaged in the synthesis of antibiotic trifolitoxin. This antibiotic features antagonistic activity against several phytopathogens [26].

Region No. 8 (*nrps_4*) contains 15 genes. It demonstrates similarity with

Table 3. **Genome regions of *P. brassicacearum* S-1 probably responsible for the synthesis of secondary metabolites with antagonistic activity**

Re-gion	Locus tag (gene name)	Size, bp.	Protein function or product	Strains with similar genes (accession number)	Iden-tity, %
1	GFU70_01215 (*nrps_1*)	3 540	non-ribosomal peptide synthetase	*P. brassicacearum* LBUM300 (CP012680.1) *P. brassicacearum* L13-6-12 (CP014693.1)	94.72 92.70
2	GFU70_02420 — GFU70_02435 (-)	3 612	aryl polyene biosyn-thesis	*P. brassicacearum* LBUM300 (CP012680.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	96.32 96.26
3	GFU70_08005 — GFU70_08015 (*naggn*)	4 983	N-acetylglutaminylglu-tamine biosynthesis	*P. brassicacearum* 3Re2-7 (CP034725.1) *P. brassicacearum* subsp. *brassi-cacearum* NFM421 (CP002585.1)	96.25 96.22
4	GFU70_09760 — GFU70_09765 (-)	3 252	β–lactone biosynthesis	*P. fluorescens* FW300-N2E2 (CP015225.1) *P. thivervalensis* BS3779 (LT629691.1)	93.00 92.38
5	GFU70_11525 — GFU70_11550 (*nrps_2*)	35 806	non-ribosomal peptide biosynthesis	*P. brassicacearum* LBUM300 (CP012680.1) *P. brassicacearum* 3Re2-7 (CP034725.1)	93.98 93.96
6	GFU70_11690 — GFU70_11700 (*nrps_3*)	5 123	polyketide synthase	*P. brassicacearum* subsp. *brassi-cacearum* NFM421 (CP002585.1) *P. brassicacearum* L13-6-12 (CP014693.1)	95.16 95.24
7	GFU70_12325 — GFU70_12330 (-)	1 920	thioamitide biosynthe-sis	*P. synxantha* KENGFT3 (CP014868.1) *P. synxantha* LBUM223 (CP011117.2)	85.02 84.97
8	GFU70_14995 — GFU70_15065 (*nrps_4*)	124 185	non-ribosomal peptide biosynthesis	*P. fluorescens* FW300-N2C3 (CP012831.1) *P. brassicacearum* DF41 (CP007410.1)	88.00 85.13
9	GFU70_15265 — GFU70_15270 (*nrps_5*)	8 451	polyketide synthase	*P. mediterranea* DSM 16733 (LT629790.1) *P. mediterranea S58* (CP046874.1)	78.65 78.43

the cluster involved in the biosynthesis of antimicrobial cyclic lipopeptides nunamycin and nunapeptin in *P. fluorescens* In5. The antimicrobial activity of nunamycin is accounted for by its ability in *Rhizoctonia solani* Ag3 mycelial growth inhibition, whereas nunapeptin was essential to suppress the growth of *Pythium aphanidermatum* [27].

P. brassicacearum S-1 demonstrates the potential to synthesize a variety of biocontrol-associated compounds, including secondary metabolites and antibiotics. Moreover, the genetic determinants encoding unique non-ribosomal peptide synthetases and responsible for antimicrobial properties of studies strain were identified. As a result, certain genetic determinants detected by genome analysis indicate significant biocontrol potential of *P. brassicacearum* S-1.

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REFERENCES

1. *Moraes Bazioli J., Belinato J. R., Costa J. H., Akiyama D. Y., Pontes J. G. M., Kupper K. C., Augusto F., de Carvalho J. E., Fill T. P.* Biological Control of Citrus Postharvest Phytopathogens. *J. Toxins*. 2019, 11 (8), 460. https://doi.org/10.3390/toxins11080460
2. *Nelkner J, Tejerizo G. T., Hassa J., Lin T. W., Witte J., Verwaaijen B., Winkler A., Bunk B., Spröer C., Overmann J., Grosch R., Pühler A., Schlüter A. A.* Genetic Potential of the Biocontrol Agent *Pseudomonas brassicacearum* (Formerly *P. trivialis*) 3Re2-7 Unraveled by Genome Sequencing and Mining, Comparative Genomics and Transcriptomics. *Genes*. 2019, 10 (8), 601. https://doi.org/10.3390/genes10080601
3. *Stockwell V. O., Stack J. P.* Using *Pseudomonas* spp. for Integrated Biological Control. *Phytopathology*. 2007, 97 (2), 244–249. https://doi.org/10.1094/PHYTO-97-2-0244
4. *Kolomiets E. I., Kuptsov V. N., Sverchkova N. V., Evsegneeva N. V., Mandryk-Litvinkovich M. N., Mishin L. T., Voitka D. V., Rapoport A. I., Khrustaly G. M.* Bacteria *Pseudomonas aurantiaca* BIM B-446 — the basis of biopesticide ecogreen intended for control of vegetable and green crop pathogens in low-scale hydroponics. *Collection of scientific papers "Microbial Biotechnology: Fundamental and Applied Aspects"*. 2012, V. 4, P. 98–107. (In Russian).
5. *Bertels F., Rainey P. B.* Within-Genome Evolution of REPINs: a New Family of Miniature Mobile DNA in Bacteria. *PLOS Genet*. 2011, 7 (6), P. e1002132. https://doi.org/10.1371/journal.pgen.1002132
6. *Decoin V., Barbey C., Bergeau D., Latour X., Feuilloley M. G., Orange N., Merieau A.* A type VI secretion system is involved in *Pseudomonas fluorescens* bacterial competition. *PloS One*. 2014, 9 (2), P. e89411. https://doi.org/10.1371/journal.pone.0089411
7. *Lee S., Kang M., Bae J. H., Sohn J. H., Sung B. H.* Bacterial Valorization of Lignin: Strains, Enzymes, Conversion Pathways, Biosensors, and Perspectives. *Front. Bioeng. Biotechnol.* 2019, V. 7, P. 209. https://doi.org/10.3389/fbioe.2019.00209
8. *Gontia-Mishra I., Sasidharan S., Tiwari S.* Recent developments in use of 1-aminocyclopropane-1-carboxylate (ACC) deaminase for conferring tolerance to biotic and abiotic stress. *Biotechnol. Lett.* 2014, 36 (5), 889–898. https://doi.org/10.1007/s10529-014-1458-9
9. *De Werra P., Püchy-Tarr M., Keel C., Maurhofer M.* Role of gluconic acid production in the regulation of biocontrol traits of *Pseudomonas fluorescens* CHA0. *Appl. Environ. Microbiol*. 2009, 75 (12), 4162–4174. https://doi.org/10.1128/AEM.00295-09
10. *Choi O., Kim J., Kim J. G., Jeong Y., Moon J. S., Park C. S., Hwang I.* Pyrroloquinoline Quinone Is a Plant Growth Promotion Factor Produced by *Pseudomonas fluorescens* B16. *Plant Physiol*. 2008, 146 (2), 657–668. https://doi.org/10.1104/pp.107.112748
11. *Guo Y. B., Li J., Li L., Chen F., Wu W., Wang J., Wang H.* Mutations that disrupt either the *pqq* or the *gdh* gene of *Rahnella aquatilis* abolish the production of an antibacterial substance and result in reduced biological control of grapevine crown gall. *Appl. Environ. Microbiol*. 2009, 75 (21), 6792–6803. https://doi.org/10.1128/AEM.00902-09
12. *Singh P., Singh R. K., Guo D. J., Sharma A., Singh R. N., Li D. P., Malviya M. K., Song X. P., Lakshmanan P., Yang L. T., Li Y. R.* Whole Genome Analysis of Sugarcane Root-Associated Endophyte *Pseudomonas aeruginosa* B18—A Plant Growth-Promoting Bacterium With Antagonistic Potential Against Sporisorium scitamineum. *Front. Microbiol*. 2021, V. 12, P. 628376. https://doi.org/10.3389/fmicb.2021.628376
13. *Mann E. E., Wozniak D. J.* *Pseudomonas* biofilm matrix composition and niche biology. *FEMS Microbiol. Rev*. 2012, 36 (4), 893–916. https://doi.org/10.1111/j.1574-6976.2011.00322.x
14. *Ertesveg H., Sletta H., Senneset M., Sun Y. Q., Klinkenberg G., Konradsen T. A., Ellingsen T. E., Valla S.* Identification of genes affecting alginate biosynthesis in *Pseudomonas fluorescens* by screening a transposon insertion library. *BMC Genomics*. 2017, 18 (1), 11. https://doi.org/10.1186/s12864-016-3467-7
15. *Lammens W., Le Roy K., Schroeven L., Van Laere A., Rabijns A., Van den Ende W.* Structural insights into glycoside hydrolase family 32 and 68 enzymes: functional implications. *J. Exp. Bot*. 2009, 60 (3), 727–740. https://doi.org/10.1093/jxb/ern333
16. *Malanovic N., Lohner K.* Gram-positive bacterial cell envelopes: The impact on the activity of antimicrobial peptides. *Biochim. Biophys. Acta*. 2016, 1858 (5), 936–946. https://doi.org/10.1016/j.bbamem.2015.11.004
17. *Ye L., Hildebrand F., Dingemans J., Ballet S., Laus G., Matthijs S., Berendsen R., Cornelis P.*

Draft genome sequence analysis of a *Pseudomonas putida* W15Oct28 strain with antagonistic activity to Gram-positive and *Pseudomonas* sp. pathogens. *PloS One*. 2014, 9 (11), e110038. https://doi.org/10.1371/journal.pone.0110038
18. *Anand A., Chinchilla D., Tan C., Mune-Saffranй L., L'Haridon F., Weisskopf L.* Contribution of Hydrogen Cyanide to the Antagonistic Activity of *Pseudomonas* Strains Against Phytophthora infestans. *Microorganisms*. 2020, 8 (8), 1144. https://doi.org/10.3390/microorganisms8081144
19. *Mandryk-Litvinkovich M. N., Muratova A. A., Nosonova T. L., Evdokimova O. V., Valentovich L. N., Titok M. A., Kolomiets E. I.* Molecular genetic analysis of determinants defining synthesis of 2,4-diacetylphloroglucinol by *Pseudomonas brassicacearum* BIM B-446 bacteria. *Appl. Biochem. Microbiol*. 2017, V. 53, P. 31–39. https://doi.org/10.1134/S0003683817010124
20. *Jenul C., Sieber S., Daeppen C., Mathew A., Lardi M., Pessi G., Hoepfner D., Neuburger M., Linden A., Gademann K., Eberl L.* Biosynthesis of fragin is controlled by a novel quorum sensing signal. *Nat. Commun*. 2018, 9 (1), 1297. https://doi.org/10.1038/s41467-018-03690-2
21. *Cimermancic P., Medema M. H., Claesen J., Kurita K., Wieland Brown L. C., Mavrommatis K., Pati A., Godfrey P. A., Koehrsen M., Clardy J., Birren B. W., Takano E., Sali A., Linington R. G., Fischbach M. A.* Insights into secondary metabolism from a global analysis of prokaryotic biosynthetic gene clusters. *Cell*. 2014, 158 (2), 412–421. https://doi.org/10.1016/j.cell.2014.06.034
22. *Sagot B., Gaysinski M., Mehiri M., Guigonis J. M., Le Rudulier D., Alloing G.* Osmotically induced synthesis of the dipeptide N-acetylglutaminylglutamine amide is mediated by a new pathway conserved among bacteria. *Proc. Natl. Acad. Sci. U. S. A.* 2010, 107 (28), 12652–12657. https://doi.org/10.1073/pnas.1003063107
23. *Chen X. H., Koumoutsi A., Scholz R., Eisenreich A., Schneider K., Heinemeyer I., Morgenstern B., Voss B., Hess W. R., Reva O., Junge H., Voigt B., Jungblut P. R., Vater J., Sьssmuth R., Liesegang H., Strittmatter A., Gottschalk G., Borriss R.* Comparative analysis of the complete genome sequence of the plant growth-promoting bacterium *Bacillus amyloliquefaciens* FZB42. *Nat. Biotechnol.* 2007, 25 (9), 1007–1014. https://doi.org/10.1038/nbt1325
24. *Kreutzer M. F., Kage H., Nett M.* Structure and biosynthetic assembly of cupriachelin, a photoreactive siderophore from the bioplastic producer *Cupriavidus necator* H16. *J. Am. Chem. Soc*. 2012, 134 (11), 5415–5422. https://doi.org/10.1021/ja300620z
25. *Connolly J. A., Wilson A., Macioszek M., Song Z., Wang L., Mohammad H. H., Yadav M., di Martino M., Miller C. E. Hothersall J., Haines A. S., Stephens E. R., Crump M. P., Willis C. L., Simpson T. J., Winn P. J., Thomas C. M.* Defining the genes for the final steps in biosynthesis of the complex polyketide antibiotic mupirocin by *Pseudomonas fluorescens* NCIMB10586. *Sci. Rep*. 2019, 9 (1), 1542. https://doi.org/10.1038/s41598-018-38038-9
26. *Breil B., Borneman J., Triplett E. W.* A newly discovered gene, *tfuA*, involved in the production of the ribosomally synthesized peptide antibiotic trifolitoxin. *J. Bacteriol*. 1996, 178 (14), 4150–4156. https://doi.org/10.1128/jb.178.14.4150-4156.1996
27. *Michelsen C. F., Watrous J., Glaring M. A., Kersten R., Koyama N., Dorrestein P. C., Stougaard P.* Nonribosomal Peptides, Key Biocontrol Components for *Pseudomonas fluorescens* In5, Isolated from a Greenlandic Suppressive Soil. *mBio*. 2015, 6 (2), e00079. https://doi.org/10.1128/mBio.00079-15

АНАЛІЗ ГЕНОМУ БАКТЕРІЙ *Pseudomonas brassicacearum* S-1 — АНТАГОНІСТІВ ФІТОПАТОГЕНІВ СІЛЬСЬКОГОСПОДАРСЬКИХ КУЛЬТУР

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Вступ. Штам *Pseudomonas brassicacearum* S-1 є основою біопрепарату «Екогрін», який застосовують для захисту від патогенів овочевих та зеленних культур в умовах малооб'ємної гідропоніки.

Метою роботи було секвенування та аналіз нуклеотидної послідовності геному бактерій *P. brassicacearum* S-1 (код доступу в базі даних ГенБанк CP045701).

Методи. Повногеномне секвенування проводили за допомогою приладів MiSeq (Illumina) та MinION (Oxford Nanopore), для аналізу послідовності геному було використано низку біоінформатичних програм.

Результати. Геном штаму *P. brassicacearum* S-1 складається з однієї кільцевої хромосоми розміром 6 577 561 пар основ зі вмістом ГЦ-пар 60,8%. Аналіз геному дав змогу виявити гени, які визначають синтез широкого спектру вторинних метаболітів, що становлять біотехнологічний потенціал штаму S-1. Крім того, було визначено локалізацію мобільних генетичних елементів, профагів і коротких повторюваних послідовностей у межах геному штаму S-1.

Висновки. Виявлені генетичні детермінанти синтезу практично значущих сполук вказують на значний потенціал штаму *P. brassicacearum* S-1 як агента біоконтролю.

Ключові слова: геном, секвенування, *Pseudomonas brassicacearum*, вторинні метаболіти, біоконтроль.

АНАЛИЗ ГЕНОМА БАКТЕРИЙ *Pseudomonas brassicacearum* S-1 — АНТАГОНИСТОВ ФИТОПАТОГЕНОВ СЕЛЬСКОХОЗЯЙСТВЕННЫХ КУЛЬТУР

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Вступление. Штамм *Pseudomonas brassicacearum* S-1 является основой биопрепарата «Экогрин», который применяется для защиты от патогенов овощных и зеленных культур в условиях малообъёмной гидропоники.

Целью работы было секвенирование и анализ нуклеотидной последовательности генома бактерий *P. brassicacearum* S-1 (код доступа в базе данных ГенБанк CP045701).

Методы. Полногеномное секвенирование проводили с помощью приборов MiSeq (Illumina) и MinION (Oxford Nanopore), для анализа последовательности генома был использован ряд биоинформатических программ.

Результаты. Геном штамма *P. brassicacearum* S-1 состоит из одной кольцевой хромосомы размером 6 577 561 п. н. с содержанием ГЦ-пар 60,8%. Анализ генома позволил выявить гены, определяющие синтез широкого спектра вторичных метаболитов, составляющих биотехнологический потенциал штамма S-1. Кроме того, была определена локализация мобильных генетических элементов, профагов и коротких повторяющихся последовательностей в пределах генома штамма S-1.

Выводы. Выявленные генетические детерминанты синтеза практически значимых соединений указывают на значительный потенциал штамма *P. brassicacearum* S-1 как агента биоконтроля.

Ключевые слова: геном, секвенирование, *Pseudomonas brassicacearum*, вторичные метаболиты, биоконтроль.