Complete genome sequence of *Pseudomonas brassicacearum* strain L13-6-12, a biological control agent from the rhizosphere of potato

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**Abstract**

*Pseudomonas brassicacearum* strain L13-6-12 is a rhizosphere colonizer of potato, lettuce and sugar beet. Previous studies have shown that this motile, Gram-negative, non-sporulating bacterium is an effective biocontrol agent against different phytopathogens. Here, we announce and describe the complete genome sequence of *P. brassicacearum* L13-6-12 consisting of a single 6.7 Mb circular chromosome that consists of 5773 protein coding genes and 85 RNA-only encoding genes. Genome analysis revealed genes encoding specialized functions for pathogen suppression, thriving in the rhizosphere and interacting with eukaryotic organisms.

**Keywords:** Short genome report, *Pseudomonadaceae*, *Pseudomonas brassicacearum* L13-6-12, Potato rhizosphere, Volatile organic compounds, Biocontrol, Plant growth promotion, Secretion systems

**Introduction**

*Pseudomonas brassicacearum* strain L13-6-12 was isolated from the rhizosphere of a field grown potato plant [1]. L13-6-12 was selected as effective biological control agent with disease-suppressing effects against *Rhizoctonia solani* Kühn in treated lettuce and potato plants in greenhouse and field trials [2]. It has additional antifungal activity against the phytopathogenic fungi *Alternaria alternata*, *Botrytis cinerea* Pers. DSM5145, *Penicillium italicum*, *Phoma betae*, *Sclerotinia sclerotiorum*, *Verticillium dahliae* Kleb. V25 (all Ascomycota) and *Rhizoctonia solani* AG2-2IIIB and AG4 and *Sclerotium rolfsii* (Basidiomycota). This biocontrol activity is linked to the production of secondary metabolites, including 2,4-diacetylphloroglucinol and hydrogen cyanide. For various strains of plant-associated pseudomonads the production of antifungal metabolites like DAPG and recombinase genes were identified as the major trait for biological control of soilborne pathogens and plant root colonization [3]. Genes in L13-6-12 predicting functions for biocontrol include factors such as secreted proteases and comprehensive secretion systems. It also supports plant growth by nutrient delivery by phosphate solubilization, production of indole-3-acetic acid as well as by aminocyclopropane-1-carboxylate deaminase activity. Additionally, L13-6-12 copes with abiotic stresses such as desiccation and high salt concentrations. To gain insight into ecological relevant traits and to improve its biotechnological applications we sequenced the complete genome of this bacterium.

**Organism information**

**Classification and features**

*P. brassicacearum* L13-6-12 is a motile, Gram-negative, non-sporulating rod in the order *Pseudomonadales* of the class *Gammaproteobacteria*. The rod-shaped cells are approximately 0.4 μm in width and 0.8–1.5 μm in length (Fig. 1 left). The strain is moderately fast-growing, forming 1 mm colonies within 1–2 days at 25 °C. Colonies formed on NBII agar plates are yellow shining, domed and moderately mucoid with smooth margins (Fig. 1 right). Cultivation for more than two weeks on NA result in a color change of the medium to dark brown. L13-6-12...
was isolated from a potato rhizosphere from plants grown in a field trial in Groß Lüsewitz, Germany, in 1997 [1].

Even though the optimal growth temperature is 30 °C, L13-6-12 can also slowly replicate at 5 °C in liquid Luria Bertani medium. Growth was observed at 37 °C and slightly at 40 °C in this culturing medium as well as on solidified medium after 24 h. The strain grows in complex media, but not in Standard Succinate Medium (pH 7.0). Optimum pH for growth in LB is pH 7.0. The bacterium is an efficient colonizer of lettuce, potato [2, 3] and sugar beet plants, where microcolonies consisted of tens to hundreds of bacterial cells, forming an interconnected network between epidermal cells in the rhizoplane [3]. It does not cause any deleterious effect on its original host plant potato or lettuce [1, 2] and sugar beet [4] or on the nematode Caenorhabditis elegans [5]. Strain L13-6-12 has natural resistance to gentamycin (10 μg mL⁻¹), trimethoprim (50 μg mL⁻¹) and is able to develop spontaneous rifampicin-resistance.

Minimum Information about the Genome Sequence of P. brassicacearum L13-6-12 is summarized in Table 1. The phylogenetic relationship of P. brassicacearum L13-6-12 to other species within the genus Pseudomonas is visualized in a 16S rRNA based tree (Fig. 2) [6].

**Genome sequencing information**

**Genome project history**

Strain L13-6-12 was originally assigned to P. fluorescens based on 16S rRNA gene sequencing and alignments with NCBI database [1, 2, 4, 5]. After average nucleotide identity [7] comparison of the genome sequence against the genomes of the type strains and proxytype strains that are already in GenBank, L13-6-12 showed 99.604% identity to the type genome of P. brassicacearum with 95.5% coverage of the genome. The genome of P. brassicacearum strain L13-6-12 was selected for sequencing based on its ability to exert biocontrol abilities against fungal pathogens and to promote plant growth [1, 3]. This whole-genome shotgun project has been deposited in the NCBI database under the accession no. CP014693. The version described in this paper is the first version (Table 2).

**Growth conditions and genomic DNA preparation**

P. brassicacearum strain L13-6-12 was grown in 50 mL of NBII (Sifin, Berlin, Germany) medium and incubated for 20 h at 30 °C. 1.0 mL was centrifuged at 2500 × g for 5 min at 4 °C and genomic DNA was extracted using the MasterPure DNA purification kit (Epicentre, Madison, WI, USA). DNA quality and quantity were validated by agarose gel electrophoresis and spectrophotometry using a UV-Vis spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, MA USA). In total, 54 μg genomic DNA (1.8 μg μL⁻¹) was sent on dry ice to the sequencing service. PacBio RS libraries with inserts of 8 to 20 kb were constructed and sequenced at GATC Biotech (Konstanz, Germany).

**Genome sequencing and assembly**

PacBio RS libraries with inserts of 8 to 20 kb were constructed and sequenced at GATC Biotech (Konstanz, Germany) using single molecule, real-time sequencing. Assembly was completed with the Hierarchical Genome Assembly Process algorithm implemented in the PacBio SMRT Analysis software (Pacific Biosciences, Menlo Park, CA, USA). The assembly of L13-6-12 genome based on 130,283 quality reads with a mean length of 4995 bp resulting in a single circular chromosome consisting of...
6,715,909 bp, with 84.9-fold overall coverage and a GC content of 60.7%.

**Genome annotation**

Automatic annotation was conducted on the RAST Web server (version 36) using RAST gene calling based on FIGfam version Release70 [8, 9], and additional annotation for using the automated assignment of COG-functions to protein-coding genes was completed on the BASys web server using Glimmer gene prediction [10, 11]. Pseudogenes were predicted using the NCBI Prokaryotic Genome Annotation Pipeline. Signal peptides and transmembrane helices were predicted using SignalP [12, 13] and TMHMM [14, 15].

**Genome properties**

The genome of L13-6-12 is composed of one circular chromosome consisting of 6,715,909 bp with an average GC content of 60.7% (Table 3 and Fig. 3), which is similar to that of other *P. brassicacearum* strains. Among the 5887 predicted genes, 5773 were identified as protein coding genes. Of the last, 4801 (83.2%) were assigned a putative function, while the other 972 (16.8%) were designated as hypothetical proteins. The classification of CDSs into functional categories according to the COG [16, 17] database is summarized in Table 4 based on BASys gene prediction. Beside the predicted genes, the genome annotation contained 65 tRNA, five rRNA loci (5S, 16S, 23S) with one additional 5S rRNA, four ncRNAs and 284 predicted SEED subsystem features.

**Insights from the genome sequence**

The genome-wide phylogenetic analysis on different *Pseudomonas* species with the L13-6-12 genome showed that strain L13-6-12 clusters closely to *P. fluorescens* Q8r1-96 (NCBI Accession no. PRJNA67537) (Fig. 2).

### Table 1

Classification and general features of *P. brassicacearum* strain L13-6-12 according to the MIGS recommendation [29]

| MIGS ID | Property                        | Term                                | Evidence code |
|---------|---------------------------------|-------------------------------------|---------------|
|         | Classification                  | Domain Bacteria                     | TAS [30]      |
|         |                                 | Phylum Proteobacteria               | TAS [31]      |
|         |                                 | Class Gammaproteobacteria           | TAS [32]      |
|         |                                 | Order Pseudomonadales               | TAS [33, 34]  |
|         |                                 | Family Pseudomonadaceae             | TAS [31, 35]  |
|         |                                 | Genus Pseudomonas                   | TAS [36–39]   |
|         |                                 | Species Pseudomonas brassicacearum  | TAS [39]      |
|         | Strain:                          | L13-6-12                            | TAS [1]       |
|         | Gram stain                       | Negative                            | IDA, TAS [39] |
|         | Cell shape                       | Rod                                 | IDA, TAS [39] |
|         | Motility                         | Motile                              | TAS [39]      |
|         | Sporulation                      | Not reported                         | NAS           |
|         | Temperature range                 | 5 °C–40 °C                          | IDA           |
|         | Optimum temperature              | 30 °C                               | IDA           |
|         | pH range; Optimum                | 5.0–9.0; 7                          | IDA           |
|         | Carbon source                    | Heterotrophic                       | TAS [39]      |
|         | Habitat                          | Potato, Rhizosphere                 | TAS [1]       |
|         | Salinity                         | 1.0–9.0% NaCl (w/v)                 | IDA, TAS [1]  |
|         | Oxygen requirement                | Aerobic                             | TAS [39]      |
|         | Biotic relationship              | Rhizospheric                        | TAS [1, 2, 4] |
|         | Pathogenicity                    | Non-pathogen                        | TAS [1, 5]    |
|         | Geographic location              | Gross Luesewitz, Germany            | TAS [1]       |
|         | Sample collection                | 2001                                | TAS [1]       |
|         | Latitude                         | 54°41′15.4704″ N                     | NAS           |
|         | Longitude                        | 12°20′19.9248″ E                     | NAS           |
|         | Altitude                         | 37 m                                | NAS           |

*Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [40].*
Recently, Q8r1-96 was described as a biological control strain that produces the antibiotic DAPG and that exceptionally colonizes the roots of wheat and pea [18, 19]. The genome of L13-6-12 contains several genes, which are important contributors to biological control. They are related to the biosynthesis of secondary metabolites or antimicrobial products that are similar to those found in the genomes of other Pseudomonads [20]. We detected genes for the biosynthesis of DAPG (Locus tags: A0U95_04640, A0U95_04655, A0U95_04660, A0U95_04665) and productions of exoproteases (A0U95_00125, A0U95_02755). The suppression of hyphal growth of S. rolfsii by volatile organic compounds produced by L13-6-12 was observed in a test system developed by Cernava et al. [21]. Volatile components have been shown to act as antibiotics and to induce plant growth [22, 23]. Hydrogen cyanide (HCN) is an inorganic volatile compound with antagonistic effects against soil microbes [24]. The production of HCN was observed in L13-6-12 (A0U95_28525) by applying an assay according to Blom et al. [25]. Genes predicting biosynthesis of other volatile components such as 2,3-butanediol (A0U95_29290) and acetoin (A0U95_29285) were found as well.

We further identified genes most probably involved in the direct promotion of plant growth, such as biosynthesis or carrier gene clusters for spermidine (A0U95_07830), pyoverdine (e.g. A0U95_07605, A0U95_25745, A0U95_25750) and aminocyclopropane-1-carboxylate (ACC) deaminase.

| Table 2 Project information |
|-----------------------------|
| **MIGS ID** | Property | Term |
| **MIGS 31** | Finishing quality | Finished |
| **MIGS-28** | Libraries used | PacBio RS libraries with inserts of 8 to 20 kb |
| **MIGS 29** | Sequencing platforms | PacBio RS II sequencer |
| **MIGS 31.2** | Fold coverage | 84.9 |
| **MIGS 30** | Assemblers | Hierarchical Genome Assembly Process algorithm implemented in the PacBio SMRT Analysis software |
| **MIGS 32** | Gene calling method | Glimmer gene prediction, NCBI Prokaryotic Genome Annotation Pipeline |
| **Locus Tag** | A0U95 |
| **Genbank ID** | CP014693 |
| **GenBank Date of Release** | September 20, 2016 |
| **GOLD ID** | Gs0118536, Gp0137088 |
| **BIOPROJECT** | PRJNA311625 |
| **MIGS 13** | Source Material Identifier | Plant-bacteria interaction, agricultural, environmental |

| Table 3 Genome statistics |
|---------------------------|
| **Attribute** | **Value** | **% of Total** |
| Genome size (bp) | 6,715,909 | 100 |
| DNA coding (bp) | 6,050,433 | 90.1 |
| DNA G + C (bp) | 4,091,158 | 60.7 |
| DNA scaffolds | 1 | – |
| Total genes | 5887 | 100 |
| Protein coding genes | 5773 | 98.1 |
| RNA genes | 85 | 1.4 |
| Pseudo genes | 29 | 0.5 |
| Genes in internal clusters | NA | – |
| Genes with function prediction | 4801 | 83.2 |
| Genes assigned to COGs | 4481 | 77.6 |
| Genes with Pfam domains | 3770 | 65.3 |
| Genes with signal peptides | 390 | 6.8 |
| Genes with transmembrane helices | 1389 | 24.1 |
| CRISPR repeats | NA | – |
ACC deaminase is suggested to be a key in the modulation of ethylene levels in plants by bacteria [26].

For secretion of extracellular proteins in the surrounding environment genes putatively encoding general secretory pathway proteins (Gsp) belonging to the type two secretion systems were found in L13-6-12 (e.g. A0U95_29195, A0U95_29200, A0U95_29205). Type six secretion systems have evolved in Gram-negative bacteria enabling them to interact with their host and to adapt to various microenvironments and specialized functions [27, 28]. Genes encoding components of the type six secretion system were found in L13-6-12 (e.g. A0U95_16935, A0U95_28720, A0U95_28755) putatively for interaction with eukaryotic organisms.
Conclusions
In this report, we describe the complete genome sequence of *Pseudomonas brassicacearum* strain L13-6-12, a strain that was originally isolated from the rhizosphere of potato grown in Groß Lüsewitz, Germany and which was originally assigned as *P. fluorescens*. This strain was selected for sequencing based on its ability to protect plants from biotic stresses and to promote plant growth. It also has a collection of genes predicting volatile components and enzymes such as a protease, ACC deaminase and spermidine enabling L13-6-12 to protect and promote its host plant. Genes, encoding putative T2SS, T4SS and T6SS, allowing interactions with the host and the environment were detected, too. Further functional studies and comparative genomics with related isolates will provide insights into mechanisms useful for novel biotechnological processes for seed and root applications since the strain represent a promising candidate for improving of plant performance.

Abbreviations
CDS: Coding DNA sequence; CLSM: Confocal laser scanning microscopy; COG: Clusters of Orthologous Groups; DAPG: 2,4-diacetylphloroglucinol; HCN: Hydrogen cyanide; HGAP: Hierarchical Genome Assembly Process; LB: Luria Bertani; NAII: Nutrient Broth II agar; NBII: Nutrient Broth II; RAST: Rapid annotations using subsystems technology; SMRT: Single molecule, real-time; SSM: Standard Succinate Medium; T2SS: Type 2 secretion system

Acknowledgements
The Authors thank Barbara Fetz for valuable assistance in DNA preparation. We are thankful to Eveline Adam and John H. Allan for performing growth experiments at different temperatures and pH values.

Funding
This work has been supported by the Federal Ministry of Science, Research and Economy (BMWFW), the Federal Ministry of Traffic, Innovation and Technology, the Styrian Business Promotion Agency SFG, the Standortagentur Tirol, the Government of Lower Austria and ZIT – Technology Agency of the City of Vienna through the COMET-Funding Program managed by the Austrian Research Promotion Agency FFG.

Authors’ contributions
CZ, HM and GB conceived and designed the experiments. CZ and JM performed the phenotypic characterization. HM and CZ performed the annotation and sequence homology searches. CZ wrote the manuscript. All authors commented on the manuscript before submission. All authors read and approved the final manuscript.

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

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Table 4 Number of genes associated with general COG functional categories

| Code | Value | %age | Description                                                                 |
|------|-------|------|------------------------------------------------------------------------------|
| J    | 2     | 0.03 | Translation, ribosomal structure and biogenesis                              |
| A    | 3     | 0.04 | RNA processing and modification                                               |
| K    | 281   | 4.21 | Transcription                                                                 |
| L    | 32    | 0.48 | Replication, recombination and repair                                          |
| B    | 545   | 8.16 | Chromatin structure and dynamics                                               |
| D    | 81    | 1.21 | Cell cycle control, Cell division, chromosome partitioning                    |
| V    | 284   | 4.25 | Defense mechanisms                                                            |
| T    | 162   | 2.43 | Signal transduction mechanisms                                                |
| M    | 211   | 3.16 | Cell wall/membrane biogenesis                                                 |
| N    | 165   | 2.47 | Cell motility                                                                 |
| U    | 442   | 6.62 | Intracellular trafficking and secretion                                        |
| O    | 153   | 2.29 | Posttranslational modification, protein turnover, chaperones                   |
| C    | 256   | 3.83 | Energy production and conversion                                               |
| G    | 158   | 2.37 | Carbohydrate transport and metabolism                                          |
| E    | 174   | 2.61 | Amino acid transport and metabolism                                           |
| F    | 239   | 3.58 | Nucleotide transport and metabolism                                           |
| H    | 112   | 1.68 | Coenzyme transport and metabolism                                             |
| I    | 468   | 7.01 | Lipid transport and metabolism                                                |
| P    | 344   | 5.15 | Inorganic ion transport and metabolism                                         |
| Q    | 263   | 3.94 | Secondary metabolites biosynthesis, transport and catabolism                   |
| R    | 50    | 0.75 | General function prediction only                                               |
| S    | 56    | 0.84 | Function unknown                                                              |
| –    | 3201  | 47.93| Not in COGs                                                                  |

The total is based on the total number of protein coding genes in the genome based on BASys gene prediction.

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Received: 24 May 2016 Accepted: 5 December 2016 Published online: 09 January 2017

References

1. Lottmann J, Heuer H, Smalla K, Berg G. Influence of transgenic T4-lysosome-producing plants on beneficial plant-associated bacteria. FEMS Microbiol Ecol. 1999;29:365–77.

2. Glosch R, Faltin F, Lottmann J, Kofofet A, Berg G. Effectiveness of 3 antagonistic bacterial isolates to control Rhizoctonia solani Kühn on lettuce and potato. Can J Microbiol. 2005;51(4):345–53.

3. Raaijmakers JM, Weller DM. Exploiting Genotypic Diversity of 24-diacetylphlorogluconol-producing Pseudomonas spp.: Characterization of superior root-colonizing P. fluorescens strain QB1-96. Appl Environ Microbiol. 2001;67(6):2545–54.

4. Zachow C, Faterhi J, Cardinale M, Tilcher R, Berg G. Strain-specific colonization pattern of Rhizoctonia antagonists in the root system of sugar beet. FEMS Microbiol Ecol. 2010;74(1):124–35.

5. Zachow C, Pirker H, Westendorf C, Tilcher R, Berg G. The Caenorhabditis elegans assay: a tool to evaluate the pathogenic potential of bacterial biocontrol agents. Europ Plant Pathol. 2009;125(3):367–76.

6. Loper JE, Hassan KA, Mavrodi DV, Davis II EW, Lim CK, Shaffer BT, et al. Comparative genomics of plant-associated Pseudomonas spp.: insights into diversity and inheritance of traits involved in multipartite interactions. PLoS Genet. 2012;8(7):e1002784.

7. Fedorhen S, Rossello-Mora R, Klenk HP, Tindall BJ, Konstantinidis KT, Whitman WB, et al. Meeting report: GenBank microbial genomic taxonomy workshop (12–13 May, 2015). Standards Genomic Sci. 2016;11(1):1.

8. Aziz RK, Bartels D, Best AA, DeJongh M, Ditz T, Edwards RA, et al. The RAST server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.

9. Overbeek R, Olson R, Puschn GD, Olsen GJ, Davis JJ, Ditz T, et al. The RAST server: the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res. 2014;42:D206–14.

10. Van Domselaar GH, Stothard P, Shrivastava S, Cruz JA, Guo A, Dong X, Lu P, Overbeek R, Olson R, Puschn GD, Olsen GJ, Davis JJ, Ditz T, et al. The RAST server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.

11. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. Improved microbial gene identification with Glimmer. Nucleic Acids Res. 1999;27:4636–41.

12. Petersen TN, Brunak S, von Heijne G, Nielsen H. SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods. 2011;8:785–6.

13. Emanuelsson O, Brunak S, von Heijne G, Nielsen H. Locating proteins in the membrane: the TMHMM server. J Mol Biol. 2000;305:567–80.

14. TMHMM Server v. 2.0. Prediction of transmembrane helices in proteins. Center for Biological Sequence Analysis. Technical University of Denmark to complete genomes. J Mol Biol. 2001;305:567–80.

15. Tatusov RL, Koonin EV, Lipman DJ. A genomic perspective on protein families. Science. 1997;278:631–7.

16. Galperin MY, Makarova KS, Wolf YI, Koonin EV. Expanded microbial genome coverage and improved protein family annotation in the COG database. Nucleic Acids Res. 2004;32:1223–30.

17. Mavrodi OV, Mavrodi DV, Park AA, Weller DM, Thomashow LS. The role of dtsA in colonization of the wheat rhizosphere by Pseudomonas fluorescens QB1-96. Microbiol. 2006;152(3):863–72.

18. Mavrodi DV, Joe A, Mavrodi OV, Hassan KA, Weller DM, Paulsen IT, Loper JE, Alfano JR, Thomashow LS. Structural and functional analysis of the type III secretion system from Pseudomonas fluorescens QB1-96. J Bacteriol. 2011;193(1):177–89.

19. Van Der Voort M, Meijer HJ, Schmidt Y, Watrours J, Dekkers E, Mendes R, Dorestein PC, Gross H, Raaijmakers JM. Genome mining and metabolic profiling of the rhizosphere bacterium Pseudomonas sp. SH-C52 for antimicrobial compounds. Front Microbiol. 2015;6.

20. Cernava T, Aschenbrenner IA, Grube M, Liebninger S, Berg G. A novel assay for the detection of bioactive volatiles evaluated by screening of lichen-associated bacteria. Front Microbiol. 2015;6. doi:10.3389/fmicb.2015.00398.