Complete genome sequence of *Pseudomonas corrugata* strain RM1-1-4, a stress protecting agent from the rhizosphere of an oilseed rape bait plant

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**Abstract**

*Pseudomonas corrugata* strain RM1-1-4 is a rhizosphere colonizer of oilseed rape. A previous study has shown that this motile, Gram-negative, non-sporulating bacterium is an effective stress protecting and biocontrol agent, which protects their hosts against abiotic and biotic stresses. Here, we announce and describe the complete genome sequence of *P. corrugata* RM1-1-4 consisting of a single 6.1 Mb circular chromosome that encodes 5189 protein coding genes and 85 RNA-only encoding genes. Genome analysis revealed genes predicting functions such as detoxifying mechanisms, stress inhibitors, exoproteases, lipoproteins or volatile components as well as rhizobactin siderophores and spermidine. Further analysis of its genome will help to identify traits promising for stress protection, biocontrol and plant growth promotion properties.

**Key words:** *Pseudomonas corrugata*, *Sphagnum magellanicum*-treated seeds, Rhizosphere of bait plant, Stress protection, Detoxification systems, Biocontrol, Plant growth promotion

**Introduction**

*Pseudomonas corrugata* Roberts and Scarlett (1981) emend. Sutra belongs to the genus *Pseudomonas* sensu stricto and it is one of the few non fluorescent *Pseudomonas* species. *P. corrugata* strain RM1-1-4 was isolated from the oilseed rape rhizosphere grown in the greenhouse, whose seeds were treated with the microbial community associated with the moss *Sphagnum magellanicum* [1]. *Sphagnum* mosses form bog ecosystems under low-nutrient and extreme conditions supported by their microbiota [2]. RM1-1-4 was selected as stress protecting agent coping high salt concentrations, reactive oxygen species and desiccation [1]. As it has a broad antagonistic spectrum exhibiting antifungal activity against phytopathogenic fungi (Ascomycota: *Alternaria alternata*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Verticillium dahliae* and Basidiomycota: *Rhizoctonia solani* AG2-2IIB, *Sclerotium rolfsii*), it is a promising candidate for biocontrol purposes. The activity putatively base on the production of exoenzymes and the emission of antimicrobial volatile organic compounds.

In this report, we summarize the complete genome sequence and annotation of RM1-1-4. We also describe its genomic properties revealing multifaceted plant beneficial features. The genome sequence of RM1-1-4 and its comparison with related published genomes will provide a framework for further functional studies of its abiotic and biotic stress protecting effectiveness in plant and rhizosphere competence.

**Organism information**

**Classification and features**

*P. corrugata* RM1-1-4 is a motile, Gram-negative, non-sporulating rod in the order *Pseudomonadales* of the class *Gammaproteobacteria*. The rod-shaped cells are approximately 0.5 μm in width and 1.5–2.0 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 nutrient broth II (NBII) agar plates [1] are yellow opaque shining, domed and moderately mucoid with smooth margins (Fig. 1 right). No fluorescence of...
the cells was visualized under UV light (312 nm) when
grown on King’s B agar. RM1-1-4 was isolated from the
roots of healthy oilseed rape plants cv. Traviata KWS
(KWS SAAT SE, Einbeck, Germany), whose seeds were
-treated with a microbial suspension of Sphagnum magellanicum [1].

Even though the optimal growth temperature is 30 °C,
RM1-1-4 can also slowly replicate at 5 °C in liquid Luria
Bertani (LB). Growth was observed at 37 °C and slightly
at 40 °C in this culturing medium and on solidified
medium after 24 h. The strain grows in complex media
(LB, NBII), but not in Standard Succinate Medium
(pH 7.0). Optimum pH for growth in LB is pH 6.0. It
does not cause any deleterious effect on its original host
(oilseed rape) or maize, sorghum and sugar beet [1].
Strain RM1-1-4 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
(MIGS) of P. corrugata strain RM1-1-4 is summarized in
Table 1. The phylogenetic relationship of P. corrugata
RM1-1-4 to other species within the genus Pseudomonas
is visualized in a 16S rRNA based tree [3] and a tree
based on the oligopeptide content of their complete
protein sequences by using CVTree showing its phylogenetic positioning (Fig. 2b) [3–5]. The
genome project is deposited in the NCBI BioProject
PRJNA309490 database with the Biosample
SAMN04453325. This whole genome shotgun project
has been deposited in the NCBI database under the ac-
cession no. CP014262 (Table 2).

Growth conditions and genomic DNA preparation
P. corrugata strain RM1-1-4 was grown in 50 mL of
NBII (Sifin, Berlin, Germany) medium and incubated for
20 h at 30 °C. 0.5 mL were centrifuged at 2500 x 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
checked by agarose gel electrophoresis and spectrophotometry using a UV-Vis spectrophotometer (NanoDrop
2000c, Thermo Fisher Scientific, Waltham, MA USA). In
total, 91 μg genomic DNA (3.1 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 (SMRT) sequencing. Assembly was completed with the Hierarchical Genome Assembly Process (HGAP) algorithm implemented in the PacBio SMRT Analysis software (Pacific Biosciences, Menlo Park, CA, USA). The assembly of RM1-1-4 genome based on 161,326 quality reads with a mean length of 5315 bp resulting in a single circular chromosome of 6,124,363 bp, with 118.0-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 [7, 8], 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 [9, 10]. Pseudo genes were predicted using the NCBI Prokaryotic Genome Annotation Pipeline. Signal peptides and transmembrane helices were predicted using SignalP [11, 12].

Genome properties

The genome of RM1-1-4 is composed of one circular chromosome consisting of 6,124,363 bp with an average GC content of 60.7% (Table 3 and Fig. 3), which is comparable to that of other P. corrugata strains. Among the 5335 predicted genes, 5189 were identified as protein coding genes of which 4110 (79.2%) were assigned as putative function, while the other 1079 (20.8%) were designated as hypothetical proteins. The classification of CDSs into functional categories according to the COG (Clusters of Orthologous Groups) [13, 14] database is summarized in Table 4 on BASys gene prediction. Beside the predicted genes, the genome annotation revealed 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 encodes genes that can be linked to detoxification mechanisms of oxygen radicals, toxins and heavy metals by efflux pumps as well as to stress response by heat and cold shock proteins and the universal stress protein A (UspA). UspA with orthologues (Locus Tags AXG94_21760, AXG94_02180, AXG94_04130, AXG94_24005, AXG94_24695) could play a significant role in protecting RM1-1-4 cells from H<sub>2</sub>O<sub>2</sub> and low pH as found in organisms inhabiting extreme environments [15] and analysed in detail for the clinical strain Acinetobacter baumannii ATCC 17978 [16]. A water stress/hypersensitive response protein (AXG94_21760) is present, which is supposed to be transferred to symbiotic or pathogenic bacteria by horizontal gene transfer from plants and can be seen as the acquisition of a function putatively related to the cell defense [17]. The genome of RM1-1-4 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: productions of exoproteases and lipoproteins [18]. We further identified genes

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Table 1 Classification and general features of P. corrugata RM1-1-4 according to the MIGS recommendation [24]

| MIGS ID | Property                     | Term                  | Evidence code |
|---------|------------------------------|-----------------------|---------------|
|         | Classification               | Domain Bacteria       | TAS [25]      |
|         |                              | Phylum Proteobacteria | TAS [26]      |
|         |                              | Class Gammaproteobacteria | TAS [27]  |
|         |                              | Order Pseudomonadales | TAS [28, 29] |
|         |                              | Family Pseudomonadaceae | TAS [26, 30] |
|         |                              | Genus Pseudomonas     | TAS [31-34]  |
|         |                              | Species Pseudomonas   | TAS [34]      |
|         | Strain: RM1-1-4              |                       | TAS [1]       |
|         | Gram stain                   | Negative              | IDA, TAS [34] |
|         | Cell shape                   | Rod-shaped            | IDA, TAS [34] |
|         | Motility                     | Mottile               | TAS [34]      |
|         | Sporulation                  | None                  | NAS           |
|         | Temperature range            | 5 °C–40 °C            | IDA           |
|         | Optimum temperature          | 30 °C                 | IDA           |
|         | pH range; Optimum            | 5–9; 6                | IDA           |
|         | Carbon source                | Heterotrophic         | TAS [34]      |
|         | MIGS-6 Habitat               | Soil, Rhizosphere     | TAS [1]       |
|         | MIGS-6.3 Salinity            | 1–9% NaCl (w/v)       | IDA, TAS [1]  |
|         | MIGS-22 Oxygen requirement   | Aerobic               | TAS [34]      |
|         | MIGS-15 Biotic relationship  | Rhizospheric          | TAS [1]       |
|         | MIGS-14 Pathogenicity        | Non-pathogen          | TAS [1]       |
|         | Host                         | Brassica napus L.     | TAS [1]       |
|         | Host taxa ID                 | 3708                  | NAS           |
|         | Biosafety level              | 1                     | NAS           |
|         | MIGS-4 Geographic location   | Graz, Austria         | TAS [1]       |
|         | MIGS-5 Sample collection     | time                  | TAS [1]       |
|         | MIGS-4.1 Latitude            | 47.065545             | NAS           |
|         | MIGS-4.2 Longitude           | 15.453093             | NAS           |
|         | MIGS-4.4 Altitude            | 1340 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 [35].
Fig. 2 Phylogenetic tree showing the position of *P. corrugata* RM1-1-14 in relationships among other strains of *Pseudomonas* spp. including *P. aeruginosa* PAO1 as outgroup. 

**a** The tree is based on 16S rRNA gene alignments and was conducted in MEGA6 [3]. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood approach, and then selecting the topology with superior log likelihood value. 

**b** The dendrogram based on protein sequences for representative strains belonging to the existing five subgroups (I-V) of the *P. fluorescens* complex including *P. aeruginosa* PAO1 as outgroup (O) and was created by using Composition Vector Approach [5].

| **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 | 118.0 |
| MIGS 30 | Assemblers | Hierarchical Genome Assembly Process (HGAP) algorithm implemented in the PacBio SMRT Analysis software |
| MIGS 32 | Gene calling method | Glimmer gene prediction, NCBI Prokaryotic Genome Annotation Pipeline |
| Locus Tag | AXG94 |
| Genbank ID | CP014262 |
| GenBank Date of Release | July 31, 2016 |
| GOLD ID | GS018516, Gp0137000, Ga0115603 |
| BIOPROJECT | PRJNA309490 |
| MIGS 13 | Source Material Identifier | RM1-1-4 |
| | Project relevance | Plant-bacteria interaction, agricultural, environmental |

| **Table 3** Genome statistics |
|-------------------------------|
| **Attribute** | **Value** | **% of Total** |
| Genome size (bp) | 6,124,363 | 100 |
| DNA coding (bp) | 5,492,379 | 89.7 |
| DNA G + C (bp) | 3,715,247 | 60.7 |
| DNA scaffolds | 1 | – |
| Total genes | 5335 | 100 |
| Protein coding genes | 5189 | 97.3 |
| RNA genes | 85 | 1.6 |
| Pseudo genes | 61 | 1.1 |
| Genes in internal clusters | NA | – |
| Genes with function prediction | 4256 | 82.0 |
| Genes assigned to COGs | 4013 | 77.3 |
| Genes with Pfam domains | 3296 | 63.5 |
| Genes with signal peptides | 434 | 8.4 |
| Genes with transmembrane helices | 1365 | 26.3 |
| CRISPR repeats | NA | – |
most probably involved in the direct promotion of plant growth, such as biosynthesis or carrier gene clusters for aminocyclopropane-1-carboxylate deaminase suggested to be a key in the modulation of ethylene levels in plants by bacteria [19], auxin, biofilm dispersion, rhizobactin siderophores and spermidine.

Genes predicting the synthesis of volatile components are present in the RM1-1-4 genome as well. Volatile components have been shown to act as antibiotics and to induce plant growth [20, 21]. An example is hydrogen cyanide (HCN), an inorganic compound with antagonistic effects against soil microbes [22]. RM1-1-4 encodes a hydrogen cyanide synthase HcnA (AXG94_04380) and orthologues of genes required for the biosynthesis of other volatile components such as 2,3-butanediol (AXG94_01200) and its precursor acetoin (AXG94_01195) were annotated too. Beside the presence of specific genes and the noticeable ability of RM1-1-4 to expose stress protection, the function of particular genes needs to be clarified in further detailed studies.

The genome-wide phylogenetic analysis on Pseudomonas species [3–5] with the RM1-1-4 genome showed that strain RM1-1-4 clusters within the P. fluorescens group (Fig. 2a, b) and most closely to P. corrugata CFBP 5454 (DDBJ/EMBL/GenBank accession ATKI01000000). The two P. corrugata strains belong to the few nonfluorescent Pseudomonas species. CFBP 5454 was originally described as the causal agent of the tomato disease called ‘pith necrosis’ and is yet considered as a biological resource in the fields of biocontrol of plant diseases and production of industrially promising microbial biopolymers like antimicrobial cyclic lipopeptides [23].
and a deaminase. Such properties likely have origins in volatile components and enzymes such as a protease actions. The genome encodes for a collection of genes traits for establishment of beneficial plant-microbe inter-stress protecting factors and other well-known bacterial abiotic and biotic stresses and to promote plant growth. sequencing based on its ability to protect plants from greenhouse in Graz, Austria. This strain was selected for originally isolated from the roots of moss microbiome- within the non-fluorescent P. corrugata strain RM1-1-4. It is a P. corrugata This report described the complete genome sequence of Conclusions

This report described the complete genome sequence of P. corrugata strain RM1-1-4. It is a “Pseudomonadales” within the non-fluorescent P. corrugata clade that was originally isolated from the roots of moss microbiome-primed oilseed rape seeds cv. Traviata KWS grown in a greenhouse in Graz, Austria. This strain was selected for sequencing based on its ability to protect plants from abiotic and biotic stresses and to promote plant growth. We could highlight genes encoding abiotic and biotic stress protecting factors and other well-known bacterial traits for establishment of beneficial plant-microbe interactions. The genome encodes for a collection of genes predicting biofilm dispersion, detoxifying compounds, volatile components and enzymes such as a protease and a deaminase. Such properties likely have origins in a repertoire of genes including efflux pumps, putative T2SS, T4SS and T6SS, and several genes presumably implicated in auxin, rhizobactin siderophore and spermidine production. Further functional studies and comparative genomics with related isolates will provide insights into naturally acquired plant stress protection and promotion of plant health.

Abbreviations

COD: Coding DNA Sequence; CLSM: Confocal Laser Scanning Microscopy; COG: Clusters of Orthologous Groups; CVTree: Composition Vector Tree; HCN: Hydrogen Cyanide; HGAP: Hierarchical Genome Assembly Process; LB: Luria Bertani; NBII: Nutrient Broth II; RAST: Rapid Annotations using Subsystems Technology; SMRT: Single Molecule, Real-Time; T2SS: Type 2 Secretion System

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Authors’ contributions

CZ, HM, RT and GB conceived and designed the experiments. CZ and CML 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    | 161   | 2.41 | Translation, ribosomal structure and biogenesis |
| A    | 3     | 0.04 | RNA processing and modification |
| K    | 375   | 5.61 | Transcription |
| L    | 162   | 2.43 | Replication, recombination and repair |
| B    | 2     | 0.03 | Chromatin structure and dynamics |
| D    | 32    | 0.48 | Cell cycle control, Cell division, chromosome partitioning |
| V    | 61    | 0.91 | Defense mechanisms |
| T    | 235   | 3.52 | Signal transduction mechanisms |
| M    | 236   | 3.53 | Cell wall/membrane biogenesis |
| N    | 126   | 1.89 | Cell motility |
| U    | 42    | 0.63 | Intracellular trafficking and secretion |
| O    | 168   | 2.52 | Posttranslational modification, protein turnover, chaperones |
| C    | 270   | 4.04 | Energy production and conversion |
| G    | 247   | 3.70 | Carbohydrate transport and metabolism |
| E    | 445   | 6.66 | Amino acid transport and metabolism |
| F    | 79    | 1.18 | Nucleotide transport and metabolism |
| H    | 152   | 2.28 | Coenzyme transport and metabolism |
| I    | 182   | 2.72 | Lipid transport and metabolism |
| P    | 197   | 2.92 | Inorganic ion transport and metabolism |
| Q    | 97    | 1.45 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 412   | 6.17 | General function prediction only |
| S    | 329   | 4.93 | Function unknown |
| –    | 2615  | 40.14 | Not in COGs |

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

Conclusions

This report described the complete genome sequence of P. corrugata strain RM1-1-4. It is a “Pseudomonadales” within the non-fluorescent P. corrugata clade that was originally isolated from the roots of moss microbiome-primed oilseed rape seeds cv. Traviata KWS grown in a greenhouse in Graz, Austria. This strain was selected for sequencing based on its ability to protect plants from abiotic and biotic stresses and to promote plant growth. We could highlight genes encoding abiotic and biotic stress protecting factors and other well-known bacterial traits for establishment of beneficial plant-microbe interactions. The genome encodes for a collection of genes predicting biofilm dispersion, detoxifying compounds, volatile components and enzymes such as a protease and a deaminase. Such properties likely have origins in a repertoire of genes including efflux pumps, putative T2SS, T4SS and T6SS, and several genes presumably implicated in auxin, rhizobactin siderophore and spermidine production. Further functional studies and comparative genomics with related isolates will provide insights into naturally acquired plant stress protection and promotion of plant health.

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Competing interests

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