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Short Communication

# Draft genome of *Pseudomonas* sp. RGM 2987 isolated from *Stevia philippiana* roots reveals its potential as a plant biostimulant and potentially constitutes a novel species

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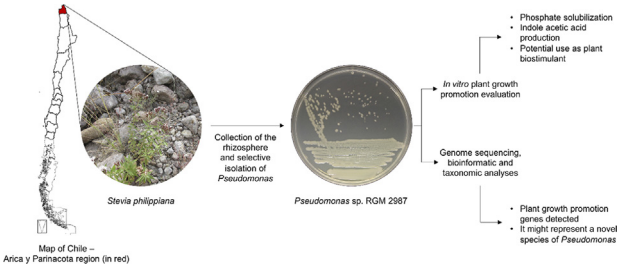
## GRAPHICAL ABSTRACT

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## ABSTRACT

*Background:* Reports on *Pseudomonas* species associated with Chilean native plants and their diversity are scarce. The draft genome of *Pseudomonas* sp. RGM 2987 isolated from the rhizosphere of *Stevia philippiana*, a native Chilean plant from the Altiplano, is presented.
*Results:* The assembled genome features 6,161,133 bp, a G+C content of 61.3%, and 5,350 predicted open reading frames. dDDH, and ANIb differences between RGM 2987 and the closest relatives support its classification as a new species within the *Pseudomonas* genus. Genome mining and functional classification revealed the presence of genes involved in cell function and metabolism as well as plant-growth promotion, including those for indole acetic acid production, phosphate solubilization, and ethylene concentration.
*Conclusions:* The draft genome of *Pseudomonas* sp. RGM 2987 provides insights on its phylogeny and classification as a new species and shed light on its potential as a plant-biostimulant, expanding our knowledge on *Pseudomonas* biodiversity worldwide and, specifically, for those strains associated with native Chilean plants.
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## 1. Introduction

*Pseudomonas* is a diverse genus of aerobic, gram-negative bacteria, present in diverse habitats, including plant rhizosphere and

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endosphere, and contains more than 200 validly published species [1]. Species of the *Pseudomonas* genus are known for exhibiting plant-growth promotion traits, such as indole acetic acid (IAA) biosynthesis, stress alleviation enzyme production, and phosphate solubilization, which are desirable properties for the development of effective plant biostimulants [2].

Chile is one of the top five plant biodiversity hotspots on the planet [3]; however, little information regarding *Pseudomonas* species associated with native Chilean plants can be found in the literature. *Pseudomonas atacamensis* is one of the formally described species isolated from the rhizosphere of desert bloom plants in the Atacama region, and its draft genome revealed important plant growth-promoting (PGP) traits including iron acquisition and alleviation of abiotic stress [4,5]. Here, we present the draft genome of *Pseudomonas* sp. RGM 2987, isolated from the rhizosphere of *Stevia philippiana*, a native Chilean plant, distributed from Arica-Parinacota to Atacama regions in northern Chile and is a relative of the worldwide known *Stevia rebaudiana*, source of the natural sweetener "stevia" [6]. We explored its taxonomic affiliation as a novel species and its biotechnological potential as a plant biostimulant.

## 2. Experimental

### 2.1. Bacteria isolation

A plant sample of *S. philippiana* was collected in 2017 from the Chilean Altiplano at Pachama, Putre, Arica-Parinacota region, Chile (-18.454733, -69.510400; altitude: 3,400 m.a.s.l.), placed inside a sterile plastic bag, and transported in a dark container to the Microbial Genetic Resources Bank at INIA. Rhizosphere soil was removed from the roots by soaking them in an epiphyte removal buffer [7]. Next, 100 µL of serial dilutions of the soil rhizosphere of *S. philippiana* were inoculated on King's B (KB) agar medium supplemented with 25 µg/mL nystatin and 50 µg/mL cycloheximide. Plates were incubated at 25°C for 48 h. A UV-fluorescent colony was streaked out on KB agar and incubated at 25°C for 48 h. This step was repeated twice to obtain an axenic culture. The isolate was deposited in the Chilean Collection of Microbial Genetic Resources (CChRGM) under the code RGM 2987.

### 2.2. In vitro assessment of plant growth promotion traits

RGM 2987 was grown in Luria Bertani broth to prepare a bacterial suspension of $10^8$ CFU/mL in sterile distilled water. IAA production was evaluated by inoculating 20 mL YMD broth, supplemented with 1% L-tryptophan, with 10 µL of the cell suspension. The culture was incubated for 72 h at 25°C and 120 rpm. IAA was quantified according to Carrasco-Fernández et al. [8]. Phosphate solubilization was evaluated by inoculating the center of a Petri dish containing NBRIP agar with 10 µL of the cell suspension. Plates were incubated at 28°C for 7 d. A clear halo around the colony was considered as positive solubilization of phosphate, and the phosphate solubilization index was calculated according to Demissie et al. [9].

### 2.3. Isolation of genomic DNA, 16S rRNA gene amplification, and whole-genome sequencing

Genomic DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega, USA), following the Gram-negative strain protocol. The 16S rRNA gene sequencing was conducted according to Carrasco-Fernández et al. [8]. DNA extraction and library construction was performed at MicrobesNG (UK), according to the provider's methodology [10]. Libraries were sequenced using Illumina sequencers (HiSeq/NovaSeq) using a 250-bp paired-end protocol.

### 2.4. Bioinformatic analyses

Nucleotide sequences from Sanger sequencing were processed using Sequencher 5.4.6 (Gene Codes Corporation, USA). EzBioCloud was used for 16S rRNA gene identification [11]. Whole-genome sequencing reads were adapter trimmed with Trimmomatic 0.30 using a sliding window quality cutoff of Q15 [12]. *De novo* assembly was performed using SPAdes 3.7 [13], and contigs were annotated with the Prokaryotic Genome Annotation Pipeline (PGAP) 6.0 [14]. The Conserved Domain Database was used for protein domain search [15]. Digital DNA-DNA hybridization (dDDH) and 16S rRNA gene phylogeny (maximun parsimony and maximun likelihood trees were evaluated with 1000 bootstrap iterations) were performed in the Genome-to-Genome Distance Calculator [16] webserver. The Average Nucleotide Identity using BLAST (ANIb) calculation was performed with JSpeciesWS [17]. Reference genomes were retrieved from GenBank [18]. ANIb values were used for Pearson correlation matrix construction, which were subsequently ordered by hierarchical clustering with the R package *corrplot* in R [19]. Comparison of the RNA polymerase sigma factor (*rpoD*) nucleotide sequence of RGM 2987 was performed against a dataset of *rpoD* sequences from reference *Pseudomonas* strains [1]. Search for particular genes within the genome annotation was performed using local BLAST analysis on Sequenceserver [20] and functional Clusters of Orthologous Groups (COGs) of proteins were obtained from Global Catalogue of Type Strain (gcType) [21].

## 3. Results and discussion

### 3.1. Genome sequence features and taxonomic analysis of *Pseudomonas sp. RGM 2987*

The RGM 2987 genome was 6,161,133 bp in length (223 contigs), with 61.3% G+C content, mean coverage of 31.79x, and $N_{50}$ of 59,539; 5,350 open reading frames and 60 tRNAs were predicted. According to the similarities of the 16S rRNA gene, RGM 2987 belonged to the genus *Pseudomonas*, sharing the highest similarity with *Pseudomonas mucoides* P154a$^T$ (98.86%). The maximum likelihood phylogenetic tree separates the strain RGM 2987 as a new branch in the *Pseudomonas* phylogeny and diverges from its closest relatives with 100% branch support (Fig 1A). dDDH values support 16S rRNA gene phylogeny, indicating that RGM 2987 corresponds to a new species (cutoff $\leq$70% [22]) as well as the ANIb values (cutoff $\leq$95% [23]) and their clustering patterns within the *Pseudomonas* representatives (Fig 1B). According to the *rpoD* gene similarities, the strain RGM 2987 was grouped within members of the *Pseudomonas corrugata* subgroup, being *Pseudomonas zanjanensis* SWRI12$^T$, a strain isolated from wheat rhizosphere, its closest relative [1].

### 3.2. In vitro assessment of plant growth promotion features

The strain RGM 2987 produced 41.05 ± 7.68 µg/mL IAA and displayed a phosphate solubilization index of 1.55 ± 0.09 (Fig 1C), which suggests its potential use as a plant-growth promoting rhizobacteria [8,24].

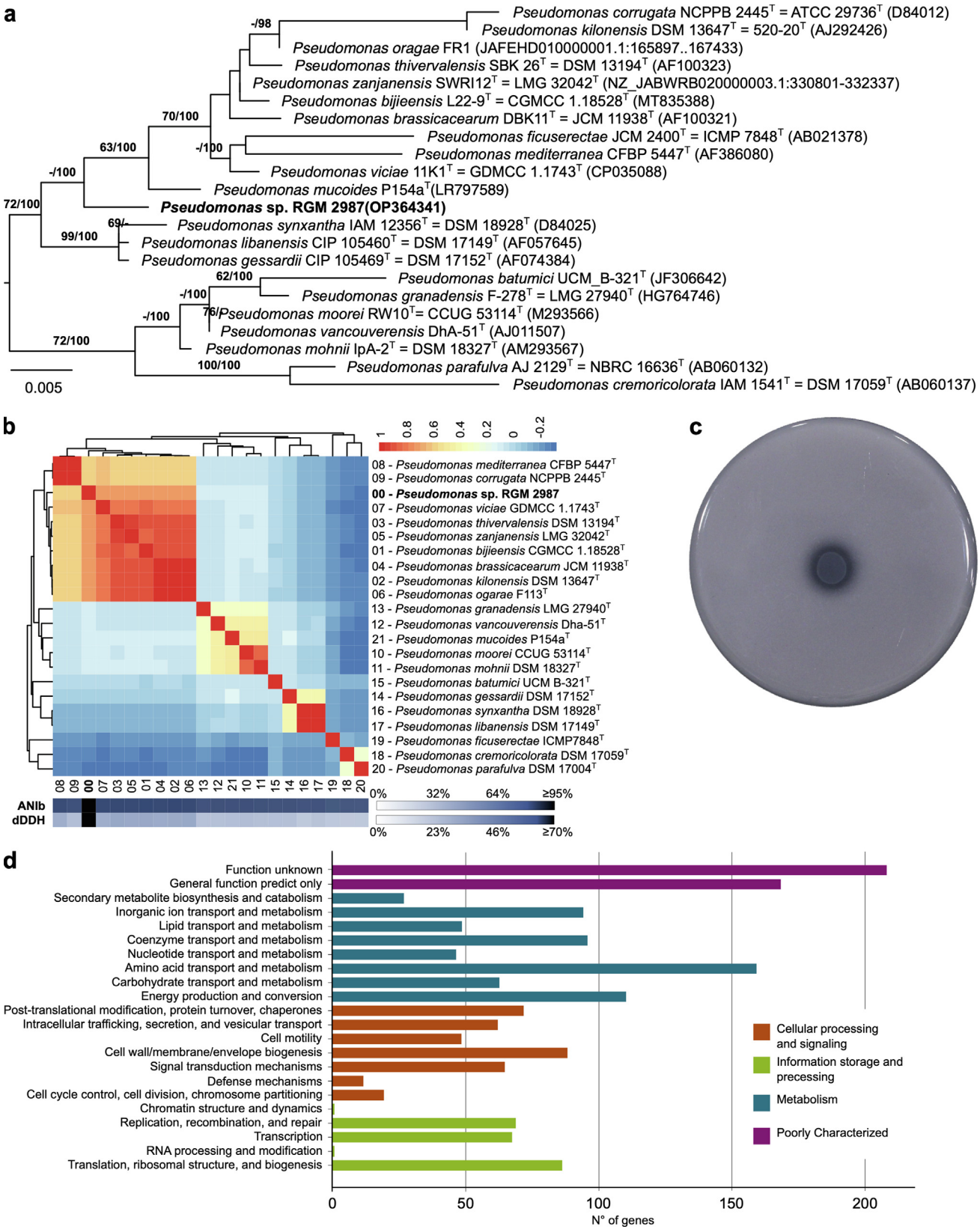


**Fig. 1.** Taxonomic, genomic, and phenotypic features of *Pseudomonas* sp. RGM 2987. (a) Maximum likelihood (ML) tree of 16S rRNA gene sequences inferred under the GTR +GAMMA model and rooted by midpoint-rooting, scale bar indicates substitutions per site. The numbers above the branches are support values when larger than 60% from ML (left) and maximum parsimony (right) bootstrapping; (b) correlation plot based on ANIb values between each species (species name is indicated with the respective key in the y-axis). ANIb and dDDH values from pairwise whole-genome comparisons between *Pseudomonas* sp. RGM 2987 and the closest type strains are indicated at the bottom; (c) clear zone (halo) produced by RGM 2987; (d) COG features retrieved from the RGM 2987 genome sequence.

**Table 1**
Plant-growth promotion traits found in *Pseudomonas* sp. RGM 2987.

| Plant-growth promotion trait | Protein name (gene name) | Protein code (identity; UniProt accession) |
|---|---|---|
| Phosphate solubilization | Quinoprotein glucose dehydrogenase (*gcd*) | RGM2987_14740 (69.34%, A0A0B6F0P5) |
| | Coenzyme PQQ synthesis protein A (*pqqA*) | RGM2987_00795, RGM2987_16840 (95.45%, 87.50%; Q3K5R0) |
| | Coenzyme PQQ synthesis protein B (*pqqB*) | RGM2987_16845 (95.71%, C3K348) |
| | Pyrroloquinoline-quinone synthase (*pqqC*) | RGM2987_16850 (96.34%, Q88QV6) |
| | PqqA binding protein (*pqqD*) | RGM2987_16855 (90.11%, Q4K4U9) |
| | PqqA peptide cyclase (*pqqE*) | RGM2987_16860 (93.57%, Q4K4U8) |
| | Coenzyme PQQ synthesis protein F (*pqqF*) | RGM2987_16835 (56.34%, P55174) |
| IAA production | Indole-3-pyruvate decarboxylase (*ipdC*); IPyA pathway | RGM2987_05960 (25.45%, A0A5E6Q147) |
| | Tryptophan 2-monooxygenase (*iaaM*); IAM pathway | RGM2987_16825 (31.26%, P06617); RGM2987_13765 (25.00%, P06617) |
| | Indoleacetamide hydrolase (*iaaH*); IAM pathway | - |
| | Nitrile hydratase (*nthAB*); IAN pathway | - |
| | Nitrilase (*nit*); IAN pathway | RGM2987_04870 (89.87%, K9NKH3) |
| ACC deaminase | ACC deaminase (*acdS*) | RGM2987_23510 (99.41%, Q51813) |
| | Leucine-responsive regulatory protein (*acdR*) | RGM2987_23515 (87.57%, K9NP20) |

-, no significant hit was found.

### *3.3. Biotechnological potential of Pseudomonas sp. RGM 2987 as a plant biostimulant*

The genome annotation of strain RGM 2987 revealed the presence of multiple open reading frames for multiple cellular function, secondary metabolism, and other of unknown function (Fig 1D); PGP traits found in the genome are summarized in Table 1. Phosphate-solubilizing bacteria are capable of solubilizing phosphate present in soil by releasing organic acids, of which gluconic acid is mostly produced by them [25]. Bacteria produce gluconic acid by the direct oxidation of glucose through the quinoprotein glucose dehydrogenase enzyme, encoded by the *gcd* gene [26]. Gcd uses pyrroloquinoline quinone as a cofactor, which is produced by the activities encoded in the *pqqA-F* operon [27]. The *gcd* gene and the six genes comprising the *pqq* operon were identified in RGM 2987, implying the presence of a well-conserved mechanism for phosphate solubilization in soil through gluconic acid synthesis.

IAA is an auxin hormone involved in plant growth and development. It can be synthesized by the plant itself and by soil bacteria, and its production levels may depend upon tryptophan availability and abiotic factors [28,29]. Soil bacteria can synthesize IAA from L-tryptophan by multiple interconnected pathways, such as: (i) indole-3-pyruvate (IPyA), (ii) indole-3-acetamide (IAM), and (iii) indole-3-acetonitrile (IAN) [29,30]. A key enzyme for IAA biosynthesis in the IPyA pathway is the indole-3-pyruvate decarboxylase enzyme, encoded by the *ipdC* gene. It belongs to the indol_phenyl_DC superfamily (cl37262) and its disruption in *Azospirillum brasilense* reduced ∼50% IAA production [31]. RGM2987_05960 was the closest ortholog to IpdC in strain RGM 2987; however, it lacks the domain cl37262. Two enzymes participate in the IAM pathway, a tryptophan 2-monooxygenase (IaaM) and an indole acetamide hydrolase (IaaH). *Pseudomonas* sp. RGM 2987 contains two ortholog proteins of IaaM, of which only RGM2987_16825 belongs to the same superfamily as IaaM (cl38049), of the plant pathogenic bacteria *Pseudomonas savastanoi* [32]. No homologues for the *iaaH* gene were detected in the genome of RGM 2987 suggesting that the IAM route may be truncated in this strain. Two sub-routes to synthesize IAA in the IAN pathway have been described: one generates IAA from the IAN substrate through a reaction catalyzed by a nitrilase enzyme encoded by the *nit* gene, and a second route transforms IAN into IAM intermediate by a nitrile hydratase, encoded by *nthAB* genes [33]. A nitrilase encoding gene was found in strain RGM 2987, which belonged to the nitrilases_CHs superfamily (cd07564) as well as the Nit protein of *Pseudomonas* sp. UW4 [33]. No *nhtAB* genes were found in RGM 2987 suggesting that IAA production in RGM 2987 may occur through a nitrilase enzyme in the IAN pathway.

The molecule 1-aminocyclopropane-1-carboxylate (ACC), the precursor of ethylene in higher plants, is a gaseous phytohormone that governs plant development and, depending on its concentration, it may inhibit or induce plant senescence under a wide range of stress conditions [34,35]. The gene *acdS* encodes for an ACC deaminase that participates in the conversion of ACC into ammonia and α-ketobutyrate [36]. The presence of *acdS* and its regulatory gene, *acdR*, in the genome of RGM 2987 suggest a potential modulatory mechanism for ethylene concentration.

## 4. Conclusions

The genome of *Pseudomonas* sp. RGM 2987 revealed important plant-growth promoting traits such as a route for IAA biosynthesis, phosphate solubilization, and ethylene concentration regulation, which are desirable features for developing plant-biostimulants. Phylogenetic and phylogenomic analysis provide strong support to claim that the strain RGM 2987 represents a novel species of the *Pseudomonas* genus, thus expanding the knowledge regarding microbial biodiversity associated with native plants in Chile.

## Author contribution

Study conception and design: JF Castro; M Guerra
Data collection: J Carrasco-Fernández
Genome sequencing and data analysis: M Guerra; JH Valdés
Analysis and interpretation of results: JF Castro, JH Valdés, M Panichini, M Guerra
Draft manuscript preparation: JF Castro; M Guerra; J Carrasco-Fernández; JH Valdés
Revision of the results and approved the final version of the manuscript: JF Castro; JH Valdés

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## Conflict of interest

None.

## Data availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JALJDX000000000. The version presented here is JALJDX000000000.1. Raw reads have been deposited in the Sequence Read Archive (SRA) under the accession numbers SRR18649284 and SRR18649285. All project data are available under BioProject accession number PRJNA820732. 16S rRNA and *rpoD* gene sequences were submitted to the NCBI GenBank under the accession numbers OP364341 and OP380681, respectively.

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