Genome insights from the Amazonian rhizobacterium Bacillus paramycoides RZ3MS14 reveal plant growth-promoting multi-traits and bioprotection against phytopathogens and environmental stresses

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Short Report

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Abstract

Bacillus paramycoides is poorly understood bacterium with potential application as plant growth promoter and agent control bacterium to a more sustainable agriculture. *B. paramycoides* strain RZ3MS14 was previously isolated from guarana´s rhizosphere on Amazonian rainforest. The obtained RZ3MS14 draft genome comprised 28 scaffolds with a genome size estimated of 5,493,110 bp and a G + C content of 34.9%. We predicted 5,637 coding sequences (CDS). From phylogenomic analysis was observed that RZ2MS14 clustered in a monophyletic group with *B. paramycoides* strains. The ANI and digital DNA-DNA hybridization comparing RZ3MS14 and the *B. paramycoides* strain type NH24A2 was 98.5% and 73-80.6% respectively. RZ3MS14 harbors different genes related with plant growth-promoting as instance phosphorus solubilization and mineralization as well as auxin production by IPA pathway and others. Moreover, the RZ3MS14 draft genome presented the putative micrococcin, cerecidin, and paeninodin gene clusters, antibiotics related with pathogen control in addition of genes to VOCs production. Our results contribute to improve the knowledge concerning the molecular basis of *B. paramycoides* related with its vast application on agriculture.

Introduction

The demand for sustainable alternatives that increase crop production has stimulated the screening of beneficial plant-associated microbes as candidates for applying as new bioinoculants. Bioinoculants may be plant growth-promoting bacteria (PGPB) that enhance plant nutrition while reducing dependence on conventional chemical fertilizers (Mitter et al. 2021). Many *Bacillus* species, resilient endospore-forming bacteria, have emerged for their multiple characteristics for plant health and development, which include pathogen suppression and host plant protection under adverse conditions (Goswami et al. 2016; Radhakrishnan et al. 2017; Saxena et al. 2020).

Bacillus paramycoides strain RZ3MS14 was isolated from the rhizosphere of guarana, a typical crop in the Brazilian Amazon. It has shown previously ability to produce indole-acetic acid (IAA) under *in vitro* assays (Batista et al. 2018). This species belongs to the *Bacillus* cereus group, a subdivision of the genus *Bacillus* (Liu et al. 2017), and certain isolates identified through 16S rDNA sequence analysis have attained notoriety as PGPB, especially in root development (Ooi et al. 2022). Activities of phosphorus (P) and potassium (K) solubilization, ACC deaminase, hydrogen cyanide, production of phytohormones (IAA, ABA, benzyl, cytokinins, and gibberellins), siderophores, and secretion of various lytic enzymes, have been carried out by this species under *in vitro* or greenhouse conditions. *B. paramycoides* strains have also been promising for the biocontrol of phytopathogens such as *Fusarium oxysporum* and *Xanthomonas oryzae* pv. oryzae (El-Sersawy et al. 2021; Ooi et al. 2022), in the degradation of acephate and methomyl widely used in agricultural pesticides (Omeiri et al. 2022; Ren et al. 2020), and the reduction of heavy metals to less toxic forms (Borah et al. 2021) or by assisting the host as phytoremediator (Liu et al. 2021). Furthermore, research efforts have demonstrated its biotechnological applications. Some strains are able to use synthetic polyethylene materials as a nutrient (Wu et al. 2023) and, conversely, synthesize eco-friendly polyhydroxybutyrate (PHB) polymers (Djerrab et al. 2022).

Currently, there are just available ten *B. paramycoides* genomes based on genomospecies assignment by 95% Average nucleotide identity (ANI) cut-off, including the genome of type strain *B. paramycoides* NH24A2 (= MCCC 1A04098 = KCTC 33709 = LMG 28876) used as reference of this species. Regardless of this amount, there have been few genome-level explorations of the species and even fewer as a PGPB. Recently a cluster of genes for polyhydroxyalkanoates (PHA) biosynthesis was detected in the genome of *B. paramycoides* strain LB_RP2 isolated from an Amazonian Blackwater River (de Castro et al. 2021). However, the relationship among genes, enzymes, and plant growth promotion or plant defense traits is still uncharted.

Here, we report the draft genome of *B. paramycoides* strain RZ3MS14, harboring several genes related to plant growth-promoting and protection against abiotic stresses and phytopathogens. This genomic study shows the potential applications of this strain to a sustainable agriculture and is the first step toward the identification of target genes for subsequent genetic improvement.

Materials And Methods

The rhizobacterium RZ3MS14 was grown in Luria-Bertani (LB) broth and incubated at 28 ºC under agitation (150 rpm) overnight. Genomic DNA was extracted from the collected cell pellet using the DNeasy® Blood & Tissue kit (Qiagen®) according to the manufacturer's protocol for Gram-positive Bacteria. The DNA libraries were prepared with Nextera XT DNA Library Preparation Kit (Illumina, San Diego, USA) and sequenced on the Illumina MiSeq Platform (2 x 250 bp paired-end reads with Illumina v3 Reagent Kit) following the manufacturer's guidelines.

We assessed the raw data quality (5,127,870 reads) using FastQC v0.11.8/ MultiQC v1.11 (Ewels et al. 2016). Adapter and PHRED quality score < 20 sequences were removed with Trimmomatic v0.39 (Bolger et al. 2014) and the remaining read pairs (4,616,236 reads) were used for genome assembly on Spades v3.15.3 (Prjibelski et al. 2020), considering –isolate and –cov-cutoff "auto" options. To verify sequencing purity, we performed a rapid taxonomic annotation of contigs using BlobTools v1.0.1 (Laetsch and Blaxter 2017) and thereafter ran scaffolding and gap-closing with Platanus v1.2.4 tools (Kajitani et al. 2014). The final assembly was polished using Pilon v1.24 (Walker et al. 2014), and scaffolds smaller than 1000 nt were excluded with Pullseq v1.0.2 (github.com/bcthomas/pullseq). Finally, CheckM v1.0.18 (Parks et al. 2015) and Benchmarking Universal Single-Copy Orthologs (BUSCO v5, ortholog set of the order Bacillales) via gVolante2 web server (Nishimura et al. 2017) were used to assess genome quality.

Gene prediction and annotation were performed with NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v6.1 (Tatusova et al. 2016). Functional annotation of genes was performed using Rapid Annotation Using Subsystem Technology (RAST) web server (Overbeek et al. 2014). Cluster of orthologous groups (COGs) assignment was carried out via WebMGA v2.1.9 (Wu et al. 2011), and Orthology assignments based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and metabolic pathways were performed by KEGG Automatic Annotation Server (KAAS) v2.1 (Moriya et al. 2007), using

bi-directional best hit (BBH) method in GHOSTZ. Secondary metabolite gene cluster prediction made by AntiSMASH v6.1.1 (Blin et al. 2021).

Genes focusing on plant growth promotion and those protecting against adverse environmental conditions and stimulating plant immunity were compared using BLAST against UniProtKB/Swiss-Prot database release 2022_03 (UniProt Consortium 2021). In some cases, conserved protein families/domains were detected using CD-Search in NCBI's interface (Lu et al. 2020).

The PhyloPhlAn v3.0.60 pipeline (Asnicar et al. 2020), which relies on a concatenated alignment of up to 400 bacterial marker proteins, was used for the phylogeny analysis. The phylogenomic tree included genomes of type strains of different *Bacillus* species retrieved from the NCBI GenBank database (as of July 18, 2022) and selected following the quality scores of Land et al. (2014) and the sampling criteria described by Zhu et al. (2019). For its building, we added the options -f a, -m GTRGAMMA, and -# 1000 (bootstrap replicates) to the raxmlHPC-PTHREADS-SSE3 setup. ANI and Digital DNA–DNA hybridization (dDDH) values were calculated using OrthoANI from the OAT v.0.93.1 software (Lee et al. 2016) and Genome-to-Genome Distance Calculator (GGDC) v3.0 (Meier-Kolthoff et al. 2022) (https://ggdc.dsmz.de/ggdc.php), respectively.

Results And Discussion

The draft genome of RZ3MS14 consisted of 28 scaffolds in a total size of 5,493,110 bp (G + C content of 34.9%), N50 and L50 values of 397,909 bp and 3, respectively (Table 1). Genome completeness scored 97.7% in Checkm analysis and 99.8% using BUSCOs. Metrics and PGAP prediction are summarized in Table 1.

Table 1
Genome metrics of *Bacillus paramycoides* RZ3MS14

Assessment	Value
Genome size (bp)	5,493,110
Scaffolds	28
Contigs	28
Largest contig (bp)	1,521,286
G + C content (%)	34.9
Quality assessment	
Completeness (%)	97.7
Contamination (%)	2.3
Complete BUSCOs (nº of core genes detected, %)	449, 99.8
Missing BUSCOs (nº of core genes detected, %)	1, 0.2
PGAP annotation	
Genes (total)	5,744
CDSs (total)	5,637
Genes (coding)	5,339
Genes (RNA)	107
rRNAs (5S, 16S, 23S)	5, 4, 1
Complete rRNAs (5S, 23S)	4, 1
Partial rRNAs (5S, 16S)	1, 4
tRNAs	92
ncRNAs	5
Pseudo genes (total)	298
Pseudo genes (ambiguous residues)	0 of 298
Pseudo genes (frameshifted)	99 of 298
Pseudo genes (incomplete)	227 of 298
Pseudo genes (internal stop)	75 of 298
Pseudo genes (multiple problems)	89 of 298

RZ3MS14 clustered in the *Bacillus paramycoides* clade, represented by the type strain *B. paramycoides* NH24A2 (Fig. 1). Within this cluster, RZ3MS14 presented a separate branch (length 0.03350), followed by *B. paramycoides* DE0103 (length 0.02653). The OrthoANI value between the genomes of RZ3MS14 and *B. paramycoides* strains (97.48 to 97.63%) provided initial support for its inclusion in this species (Fig. 1). Moreover, dDDH metrics ranged from 73-80.6% when compared to strain type NH24A2. Both measurements were higher than ANI (95 ~ 96%) and dDDH (70%) thresholds, widely used and supported for bacterial species delineation based on genomic analyses (Chun et al. 2018). Therefore, this study refers to this strain as *B. paramycoides* RZ3MS14.

The RAST provided an overview of the biological features of RZ3MS14, categorizing 2.408 (44%) genes (including both protein- and RNA-coding genes) into 466 SEED subsystems (Fig. 2A). The draft genome contained genes primarily involved in the metabolism of carbohydrates (498), proteins (359), amino acids, and derivatives (580), as well as in the cofactors, vitamins, prosthetic groups, and pigments category (274). Metabolism of phosphorus (102), sulfur (43), nitrogen (34), and iron (19) were other notable categories, as these are essential nutrients, and PGPR are directly tied to their recycling in the soil. The highest number of protein-coding genes (84.24%) was annotated using COG database. According to the distribution in COG classes, 1683 of these genes (30.48%) were associated with metabolic processes, predominantly within the amino acid transport and metabolism subclass, 932 genes (16.88%) in Information storage and processing, and 775 (14.03%) for Cellular processes and signaling (Fig. 2B). With KEGG, 1.46% of protein-coding genes were mapped in 210 metabolic pathways, mainly in carbohydrate (427) and amino acid (361) metabolism, alongside protein families related to genetic information processing (633) and cellular processes and signaling (683) (Fig. 2C).

The presence of genes involved in ammonia assimilation (*glnR*, *glnA*, and *gltB*) and in aerobic denitrification (*narG*, *narH*, *narJ*, and *narI*) revealed the ability of RZ3MS14 to regulate nitrogen (N) in the rhizosphere (Table S1). Furthermore, this rhizobacterium exhibited genetic potential in the cycling of other nutrients essential for plant growth and development. A repertoire of genes associated with inorganic phosphorus (Pi) solubilization through the synthesis of organic acids such as citric, formic, glycolic, glyoxylic, malic, oxalacetic, 2-oxoglutaric, pyruvic, and succinic acids, were found in RZ3MS14 genome; along with genes encoding the Pi transport system (*pstSCAB*), the two-component PhoP/PhoR system, and its regulon (Table S1). Regarding the release of P from organic sources for plant uptake, three genes with phosphatase activity were identified, among others related to phosphonoacetate and 2-aminoethylphosphonic acid (ciliatin) mineralization (Table S1). The RZ3MS14 genome also harbors a biosynthetic cluster of catecholate-type bacillabactin siderophore (*dhbFBECA*). However, it might be able to internalize iron (Fe) bound to other hydroxamate-type siderophores or by direct Fe2+ (*Feo* genes) iron transport system (Table S2).

Auxin production is another important mechanism to increase plant growth, uptake of nutrients, and even alleviate environmental stresses. We detected the indole-3-pyruvate (IPA) pathway tryptophan-dependent composed of the key genes *ipdC* (encoding for indole-3-pyruvate decarboxylase) and *aldA* (encoding an aldehyde dehydrogenase). These results would uncover the genes responsible for the in vitro IAA

production reported previously for strain RZ3MS14 (Batista et al. 2018). The tryptophan biosynthesis genes *trpA*, *trpB*, *trpC*, *trpF*, *trpD*, *trpG* and *trpE*, were also found.

Several protective mechanisms for plant health were widely distributed in the genome of the Amazonian isolate RZ3MS14; among them, genes responsible for production of hydrogen sulfide (Table S3). This gas-signaling molecule plays an emerging role in regulating plant senescence and maturation, as well as providing resistance against fungal pathogens and protection against drought, extreme temperatures, toxic metals, and salinity (Aroca et al. 2018; Corpas and Palma 2020; Liu et al. 2021). The genome also contains genes involved in synthesizing, transporting, and utilizing polyamines putrescine and spermidine (Table S3). Various roles have been ascribed to bacterial polyamines, including induction of positive physiological changes in plants and protection against various abiotic stresses and pathogen attacks. In particular, spermidine has been linked to biofilm formation in Bacillus subtilis (Hobley et al. 2017). Additionally, genes associated with the formation of γ-aminobutyrate (GABA), butanoic acid, acetoin, 2,3-butanediol, and 2,3-butanedione (Table S3) evidence the potential of the strain to produce volatile organic compounds (VOCs), that elicit induced systemic resistance in plants and responses against environmental stresses (Dias et al. 2021; Yi et al. 2016).

By AntiSMASH v6.1.1 were predicted six biosynthetic gene clusters for relevant natural products: three biosynthetic clusters of antibiotics micrococcin, cerecidin, and paeninodin; a cluster for the root elongation promoter trehangelin; and a biosynthetic cluster of antifungal lipopeptide fengycin (Table S4). This analysis also detected one last cluster for NRPS siderophore bacillibactin, previously described.

The bioinformatics analysis of the draft genome of *B. paramycoides* strain RZ3MS14 unveils several mechanisms contributing to the nutrient dynamics in the rhizosphere and plant growth-promoting multitraits. RZ3MS14 also harbors genes related with antifungal activities and responses to environmental stress through VOCs among a wide range of metabolites. Overall, this study provides new insights into the species and its potential as microbial bioinoculants.

Declarations

Nucleotide sequence accession numbers. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAOVZP000000000. The version described in this paper is version JAOVZP010000000 (BioProject accession number PRJNA889174 and Biosample numbers SAMN31232808).

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AUTHOR CONTRIBUTIONS

GAAC: conceptualization, investigation, writing— original draft, writing—review and editing.
GKH: conceptualization, investigation, writing— original draft, writing—review and editing contributed to.
MCQ: writing—review and editing.

COMPLIANCE WITH ETHICAL STANDARDS

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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Figures

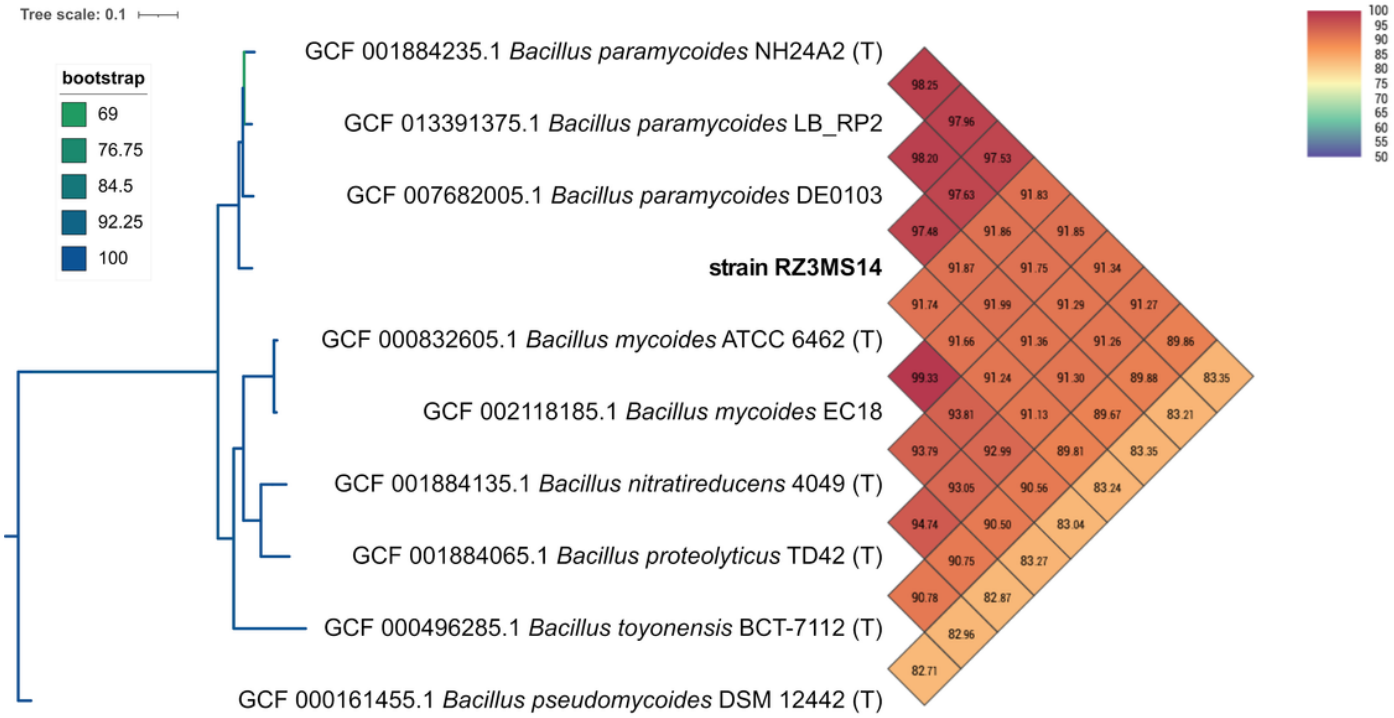

Figure 1

Phylogenomics of *Bacillus paramycoides*RZ3MS14 based on 381 bacterial marker proteins and heatmap of OrthoANI values obtained from OAT software. The phylogeny tree was graphed using Interactive Tree Of Life (iTOL) v6 (Letunic and Bork, 2021).

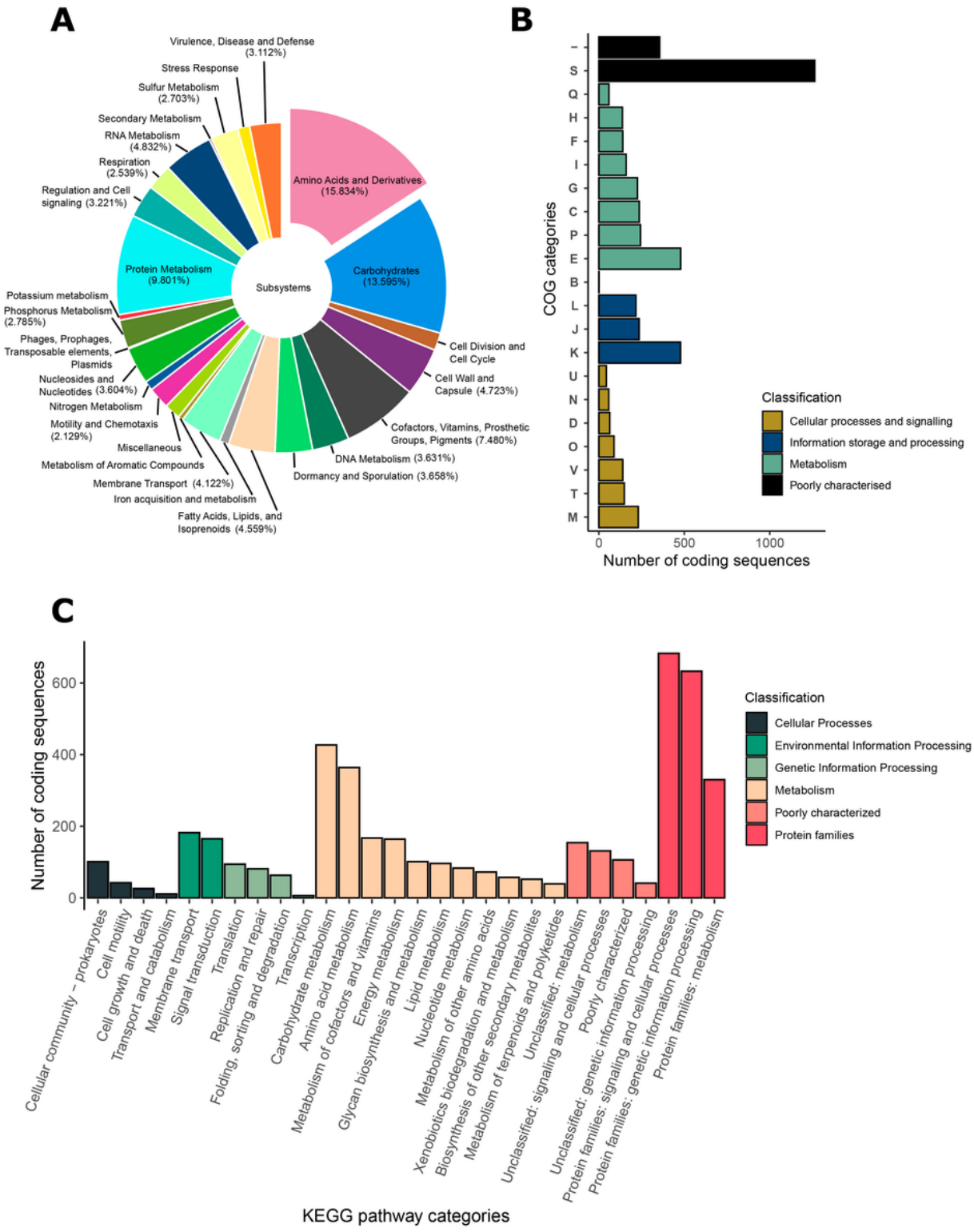

Figure 2

Functional annotation of the draft genome of *Bacillus paramycoides* RZ3MS14. A. Classification of coding sequences into RAST subsystems; B. into COGs categories; C. and by KEGG metabolic pathways. COG categories: (B) Chromatin structure and dynamics; (C) Energy production and conversion; (D) Cell cycle control, cell division, chromosome partitioning; (E) Amino acid transport and metabolism; (F) Nucleotide transport and metabolism; (G) Carbohydrate transport and metabolism; (H) Coenzyme

transport and metabolism; (I) Lipid transport and metabolism; (J) Translation, ribosomal structure and biogenesis; (K) Transcription; (L) Replication, recombination and repair; (M) Cell wall/membrane/envelope biogenesis; (N) Cell motility; (O) Posttranslational modification, protein turnover, chaperones; (P) Inorganic ion transport and metabolism; (Q) Secondary metabolites biosynthesis, transport and catabolism; (S) Function unknown, (T) Signal transduction mechanisms; (U) Intracellular trafficking, secretion, and vesicular transport; (V) Defense mechanisms; (-) Not in COGs.

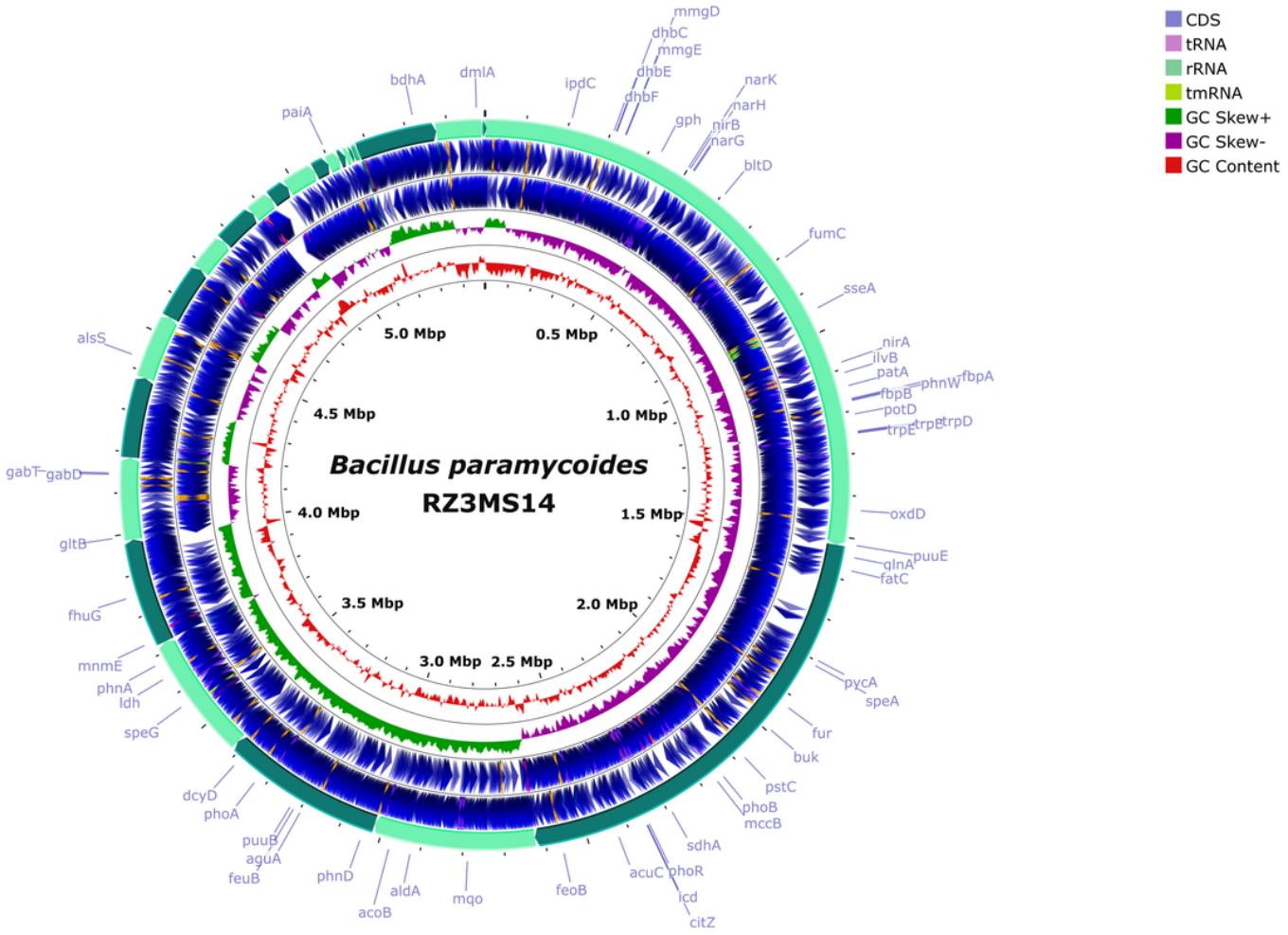

Figure 3

Circular representation of draft genome of *Bacillus paramycoides* RZ3MS14 and genes involved in plant growth promotion and defense mechanisms. Genome characteristics represented in circles and described from the outside to the center. (Circle 1) distribution of scaffolds, (Circle 2 and 3) CDSs in forward and reverse strands, (Circle 4) GC skew and (Circle 5) GC content plot. The figure was created using CGView ServerBETA (http://cgview.ca/).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CastilloSupplementaryMaterial.docx