Detailed transcriptome analysis of the plant growth promoting *Paenibacillus riograndensis* SBR5 by using RNA-seq technology

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**Abstract**

**Background:** The plant growth promoting rhizobacterium *Paenibacillus riograndensis* SBR5 is a promising candidate to serve as crop inoculant. Despite its potential in providing environmental and economic benefits, the species *P. riograndensis* is poorly characterized. Here, we performed for the first time a detailed transcriptome analysis of *P. riograndensis* SBR5 using RNA-seq technology.

**Results:** RNA was isolated from *P. riograndensis* SBR5 cultivated under 15 different growth conditions and combined together in order to analyze an RNA pool representing a large set of expressed genes. The resultant total RNA was used to generate 2 different libraries, one enriched in 5′-ends of the primary transcripts and the other representing the whole transcriptome. Both libraries were sequenced and analyzed to identify the conserved sequences of ribosome binding sites and translation start motifs, and to elucidate operon structures present in the transcriptome of *P. riograndensis*. Sequence analysis of the library enriched in 5′-ends of the primary transcripts was used to identify 1082 transcription start sites (TSS) belonging to novel transcripts and allowed us to determine a promoter consensus sequence and regulatory sequences in 5′ untranslated regions including riboswitches. A putative thiamine pyrophosphate dependent riboswitch upstream of the thiamine biosynthesis gene *thiC* was characterized by translational fusion to a fluorescent reporter gene and shown to function in *P. riograndensis* SBR5.

**Conclusions:** Our RNA-seq analysis provides insight into the *P. riograndensis* SBR5 transcriptome at the systems level and will be a valuable basis for differential RNA-seq analysis of this bacterium.

**Keywords:** *Paenibacillus riograndensis*, RNA sequencing, Transcriptional start sites, Promoter motifs, Ribosome binding sites, Operon structures, Thiamine pyrophosphate riboswitch

**Background**

Members of *Paenibacillus* genus are Gram-positive, spore-forming, motile and facultatively anaerobic bacteria [1]. This group is biochemically and morphologically diverse and is found in various environments, such as soil [2], rhizosphere [3], insect larvae [4], and clinical samples [5]. Originally, *Paenibacillus* belonged to the genus *Bacillus*, however, in 1993 it was reclassified as a separate genus [6]. The important plant growth promoting (PGP) species *P. polymyxa*, *P. macerans* and *P. azotofixans* were included in the new genus when it was proposed [6]. The genus *Paenibacillus* currently comprises more than 150 named species; approximately 6% of these are able to fix nitrogen and possess some other plant growth promotion abilities [7]. *Paenibacillus riograndensis* SBR5 is the type strain of this species and was isolated from rhizosphere of wheat (*Triticum aestivum*) fields in the south of Brazil (Rio Grande do Sul) [8]. It was shown that *P. riograndensis* SBR5 is a promising candidate for crop inoculation because of its nitrogen fixation ability and other plant growth promotion characteristics such as production of phytohormones and antimicrobial substances. 

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For each condition tested, four biological replicates were grown in 500 mL flasks containing 50 mL of medium. In all experiments, the bacterial cells were harvested in the middle of the exponential phase. The initial OD600 nm in all cultivations was approximately 0.05.

The first experiment was performed with lysogeny broth (LB) as growth medium; the cells were grown under 3 different temperatures: 20 °C, 30 °C or 37 °C. Cells were also cultivated at 30 °C for further application of 5 min-cold shock (from 30 °C to 4 °C) or heat shock (from 30 °C to 50 °C) when the middle of the exponential phase was reached. The PbMM (P. riograndensis minimal medium) with 20 mM glucose as carbon source was used for application of the remaining stress conditions. Minimal PbMM medium contained the following, in 1 L of RO-water: K2HPO4, 4.09 g; NaH2PO4 1.3 g; (NH4)2SO4, 2.11 g; biotin, 0.1 mg; concentrated trace element (TE) solution, 1 mL. The concentrated TE solution contained the following, in 1 L of RO-water: FeSO4*7H2O, 5.56 g; CuCl2*2H2O, 0.027 g; CaCl2*2H2O, 7.35 g; CoCl2*6H2O, 0.04 g; MnCl2*4H2O, 9.90 g; ZnSO4*7H2O, 0.288 g; Na2MoO4*2H2O, 0.048 g; H3BO3, 0.031 g. The growth of SBR5 was carried with addition of 100 mM KCl or NaCl or addition of 2 g L−1 of ethanol or methanol to the medium. Moreover, growth in PbMM with addition of 3 different carbon sources of was compared: 20 mM of glucose, 40 mM of glycerol or 10 mM of sucrose. Finally, the cells were cultivated in 3 different pHs: 5, 7 or 8, buffered with 10 mM of sucrose. The growth of SBR5 was carried with addition of 3 different carbon sources of was compared: 20 mM of glucose, 40 mM of glycerol or 10 mM of sucrose. Finally, the cells were cultivated in 3 different pHs: 5, 7 or 8, buffered with 10 mM of sucrose. The growth of SBR5 was carried with addition of 3 different carbon sources of was compared: 20 mM of glucose, 40 mM of glycerol or 10 mM of sucrose. Finally, the cells were cultivated in 3 different pHs: 5, 7 or 8, buffered with 10 mM of sucrose.
minimal medium were used to inoculate fresh PbMM medium containing its respective thiamine concentration.

**RNA isolation and preparation of cDNA libraries for sequencing**

In order to isolate total RNA from SBR5 cells, bacterial cell pellets previously harvested and kept at −80 °C were thawed in ice and RNA was extracted individually for each cultivation condition using NucleoSpin RNA isolation kit (Macherey-Nagel, Düren, Germany). Polymerase Chain Reactions (PCRs) utilizing Taq polymerase (New England Biolabs) and 2 pairs of primers amplifying 2 different genome regions was perform to detect the presence of remaining genomic DNA in the samples. Primer characteristics and sequences are listed in Additional file 1: Table S1 and the reactions were carried according to the Taq polymerase manufacture’s recommendations. RNA samples with genomic DNA contamination were treated with the RNase-free DNase set (Qiagen, Hilden, Germany). The concentration of isolated RNA was determined by DropSense™ 16 (Trinean, Ghent, Belgium; software version 2.1.0.18). To verify the quality of RNA samples, we performed capillary gel electrophoresis (Agilent Bioanalyzer 2100 system using the Agilent RNA 6000 Pico kit; Agilent Technologies, Böblingen, Germany). All procedures to obtain high quality RNA were done according to manufacturer’s recommendations. The extracted RNA samples were pooled in equal parts and the pool of total RNA was subsequently used for the preparation of 2 different cDNA libraries.

The cDNA libraries of SBR5 were prepared according to 2 different protocols. One library followed the protocol for the enrichment of 5′-ends of primary transcripts, while the other method allowed the analysis of the whole transcriptome [15, 16]. The libraries were prepared and sequenced according to Irla et al. 2015 [16]. The generated whole transcriptome and 5′-end enriched cDNA libraries were sequenced on a single flow cell of a MiSeq Desktop Sequencer system.

**Mapping sequenced reads onto the genome of P. riograndensis SBR5**

Before mapping to the reference genome, the reads obtained during sequencing of the whole transcriptome and 5′-end enriched library were trimmed to a minimal length of 20 base pairs with the Trimmomatic ver. 0.33 [23], with three first base pairs cut off at the start and bad quality bases at the end of the reads. The reads of 5′-end enriched library were trimmed in the single end mode, whereas those of whole transcriptome library in paired end mode. Trimmed reads were mapped to the reference genome of P. riograndensis SBR5 (accession number LN831776.1) by using the software for short read alignment Bowtie [24].

**Determination of transcription start sites (TSS) based on 5′-end enriched library**

To determine and classify the TSS based on mapped 5′-end enriched RNA-seq data, we used the software for visualization of mapped sequences ReadXplorer [25]. This determination was done in 2 steps, automatic TSS determination and manual data set curing. First, the TSS were automatically detected by ReadXplorer Transcription Analysis Parameter Wizard, following 2 different selected sets of criteria described in Table 1. In the generated data, to each TSS detected, several characteristics were reported; including: 70 base pairs sequence upstream the TSS, the assigned gene name and product, the DNA strand to which the assigned gene belongs, the assigned gene start and end position, the distance between the given TSS and its assigned translation start sites (TLS) and its classification regarding a TSS assigned to tRNA, mRNA or a novel transcript. As second step, the data generated through the 2 parameter sets were combined and manually cross-checked to classify the novel transcripts as antisense, intergenic or intragenic, and also to eliminate false positives, as previously described by Irla et al. 2015 [16].

**Determination of 5′ UTR length and identification of cis-regulatory elements in 5′ UTRs of P. riograndensis SBR5 genes**

A genome-wide analysis was performed in order to identify putative RNA motifs in the genome of SBR5. To this end, we used the Infernal tool [26]. The RNAs were annotated to the genome of SBR5 in conjunction with the Rfam database [27]. Furthermore, based on the difference between the position of the analyzed TSS and its assigned TLS, we could determine the 5′ UTR length of each TSS belonging to an annotated gene. The 5′ UTRs which were longer than 100 base pairs were used as candidates to evaluate whether they contain cis-regulatory elements. In total, 209 5′ UTRs were analyzed by comparison to Rfam database [28]. Because thiamine is involved in the interaction of plants with plant growth promoting rhizobacteria [29], a TPP riboswitch was

| Parameter sets selected for transcription analysis of P. riograndensis SBR5 | 1 | 2 |
|---------------------------------------------------------------|---|---|
| Transcription start site detection parameters | 1 | 2 |
| Minimum number of read starts | 5 | 3 |
| Minimum percent of coverage increase | 48 | 48 |
| Maximum low coverage read start count | 0 | 20 |
| Minimum low coverage read starts | 0 | 3 |
| Minimum transcript extension coverage | 20 | 5 |
| Maximum distance to feature of leaderless transcripts | 300 | 5500 |
| Associate neighboring TSS in a base pair window of | 3 | 3 |
selected among the detected riboswitches for further analysis; a 313 base pairs sequence of the TPP riboswitch present in the 5′ UTR of the thiC gene was analyzed in the ARNold tool for identification of transcriptional terminators [30] and in the RNAfold tool for determination of its secondary structure [31].

Detection of conserved ribosomal binding site (RBS) and promoter motifs sequences
To identify the conserved promoter motifs, 70 base pairs sequences upstream the TSS assigned to annotated genes were analyzed. All the genes with identified TSS were considered in the analysis of TLS and RBS motifs, for this analysis 50 base pairs upstream of TLS were considered. The Improbizer [32] program was used to find the motifs and the tool WebLogo [33] was used to generate the visualization charts. In both programs, the default settings were applied for the analysis. In the text representations, the conserved motifs are represented in upper or lower case depending on its conservation, as follows: nucleotides and amino acids in upper case letters represent the occurrence of more than 80% of occurrence among all analyzed sequences, nucleotides in lower case letters represent occurrence of more than 40%, but less than 80% of all cases. If a base occurs less often than 40%, the letter “n” in lower case appears.

Determination of most abundant genes transcribed in P. riograndensis SBR5
In order to determine the most abundant genes transcribed in the applied cultivation conditions in SBR5, the whole transcriptome RNA-seq data set was used. The data was normalized by calculation of Reads Per Kilobase per Million mapped reads (RPKM) [34]. The calculation of abundances was automatically generated by the ReadXplorer software [25] as described in Irla et al. 2015 [16]. When the transcripts of proteins of unknown function were automatically defined as the most abundant, the gene sequences were submitted to BLASTx analysis to identify the family to which the protein in question belongs [35].

Identification of operon structures in P. riograndensis SBR5
The operon structures present in this transcript analysis were automatically detected in the ReadXplorer software [25]. The same approach was previously shown in Irla et al. 2015 [16]. The classical operon has multiple genes transcribed as a single mRNA molecule having a single promoter to drive its expression, but transcription start sites internal to the operon sequence pointed to the presence of suboperons which often respond to different conditions [15, 16]. Based on the whole transcriptome RNA-seq data set, an operon structure was identified if the intergenic space of 2 genes positioned in same orientation linked those genes by a bridge of at least 2 paired mappings. Among the detected operon structures, the operons and suboperons were classified separately: a primary operon was considered when a TSS was assigned to the first gene of the operon; and a suboperon was detected when a TSS was assigned within primary operons. Furthermore, the automatically generated operon set was manually cross-checked with the complete whole transcriptome RNA-seq data. Finally, the difference between the position in the genome of the first nucleotide and the last nucleotide of the suboperons/operons was calculated to determine the approximated suboperons/operons length distribution. This calculation does not take the lengths of 5′ UTRs and 3′ UTRs into account.

Strains, plasmid construction and primers
P. riograndensis SBR5 was used as host for heterologous expression of gfpUV. Information about the plasmids constructed in this work and primer sequences is available in Additional file 1: Table S1. Molecular cloning was performed as described by Sambrook (2001) [36]. Chemically competent cells of E. coli DH5α were prepared for cloning [37]. Genomic DNA of P. riograndensis SBR5 was isolated as described by Eikmanns et al. (1994) [38]. The NucleoSpin® Gel and PCR Clean-up kit (Machery-Nagel, Düren, Germany) was used for PCR clean-up and plasmids were isolated using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific, Waltham, USA). Plasmid pNW33Nkan backbone was cut with restriction enzyme BamHI (Thermo Fisher Scientific, Waltham, USA) and inserts were amplified using Allin HiFi DNA polymerase (HighQu, Kraichtal, Germany) and the overlapping regions joined by Gibson assembly [39]. Taq polymerase (New England Biolabs) was used as mentioned above for colony PCR and primer characteristics and sequences are stated in Additional file 1: Table S1. The correctness of inserted DNA sequences was confirmed by sequencing. The constructed plasmids were named pP2pyk-gfpUV or pP2pyk_TPP-gfpUV and transformed to P. riograndensis SBR5 via magnesium-aminoclay method as described by Brito et al. (2016) [22].

Fluorescence-activated cell scanning analysis
To quantify the fluorescence intensities, SBR5 cells were analyzed by using flow cytometry. Routinely, the SBR5 transformants were grown until reaching the middle of the exponential growth phase and centrifuged for 15 min at 4000 rpm. The pellets were washed 3 times in NaCl 0.9% solution and the OD 600nm was adjusted to 0.3. The fluorescence of the cell suspension was measured by using flow cytometer (Beckman Coulter, Brea, US) and the data analyzed in the Beckman Coulter Kaluza Flow Analysis Software. The settings for the emission signal and filters within the flow cytometer for
detection of GfpUV were 550 short pass and 525 band pass in FL9 filter. In order to compare the obtained values of median fluorescence intensity (MFI), the results were tested for significance using one-way ANOVA followed by post hoc comparisons using the Tukey's honest significant difference (HSD) test. The level of significance of the differences observed in each strain between the control (0 μM of thiamine) and test conditions (5, 10, 15, 20 and 25 μM of thiamine) was expressed as one star for \( p \leq 0.05 \). Nonsignificant differences, when \( p > 0.05 \), were not pointed.

Results

Cultivation of \textit{P. riograndensis} SBR5 under various growth conditions

Apart from a core subset of constitutively expressed genes, most genes are transcribed only under certain conditions. In order to obtain a broad representation of the whole transcriptome, we performed several shaking flasks cultivations of SBR5 under different conditions for subsequent RNA extraction. In standard conditions, such as growth in PbMM medium with pH 7 and growth in LB medium at 30 °C, the biomass (ΔOD) was approximately 1.35 and the growth rate (μ) approximately 0.5 h⁻¹ (Additional file 2: Table S2). Compared to standard conditions, growth of SBR5 under stress conditions was in general slower (Additional file 2: Table S2). Hyperosmotic stress, low or high pH and low temperature (20 °C) affected growth of SBR5 to the largest extent (Additional file 2: Table S2). Under all conditions, exponentially growing cells were harvested for RNA isolation.

RNA-seq experiment of \textit{P. riograndensis} SBR5

After confirmation of RNA integrity and absence of DNA contamination, the prepared RNA samples were pooled. The total number of reads generated from whole transcriptome and 5’-end enriched libraries were 11.57 million and 1.40 million, respectively (Table 2). Trimming of the reads with a length threshold of 20 base pairs resulted in 5.87 million (51% of the total reads) remaining reads for the whole transcriptome library and 827,376 (59% of total reads) for the 5’-end enriched library (Table 2). The trimmed reads were mapped to the genome of \textit{P. riograndensis} SBR5, and 1.22 million whole transcriptome library reads and 345,313 reads of the 5’-end enriched library were uniquely aligned to the genome of SBR5 while 122,980 and 31,899 reads were aligned to multiple genome regions, respectively (Table 2).

Identification of transcription start sites (TSS) based on the mapped 5’-end enriched data

In order to detect putative TSS in the mapped 5’-end enriched data; 2 TSS analysis parameter sets were chosen (Table 1). The use of the parameter set 1 led to the automatic detection of 849 TSS and 1951 TSS were detected by using parameter set 2 (Table 1). Subsequently, these results were merged. Figure 1 shows the scheme of the manual review of the automatically detected TSS which led to the identification of 86 TSS belonging to rRNA or tRNA genes. Moreover, 363 elements were considered not to be TSS or to be false positives. TSS were considered false-positives if no clear accumulation of read starts was observed at the particular genomic position and additionally the putative TSS was detected within an uneven gradient of accumulated read starts [16]. The 2351 remaining TSS were classified as either belonging to 5’ UTRs of annotated genes or of novel transcripts. Out of the 6705 genes annotated in the genome of SBR5 [12], 1173 were found to possess TSS. The detected TSS were classified as single when only one TSS was present upstream a gene (1102) or multiple when more than one TSS were present upstream a gene (166). The remaining 1082 TSS were classified as belonging to novel transcripts, divided into the groups of antisense when the transcript was located in the antisense orientation to an annotated gene (170), intergenic when the transcript was located between annotated genes (77) or intragenic when a TSS was located within annotated genes in sense orientation (835) (Fig. 1).

Distribution of 5’ UTR length in \textit{P. riograndensis} SBR5

The sequences located between TSS and the gene start codons were used for the analysis of 5’ UTR lengths. For this purpose, only the 5’ UTRs assigned to annotated genes were considered. The length of 5’ UTRs in \textit{P. riograndensis} varied from 0 to 799 base pairs. Only 2 of the genes with annotated TSS were considered leaderless (no 5’ UTR present): P.riograndensis_final_2873 coding for stress-induced protein and P.riograndensis_final_5691 coding for hypothetical protein (Additional file 3: Table S3). Moreover, 10 of the analyzed 5’ UTRs were found to be shorter than 10 base pairs (Additional file 3: Table S3). Figure 2 shows the distribution of the 5’ UTR lengths indicating that the majority of 5’ UTRs are 25 to 50 base pairs long. Among the 1269 analyzed 5’ UTRs, 209 (16.4%) were longer than 100 base pairs (Fig. 2). Those 5’ UTRs were further used in a screen for cis-regulatory RNA elements.

| Table 2 | Sequencing and mapping features of cDNA libraries of \textit{P. riograndensis} SBR5 |
|---------|-------------------------------|
|          | Whole transcriptome | 5’-end enriched ends |
| Total reads | 11,577,588 | 1,401,776 |
| Reads after trimming | 5,876,240 | 827,376 |
| Mapped reads | 1,351,334 | 345,313 |
| Mapped at single position | 1,228,354 | 313,414 |
| Mapped at multiple position | 122,980 | 31,899 |
Identification of consensus promoter motif sequences in *P. riograndensis* SBR5

The 1269 TSS identified as belonging to annotated genes were used in a search for the conserved promoter motifs (Fig. 1). The software Improbizer was applied to predict the motifs in a DNA region 70 base pairs upstream of each of those TSS [32]. Conserved −35 and −10 promoter sequence motifs were found in 1220 (96.1%) and 1217 (95.9%) of the analyzed sequences, respectively (Fig. 3A). Figure 3A shows the −10 and −35 motif sequence logos, which were ttgaca for −35 hexamer motif and TAtaaT for the −10 hexamer motif. The mean spacer lengths between the −35 and −10 motifs and −10 motifs and TSS were 17.6 base pairs and 4.1 base pairs, respectively (Fig. 3A).

Identification of RBS (ribosome binding site) and TLS (translation start site) consensus sequences in *P. riograndensis* SBR5

Similarly to the analysis of the promoter motifs, the Improbizer software [32] was used to determine the consensus sequence of RBS and TLS in the sequence 50 base pairs upstream of the translation start codon of genes associated to the 1269 previously identified TSS (Fig. 1). Some genes were characterized as associated to multiple TSS (Fig. 1), therefore the upstream sequence of these genes was only included once in the analysis. Hence, the 1173 remaining sequences were extracted from the genome of SBR5 and submitted to Improbizer [32] and WebLogo [33] for the identification of the conserved motifs of RBS and TLS (Fig. 3B). RBS motifs were identified in 98% (1155) of analyzed sequences. The determined RBS motif aGGaGg of *P. riograndensis* SBR5 includes 3 conserved guanines in approximately 90% of the analyzed sequences (Fig. 3B). Translational start codons were identified in all the analyzed sequences (Fig. 3B). The TLS found in the analyzed sequences were ATG (924; 79%), GTG (138; 12%) and TTG (111; 9%). The lengths of the spacer sequence between RBS and TLS varies between 5 and 13 base pairs, with an average of 7.8 ± 2.0 base pairs (Fig. 3B).
Identification of cis-regulatory elements in 5′ UTRs of *P. riograndensis* SBR5 genes

In order to identify putative RNA motifs in the genome sequence of *P. riograndensis* SBR5, we used the Infernal tool [26] and the Rfam database, which contains hundreds of RNA families [27]. This approach revealed 327 RNA motifs that subsequently were manually cross checked. Matches to tRNAs, ribosomal RNAs and RNA motifs from Eukaryotes or different bacterial groups were not considered. As result, 98 RNA motifs among 31 Rfam families were identified (Additional file 4: Table S4).

In an alternative approach based on the RNA-seq data, we analyzed 209 5′ UTRs longer than 100 base pairs (Fig. 2) for the presence of cis-regulatory elements by comparison to the Rfam database. This analysis revealed the presence of 11 putative cis-regulatory elements grouped in 9 types of riboswitch families (Table 3). Thus, based on the RNA-seq data, the existence of 11 out of 98 putative 5′ UTR RNA motifs upstream of annotated genes was confirmed. A TPP (thiamine pyrophosphate) sensitive riboswitch was predicted to be present in the 5′ UTR of the gene *P. riograndensis_final_150* (*thiC*) encoding phosphomethylpyrimidine synthase, which is putatively involved in thiamine biosynthesis, and in the 5′ UTR belonging to the operon *P. riograndensis_final_504–502*. Although *P. riograndensis_final_503* gene is automatically annotated as a hypothetical protein, BLASTX analysis revealed that it belongs to the thiamine-biding protein superfamily. More vitamin and amino acid related riboswitches were found: a pantothenate related *pam* riboswitch in the 5′ UTR of putative pantothenate synthesis operon and a riboswitch recognizing S-adenosylmethionine (SAM) in the 5′ UTR of an operon encoding homoserine O-succinyltransferase and cystathionine gamma-lyase proteins. The T-box regulatory elements were found in 5′ UTR of the genes coding for D-3-phosphoglycerate dehydrogenase (*serA*) and valine tRNA ligase (*valS*). Furthermore, the protein dependent L20 leader and L21 leader riboswitches, the metabolite dependent *ydaO-yuaA* riboswitch, the *pfl* riboswitch and the glycine dependent riboswitch were identified in this work (Table 3).

**A TPP riboswitch influences gfpUV expression in *P. riograndensis* SBR5**

Riboswitches are regulatory elements found in the 5′-UTR of genes and they perform the regulatory control over the gene transcript by directly binding a small ligand molecule. In the riboswitch sequence, an aptamer domain recognizes and binds to that ligand which leads to adopting a new conformation that interfaces with the gene transcriptional (presence of terminator sequence) or translational machinery (sequestration of the RBS by a stem) [40]. The prediction of the secondary structure of the TPP riboswitch in the 5′ UTR of *thiC* gene, with length of 313 base pairs, showed that it contains no terminator sequence. However, a 5′-GAUAA-3′ sequence and its complementary 5′-UUAUC-3′ is present in many
Table 3: Riboswitches detected in the transcriptome of *P. riograndensis* SBR5 and their transcriptional organization

| No. | Accession | Riboswitch and its transcriptional organization | Related function | Locus tag |
|-----|-----------|-----------------------------------------------|-----------------|----------|
| 1   | RF00059   | (TPP)-thc | Phosphomethylpyrimidine synthase | *P. riograndensis_final_150* |
| 2   | RF00059   | (TPP)-*P. riograndensis_final_504*-P. riograndensis_final_503-P. riograndensis_final_502 | Conserved hypothetical protein- Hypothetical protein- Biding protein dependent transport system inner membrane component | *P. riograndensis_final_504*-P. riograndensis_final_503-P. riograndensis_final_502 |
| 3   | RF00162   | (SAM)-metaA- P. riograndensis_final_2059 | Homoserine O-succinyltransferase-Cystathionine gamma-lyase | *P. riograndensis_final_2058*-P. riograndensis_final_2059 |
| 4   | RF00230   | (T-box)-serA | D-3-phosphoglycerate dehydrogenase | *P. riograndensis_final_4453* |
| 5   | RF00230   | (T-box)-valS | Valine tRNA ligase | *P. riograndensis_final_5318* |
| 6   | RF00379   | (ydaO-yuaA)- P. riograndensis_final_93 | Cell wall-associated hydrolase (invasion- associated protein) | *P. riograndensis_final_93* |
| 7   | RF00504   | P. riograndensis_final_6104-gcv9A-gcv1B | Glycine utilization | *P. riograndensis_final_6104*-P. riograndensis_final_6105-P. riograndensis_final_6106 |
| 8   | RF00558   | (L20 leader)-infC- P. riograndensis_final_1528-P. riograndensis_final_1529 | Translation initiation factor IF-3- Conserved hypothetical protein- Ribosomal protein L20 | *P. riograndensis_final_1527*-P. riograndensis_final_1528-P. riograndensis_final_1529 |
| 9   | RF00559   | (L21 leader)-rplU- P. riograndensis_final_5299-P. riograndensis_final_5300 | 50S ribosomal protein L21- Conserved hypothetical protein- 50S ribosomal protein L27 | *P. riograndensis_final_5298*-P. riograndensis_final_5299-P. riograndensis_final_5300 |
| 10  | RF01749   | (pan)-panB- panC- P. riograndensis_final_4379 | 3-Methyl-2-oxobutanoatehydroxymethyltransferase- Pantothenate synthetase- Aspartate l-decarboxylase alpha | *P. riograndensis_final_4381*-P. riograndensis_final_4380-P. riograndensis_final_4379 |
| 11  | RF01750   | (pfl)-P. riograndensis_final_6217 | Hypothetical protein | *P. riograndensis_final_6217* |

of the predicted stems, including the stems of the aptamer region (Fig. 4A). This indicates the existence of antisequestering stems in this molecule, as showed schematically in Fig. 4A. Furthermore, we aimed to detect the influence of the *P. riograndensis* TPP riboswitch on gene expression in the presence of different concentrations of its ligand thiamine by measuring the GfpUV fluorescence. SBR5 cells were transformed with the plasmid pP2pyk_TPP-gfpLV which carries the constitutive promoter Ppyk with its native 5′ UTR replaced by the 5′ UTR of the *P. riograndensis_final_150*. The so changed Ppyk promoter drives the expression of the reporter gene gfpLV (Additional file 1: Table S1). As shown before, the 5′ UTR of the *P. riograndensis_final_150* gene contains the sequence of a TPP riboswitch (Table 3). As control for this assay, the plasmid pP2pyk-gfpLV, containing Ppyk native 5′ UTR was used to transform SBR5 cells and the resultant strain was also cultivated in glucose PbMM, but supplied with 0 or 25 μM of thiamine. The MFI of the control strain SBR5(pP2pyk-TTP-gfpLV) remained the same when the cells were in absence or in presence of 25 μM of thiamine (Fig. 4B). The GfpUV MFI of SBR5(pP2pyk-TTP-gfpLV) was similar to the control strain when no thiamine was added to the growth medium (Fig. 4B). In contrast, there was a significant effect of thiamine on MFI of SBR5(pP2pyk_TTP-gfpLV) at the p < 0.05 level for the tested conditions [F (5, 12) = 17.8, p = 0.00004]; post hoc comparisons using the Tukey’s HSD test indicated that the mean score for the 0 μM thiamine was significantly different than the 5, 10, 15, 20 and 25 μM thiamine conditions. However, the MFI of SBR5(pP2pyk_TPP-gfpLV) in 5, 10, 15, 20 and 25 μM thiamine conditions did not significantly differ from one another (Fig. 4B).

Identification and characterization of novel transcripts

Here, we performed the characterization of *P. riograndensis* novel transcripts based on the 5′-end enriched data set. Among the 2351 manually verified TSS, 1082 were classified as belonging to novel transcripts. Depending on their position in genes or untranslated regions, these TSS belonged to antisense transcripts (170), transcripts intragenic (835) to annotated genes, they were manually annotated as intergenic (77) transcripts (Fig. 1). Additional file 5: Table S5 shows the intragenic transcripts which were organized according to their position and associated gene. As intergenic novel transcripts could not be assigned to annotated genes, they were manually annotated as unknown transcripts (Additional file 6: Table S6). BLAST analysis of the intergenic novel transcripts resulted in discovery of 34 small proteins and 27 small RNAs. Small RNAs were analyzed in the Rfam database and 3 of them were annotated as Small SRP (*P. riograndensis_final_s0002*), BsrC sRNA (*P. riograndensis_final_s0008*) and RNase
Gene expression ranked according to transcript abundances

The abundance of transcripts in the analyzed RNA samples was quantified on the basis of the whole transcriptome dataset using RPKM values. Transcripts were detected in 6367 of the coding sequences during the analysis, corresponding to 94% of the total number of genes annotated in the genome of *P. riograndensis*. Transcript abundance varied over 6 orders of magnitude with RPKM values ranging from 0.11 to 71,849.57. Transcripts were categorized arbitrarily as follows: low for approximately 70% of transcripts (with RPKM values <100), intermediate (RPKM between 100 and 1000) for around 25% of the detected transcripts and high for approximately 5% of the transcripts (RPKM between 1000 and 10,000). Twenty one transcripts showed RPKM values exceeding 10,000 and these were considered as transcripts with very high transcript abundance and are listed in Table 5.

BLASTx analysis was performed to predict the functions (conserved protein domains) of the 14 genes which were automatically annotated as hypothetical proteins or as proteins with unknown function. However, a function could not be predicted for 5 genes with very highly abundant transcripts (Table 5). Part of the very highly abundant transcripts code for ribosomal proteins (6 genes). Remarkably, 3 genes related to bacterial sporulation had very highly abundant transcripts (Table 5).
file 3: Table S3). However, known operons comprising nitrogen fixation genes [10] were not detected in the present study. Notably, riboswitches were found in the 5′ UTRs of 6 operons P.riograndensis_final_502–504, infC-P.riograndensis_final_1528–1529, metaA-P.riograndensis_final_2059, panB-panC-P.riograndensis_final_4379, rplU-P.riograndensis_final_5299–5300 and P.riograndensis_final_6104–gcvPA–gcvPB (Table 3).

Discussion

In the present study, we performed for the first time a detailed transcriptome analysis of P. riograndensis SBR5. This work lays a foundation for understanding of gene expression in this bacterium and complements differential gene expression analysis. To enable the comprehensive characterization of ‘static’ bacterial transcriptomes it is necessary to generate a pool of different transcripts in order to obtain the expression of as many genes as possible [16]. This was largely achieved by cultivation of P. riograndensis SBR5 under 15 distinct conditions and pooling RNA samples prior to sequencing, since we found 94% genes expressed under these conditions. However, the absolute number of TSS (present in 5′ UTR of annotated genes together with TSS belonging to novel transcripts) in P. riograndensis SBR5 was comparable to that in B. methanolicus MGA3 [16] and C. glutamicum [15, 42]. This observation may also reflect the fact that RNA was pooled from cells cultivated under various growth and stress conditions that were chosen with a similar rationale for the 3 bacteria and may indicate that a similar set of genes is transcribed under the chosen conditions.

In this study, the 5′ UTR length of P. riograndensis SBR5 transcripts was shown to be equal or longer than 10 nucleotides in 99.2% of the cases and it peaked at around 30 base pairs (Fig. 2). A similar 5′ UTR length distribution can be found in Actinoplanes sp., C. glutamicum and B. methanolicus [15, 16, 43]. However, leaderless transcripts are rare in P. riograndensis SBR5 as the present study only revealed 2 transcripts (P.riograndensis_final_2873 and P.riograndensis_final_5691) to be leaderless in this firmicute (Additional file 3: Table S3). In silico analysis performed by Zheng et al. (2011) [44] showed that 207 among 953 analyzed bacterial genomes possess leaderless genes including species of the Firmicutes and Actinobacteria phyla. The scarcity of leaderless transcripts in the transcriptomes of the low-GC Gram-positives B. methanolicus [16] and P. riograndensis contrasts with the large proportion of leaderless transcripts present in the high-GC Gram-positive actinobacteria Actinoplanes sp. (20%) [43] and C. glutamicum (33%) [15].

The analysis of the promoter, RBS and TLS motives in P. riograndensis SBR5 transcriptome revealed that the RBS consensus sequence aGGaGg, TLS frequency (ATG

Table 4 Novel transcripts with known function in P. riograndensis SBR5

| Feature      | Class  | Locus tag                  | Feature start | Feature stop | Length | Strand |
|--------------|--------|----------------------------|---------------|--------------|--------|--------|
| Small SRP    | Small RNA | P.riograndensis_final_s0002 | 130,367       | 130,639      | 272    | +      |
| BsrC sRNA    | Small RNA | P.riograndensis_final_s0008 | 688,067       | 687,745      | 322    | –      |
| RNase P      | Small protein | P.riograndensis_final_s0039 | 6,002,090     | 6,001,625    | 465    | –      |

Fig. 5 Operon analysis in P. riograndensis SBR5. a. Length distribution (in base pairs) of detected operons and suboperons; b. Analysis of feature number in monocistronic transcripts, operons and suboperons in P. riograndensis SBR5
represented 79% of the analyzed sequences and GTG and TTG represented 12% and 9%, respectively) and spacing between RBS and TLS (7.8 base pairs) in \(P.\) \(riograndensis\) SBR5 corresponds well to the conserved sequences motifs historically found in bacteria \([45–47]\). While the \(-10\) region (TAtaaT), spacing between \(-10\) and \(-35\) boxes (17.6 base pairs) and between \(-10\) box and TSS (4.1 base pairs) are conserved between \(P.\) \(riograndensis\) SBR5, \(E.\) \(coli\), \(B.\) \(subtilis\) and \(B.\) \(methanolicus\) \([16, 48, 49]\), the conserved \(-35\) region (ttgaca) in SBR5 was similar only to the \(-35\) box described for other bacilli \([16, 50]\).

Riboswitch-mediated control of expression of a variety of genes in bacteria could have practical implications, such as development of new antibacterial drugs \([21]\), or more generally contribute to improvement of the understanding of bacterial metabolism. Here, a genome-based riboswitch analysis revealed 98 putative RNA motifs, 11 of which were also detected in the sequenced RNAs (Additional file 4: Table S4). In \(Firmicutes\), the SAM riboswitch is part of the S-box group of riboswitches which are involved in regulation of SAM, cysteine and methionine biosynthesis, and sulfur metabolism \([51, 52]\). This type of riboswitch has been well characterized in bacilli, for example in \(B.\) \(subtilis\), which has at least 11 operons and 26 genes under control of S-box RNA \([53]\). S-box RNA from \(B.\) \(subtilis\) directly senses the level of SAM and functions as SAM dependent riboswitch \([54]\). However, the most frequent mechanism of riboswitch regulation of amino acid operons in the \(Firmicutes\) is the T-box regulatory system \([55, 56]\). In \(B.\) \(subtilis\) and other \(Firmicutes\), the T-box can regulate many genes encoding amino acid biosynthetic enzymes and transporters \([57]\). The \(ydaO-yuaA\) riboswitches occur upstream of these 2 genes in \(B.\) \(subtilis\) and operate as a genetic “off” switch \([58]\). Furthermore, recognition of the cyclic di-AMP by \(ydaO-yuaA\) was characterized and also shown to exist in \(B.\) \(subtilis\) \([59, 60]\). The riboswitches in \(P.\) \(riograndensis\) SBR5 identified in the present study need to be investigated to some detail to unravel their regulatory function.

In order to analyze the function of 1 exemplary riboswitch found in the transcriptome of \(P.\) \(riograndensis\) SBR5, we selected the TPP dependent riboswitch present upstream of the \(thiC\) gene (Table 3) to control the expression of \(gfpUV\). The gene \(thiC\) encodes a phospho-methylpyrimidine synthase involved in TPP biosynthesis \([61]\). The \(E.\) \(coli\) \(thiC\) riboswitch controls translation initiation and, in the presence of TPP \(thiC\) product is not translated \([62, 63]\). The thiamine analog triazolethiamine showed a concentration dependent reporter gene repression by the TPP riboswitch in the 5′ UTR of the

| Gene Product | RPKM Value |
|--------------|------------|
| rpsH 30S ribosomal protein S8 | 71,849.57 |
| N-acetyltransferase superfamily | 70,789.99 |
| Veg protein; sporulation; Stimulates biofilm formation via transcriptional activation of extracellular matrix genes | 53,361.67 |
| Hypothetical protein | 39,913.22 |
| Hypothetical membrane protein | 28,462.66 |
| Small, acid-soluble spore protein superfamily | 24,204.00 |
| PTS maltose transporter subunit IIIBC | 21,134.31 |
| 50S ribosomal protein L24 | 20,187.98 |
| Hypothetical protein | 18,591.09 |
| Hypothetical protein | 17,946.18 |
| Recombinase RecA | 17,771.90 |
| Ribosomal S21 superfamily | 17,463.97 |
| Small, acid-soluble spore protein superfamily | 16,757.65 |
| Protein of unknown function DUF1292 superfamily | 15,456.74 |
| ATP-dependent zinc metalloprotease FtsH | 15,355.15 |
| 30S ribosomal protein S19 | 15,060.69 |
| Conserved hypothetical protein | 14,247.19 |
| 50S ribosomal protein L7A | 14,218.27 |
| Crossover junction endodeoxyribonuclease RuvA | 11,659.67 |
| Transcriptional regulator, TetR family | 11,466.86 |
| 50S ribosomal protein L16 | 10,826.18 |

| Gene products in italics were predicted with BLASTx analysis |
thiamine kinase \( \text{thiK} \) [62]. In the present study, thiamine was added to the growth medium and already 5 \( \mu \text{M} \) thiamine fully reduced \( \text{gfpUV} \) expression (Fig. 4B). \( \text{P. riograndensis} \) SBR5 is a thiamine prototroph capable of growing in minimal medium without added thiamine (data not shown). The \( \text{gfpUV} \) expression without additional thiamine remained high (Fig. 4B) suggesting that the amount of thiamine synthesized by SBR5 did not activate the TPP riboswitch. Riboswitch aptamers remain highly conserved through evolution because each one must preserve selective binding of its target metabolite. Hence, the conserved TPP riboswitch consensus regions of \( \text{B. subtilis} \) were also present in the TPP riboswitch aptamer sequence targeted in this study (Fig. 4A) [21]. The secondary structure analysis showed that, in contrast to the \( \text{tenA} \) TPP riboswitch in \( \text{B. subtilis} \) [21], the SBR5 \( \text{thiC} \) TPP riboswitch does not possess a transcriptional terminator sequence. This led us to investigate the presence of sequestering/anti-sequestering stems that could participate in the “on/off” state of the \( \text{P. riograndensis} \) SBR5 \( \text{thiC} \) TPP riboswitch. The schematic representation of the \( \text{thiC} \) TPP riboswitch secondary structure shows that the RBS is sequestered within a stem-loop structure predicted to inhibit translation initiation (Fig. 4A). We could detect the sequence 5’-GATAA-3’ and its complementary region 5’-UUAUC-3’ inserted in the TPP aptamer sequence, in the stem-loop containing the \( \text{thiC} \) RBS and also in the sequence of 1 stem-loop located before RBS containing stem. The locations of these sequences inside the TPP riboswitch structure are depicted in red in the schematic representation of Fig. 4A. Comparable secondary structures and gene expression control have very recently been described for the \( \text{E. coli} \) \( \text{thiC} \) TPP riboswitch [63]. \( \text{P. riograndensis} \) possesses 3 further putative TPP riboswitches, one of which was expressed under the growth conditions of the RNA-seq analysis presented here (Table 3; Additional file 4: Table S4). Although they share conserved sequences and secondary structure predictions (data not shown) it remains to be studied if these putative TPP riboswitches are indeed responsive to TPP and if they operate as transcriptional or translational riboswitches.

Landscape transcriptome analyses are suitable to identify antisense, intragenic or intergenic novel transcripts [15, 16]. Sixteen percent of the 1082 novel transcripts identified in the transcriptome of \( \text{P. riograndensis} \) SBR5 were shown to be antisense (Fig. 1). The number of reported antisense RNAs varies between bacteria and the biological advantages of such overlapping transcription remains unclear, but antisense RNAs may play important roles in regulation e.g. by transcription interference [64]. Commensurate with this notion, we could identify that transcription of 3 antisense RNAs initiates in the 5’ UTRs of the genes on the complementary strand and, thus, antisense transcription may interfere or attenuate with their transcription (\( \text{P.riograndensis}_\text{final}_\text{5580} \), \( \text{P.riograndensis}_\text{final}_\text{6016} \) and \( \text{P.riograndensis}_\text{final}_\text{6182} \); Additional file 5: Table S5). Moreover, 77 novel transcripts were identified in the intergenic regions between previously known genes, part of the identified intergenic transcripts represent small RNA and small protein genes (Additional file 7: Table S7). Of these, 34 were predicted to encode small proteins, for example, small signal recognition particle (or small SRP; \( \text{P.riograndensis}_\text{final}_\text{s0002} \)), which is known to be involved in protein targeting in other bacteria [65]. Twenty seven small RNA genes were found, e.g. the small RNA \( \text{bsrC} \) (\( \text{P.riograndensis}_\text{final}_\text{s0008} \)), which is present also in \( \text{B. subtilis} \) [66] and RNase P (\( \text{P.riograndensis}_\text{final}_\text{s0013} \)), the ubiquitous endonuclease that catalyzes the maturation of the 5’ end of the tRNAs [48]. Overall, the function of the novel intergenic transcripts representing small protein and small RNA genes still has to be elucidated.

In the transcriptome of \( \text{P. riograndensis} \) SBR5, the abundantly transcribed genes could be grouped by their presumed functions: ribosomal proteins, sporulation related proteins, proteins related to carbon metabolism and others. Many abundantly transcribed genes encode proteins of unknown function (Table 5). Among the highly expressed genes, we have detected 1 gene coding for a subunit of a maltose phosphotransferase system (\( \text{P.riograndensis}_\text{final}_\text{1999} \), Table 5). Maltose is a disaccharide formed from two units of glucose and this organic compound can be identified in root exudates of different plant species [65]. Although maltose was not utilized as carbon source in the present study, the transcription of this carbohydrate phosphotransferase system gene may be due to the fact that we mostly used glucose as carbon source. The gene encoding a glucose specific phosphotransferase system (\( \text{P.riograndensis}_\text{final}_\text{1998} \)) had RPKM value of 156.17 (data accession GSE98766) which places it in the group of intermediately abundant transcripts. Thus, the affinity of the highly transcribed maltose transporter to glucose still remains to be studied. A transcriptome analysis of carbon source utilization (\( \beta \)-glucan, starch, cellulose, maltose, glucose, xylose and arabinose) by \( \text{Paenibacillus} \) sp. JDR-2 revealed a regulatory connection for the utilization of the polysaccharides \( \beta \)-glucan, starch and xylans, while transcription of genes coding for proteins involved in monosaccharide (e.g. arabinose and glucose) utilization was less apparent [13]. BLASTx analysis revealed 3 sporulation-related genes (\( \text{P.riograndensis}_\text{final}_\text{4321} \), \( \text{P.riograndensis}_\text{final}_\text{2316} \) and \( \text{P.riograndensis}_\text{final}_\text{5601} \)) among the most abundantly expressed genes (Table 5). This result might be due to the fact that different stress conditions were applied during cultivations of SBR5, which included 5 min of cold and heat shock but also exposure to salinity, solvent, low temperature and low pH along the bacterial
growth. The exposure to stress conditions affected growth rates in comparison to the optimal growth conditions, and might have also induced expression of sporulation related genes (Additional file 3: Table S2). Very recently, sporulation genes spoVT and spoIIAH were shown to be transcribed by P. riograndensis SBR5 under iron-limiting conditions [67]. Moreover, the related P. polymyxa SC2 expressed sporulation genes (spoOA, spoIE, spoIIAA, spoIAB, sigE and sigF) when cultivated under sporulation conditions: on LB agar for 24 h at 37 °C, when most of SC2 step into the progress of sporulation [68].

The transcriptional organization of 1776 genes of P. riograndensis SBR5 in 622 operons including 248 suboperons and 919 monocistronically transcribed genes (Fig. 5B) was comparable to that found in B. methanolicus, in which 1164 genes were assigned to 381 operons and 94 suboperons, and also ~ 900 monocistronic transcripts were detected [16]. Similarly, in B. subtilis 736 regulated operons were found [69] and 1013 genes were organized in 616 operons (including 565 suboperons) for the actinobacterium C. glutamicum [15]. Most operons detected here were composed of only 2 genes and were between 1000 and 3000 base pairs in length (Fig. 5). Accordingly, most suboperons comprised only 1 gene and were smaller than 2000 base pairs (Fig. 5). The length distribution of suboperons/operons and the number of genes constituting these are commensurate with the average length of the genes in P. riograndensis SBR5 genome of 1008 base pairs (data not shown).

Only two differential gene expression analyses on nitrogen fixation [10] and iron metabolism [67] of P. riograndensis SBR5 have been published. The nitrogen fixation genes present in 3 genome clusters were characterized by transcript analysis by quantitative real-time qPCR and shown to be transcribed in the operons nifB1H1D1K1E1N1X1-orf1-hesA-V, nifE2N2X2 and anfHDGK [10]. In the present study, these operons that are generally transcribed under poor nitrogen supply conditions [14, 70, 71] were not found to be expressed under the chosen growth conditions since all growth conditions were characterized by sufficient nitrogen concentrations (in LB medium or minimal media with 16 mM ammonium sulfate). By contrast, a different gene related to nitrogen fixation encoding putative nitrogenase (flavodoxin; P. riograndensis_final_4327) was found to be expressed. In the second study, 150 genes of P. riograndensis SBR5 were shown to be differentially expressed under iron-replete in comparison to iron-limiting conditions [67]. Surprisingly, a high expression level of the Fe\(^{3+}\) siderophore transporter gene fecE was observed suggesting that P. riograndensis SBR5 can uptake Fe\(^{3+}\) siderophore from the environment although it is not able to produce those siderophores itself [67].

Here, we could identify 2 operon structures putatively involved in Fe\(^{3+}\) siderophore uptake and transport: the operon fluuB - P. riograndensis_final_3660 which encodes a putative Fe\(^{3+}\) hydroxamate import system permease and a component of an ABC type Fe\(^{3+}\) siderophore transport system, respectively, and the operon P. riograndensis_final_5688 - P. riograndensis_final_5687 which comprises a gene encoding the Fe\(^{3+}\) siderophore ABC transporter permease (Additional file 3: Table S3).

Conclusions
The examination of the whole transcriptome of P. riograndensis, which was reclassified recently as P. sonchi [72], is a valuable contribution to the understanding of biology of this organism. Moreover, our data validated the uncovering of novel transcripts and the presence of hundreds of operons. Although our study has revealed a functional TPP riboswitch in gene regulation of SBR5, further effort is required to fully elucidate the function of this riboswitch. Finally, the data generated in this study should be valuable for future development of genetic tools for this poorly characterized species as much as for the genus Paenibacillus. As our RNA-seq analysis provides new insight into the P. riograndensis SBR5 transcriptome at the systems level, it will be a valuable basis for further differential RNA-seq analysis exploring agronomical/physiological aspects of this bacterium, e.g. phosphate solubilization.

Additional files

**Additional file 1**: Table S1. Bacterial strains, plasmids and oligonucleotides used in this study. *Overlap regions in bold; oligonucleotide sequences in low caps were used in RNA samples to detect genomic DNA contamination. (XLSX 9 kb)

**Additional file 2**: Table S2. Delta OD, growth rate and OD of harvested cells of P. riograndensis SBR5 when cultivated in Paenibacillus minimal medium (PbMM) or lysogeny broth (LB) with a variation of growth parameters. Cells cultivated at 30 °C and transferred to LB medium at 4 °C or 50 °C for 5 min for application of treatment of *cold shock or *heat shock, respectively. (XLSX 14 kb)

**Additional file 3**: Table S3. List of CDS of P. riograndensis SBR5 with corrected translational start sites. (XLSX 288 kb)

**Additional file 4**: Table S4. Putative RNA motifs present in the genome of P. riograndensis SBR5. Highlighted cells are referent to riboswitches detected in the transcriptome analysis. (XLSX 22 kb)

**Additional file 5**: Table S5. Novel antisense transcripts of P. riograndensis SBR5. (XLSX 16 kb)

**Additional file 6**: Table S6. Novel intragenic transcripts of P. riograndensis SBR5. (XLSX 21 kb)

**Additional file 7**: Table S7. Novel intergenic transcripts of P. riograndensis SBR5. (XLSX 12 kb)

**Abbreviations**
5′ UTR: 5′ Untranslated region; BLAST: Basic local alignment search tool; CDS: Coding DNA sequences; HSD: Honest significant difference; LB: Lysogeny broth; MES: 2-(N-morpholino)ethanesulfonic acid; MFI: Median
fluorescence intensity; MOPS: 3-Morpholinopropane-1-sulfonic acid; OD: Optical density; PoMV: Paenibacillus minimal medium; PGP: Plant growth promoting; RBS: Ribosome binding site; RPMK: Reads per kilobase per million mapped reads; SAM: S-adenosylmethionine; TAPS: 3-{[(1,3-Dihydroxy-2-hydroxymethylpropan-2-yl)amino]propane-1-sulfonic acid; TE: Trace element; TLS: Translation start sites; TTP: Thiamine pyrophosphate; TSS: Transcription start sites

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Availability data and materials

The data sets supporting the results of this article are available in the NCBI Gene Expression Omnibus database; under the accession number GSE98766. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE98766.

Authors’ contributions

LFB and MI performed experimental procedure and the complete data analysis of the present study. LFB prepared a draft of the manuscript. VFW and MI performed experimental procedure and the complete data preparation and sequencing of the cDNA libraries and Dr. Alexander Sczyrba and Maximilian Wiens for the bioinformatics advice. We thank Anika Winkler and Tobias Busche for the kind assistance with the preparation and sequencing of the cDNA libraries and Dr. Alexander Sczyrba and Maximilian Wiens for the bioinformatics advice.

Competing interests

The authors declare that they have no competing interests.

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