Short Research Article

Serratia nevei 9rpt1, a potential microorganism for phosphorus recovery

ABSTRACT

Aims: Phosphorus (P) is an essential element for life, finite and irreplaceable. Its constant exploration on a global scale has motivated frequent alerts regarding an eventual crisis due to the shortage of this nutrient. However, it is possible to recycle it and reintroduce it into the ecological cycle. One viable alternative is the microbial recovery of phosphate.
Study design: This study is based on systematic bioprospection of bacteria in phosphate-deficient Amazon regions.
Place and Duration of Study: Bacteria were isolated from black water samples, collected in the Rio Pretinho, located at Serra do Aracá, Barcelos, Amazonas, Brazil, from January to July 2019.
Methodology: Microbial isolation was performed in Luria Bertani agar medium. For the genomic study, the isolate with the best performance in the phosphate uptake test was chosen. The WGS was carried out in a Illumina HiSeq 2500 System. The assembly of the draft genome was carried out with the SPAdes and the annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP).
Results:*Serratia nevei* 9rpt1 recovers 90% of the phosphate available in the culture medium. Its draft genome comprises 5.4 MB, the GC content is 59.52% and 4,922 coding sequences were identified, among these, two pst operons: one complete, containing the five pst genes and one missing *pst*S, *pst*C and *pho*U genes.
Conclusion: *Serratia nevei* 9rpt1, isolated from an Amazonian environment poor in phosphate, is very efficient to uptake this nutrient in a P_i starvation condition. The genomic findings revealed this strain has an additional high affinity P_i uptake *pst* system containing the ATP-binding protein PstB, the canonical permease PstA, a putative permease other than PstC, upstream of the PstA and two essential enzymes in the polyphosphate metabolism: polyphosphate kinase 1 and exopolyphosphatase.

Keywords: Phosphate-specific transport, phosphate regulon, polyphosphate, orthophosphate.

1. INTRODUCTION

Phosphorus (P) is an essential element in the composition of major biomolecules such as proteins, carbohydrates, lipids and nucleic acids. It is also important for the metabolism of carbohydrates, as well as energy stores such as ATP and polyphosphate [1]. The main use of phosphorus is for the production of fertilizers and the industrial agriculture had an important impact on the ecological cycle of this element [2]. There is no substitute for phosphorus in nature. However, unlike petroleum, phosphorus can be recycled. In bacteria, inorganic phosphate (P_i) is transported into the cell by the constitutively expressed PitA low-affinity transporter, however, at low P_i concentrations the high-affinity Pst system (*pst*SCAB) is expressed by the pho *regulon* [3]. The PstSCAB transporter is well characterized in *Escherichia coli* and *Pseudomonas aeruginosa*, but less documented for bacteria of the *Serratia* genus.Lee et. al. (2009) published a partial operon containing only three genes, *pst*A, *pst*B and *pst*C. Considering the limitation of the methodology used by authors, only the incomplete operon was reported. With the massive sequencing technology, bacterial genome sequences are readily obtained and the analyses of such data shown some bacteria have more than one copy of the *pst* operon, one of these containing all five genes and the second one missing some genes.

Considering bacteria is able to store phosphate in polyphosphate granules, the microbial phosphorus recycling strategy is being extensively tested. Our strategy is based on the null hypothesis that nutrient-poor environments should select microorganisms harboring efficient absorption systems. Amazon black water river (and lakes) are the best place to test this hypothesis. Rio Pretinho is a tributary of the Rio Negro, and the entire Rio Negro basin is considered to have low concentrations of nutrients, such as phosphate, magnesium, and potassium [5].In this sense the present study reports the genomic searches for*Serratia nevei* 9rpt1, driving the discussion to the high-affinity system for phosphate uptake.

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 SAMPLING

Bacteria investigated in this study was collected from Rio Pretinho, located at Serra do Aracá (0.8737N, 63.3397W), Barcelos, Amazonas, Brazil in June 2019. The site is characterized by low nutrient content, slightly acidic water (pH<5) and low electrical conductivity, which reflects the poverty in alkaline elements, alkaline earth elements and nutrients such as phosphorus.

To obtain isolated colonies, the raw water was diluted ten-fold in sterile 1X phosphate-buffered saline (Corning ®, New York, USA). This dilution was used to prepare a serial ten-fold dilutions (10^{-1} – 10^{-3}), and 100 µL of each one were hand plating (Drigalski-spatula technique) in Luria-Bertani agar plates, followed by incubation up to 7 days at 30 ^{o}C. Some of these colonies were random isolated and tested to microdetermnation of remaining phosphorus [6].*Serratia nevei* 9rpt1 showed the best result in this test and was selected to further analysis (data not shown).

2.2 WHOLE GENOME SEQUENCING

Serratia nevei 9rpt1 genomic DNA was prepared with PureLink® Genomic DNA Mini Kit (Invitrogen™ by Thermo-Fisher, USA) and quantified in Qubit™ 2.0 Fluorometer using the dsDNA BR Assay kit (Invitrogen™ by Thermo-Fisher, USA). One microgram of each genomic DNA was sent to Genone Soluções em Biotecnologia (Rio de Janeiro, Brazil) facility. It was purchased short inserts genomic DNA library construction and 2 GB of (150 paired-end reads, Q30>80%) whole-genome sequencing in an Illumina HiSeq 2500 System(Illumina, USA). De novo assembly was carried out with SPAdes genome assembler

(3.15.4) from Pathosystems Resource Integration Center (PATRIC), and the genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The genome and annotation are deposited in the GenBank under the accession number JAMYWQ000000000.

2.3 SEARCHING FOR HOMOLOGY AND PHYLOGENETIC ANALYSES

The searches form homology of *pst*SCAB in the nucleotide databases were carried out using the Based Local Alignment Search Tool (BLASTn)[7] with default parameters. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [8]. The bootstrap consensus tree inferred from 1000 replicates [9] was taken to represent the evolutionary history of the taxa analyzed [9]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches [9]. Initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites: 5 categories +G, parameter = 0.3713. This analysis involved 8 complete *pst* operon nucleotide sequences: *S. nevei* 9rpt1 and. There were a total of 4688 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [10, 11]

3. RESULTS AND DISCUSSION

Serratia nevei is a Gram-negative Gammaproteobacteria, motile and rod shaped, recently reclassified from *Serratia marcescens* subspecies[12]. It is a chemoorganotroph aerobe microorganism found in a broad range of habitat, including water. The are six genomes, so far, deposited in GenBank, ranging from 4,95 MB to 5.45 MB and GC% content raging from 59,2% to 59,7%. *S. nevei* draft genome spans 5,302,788 bp and GC% of 59,52%, consistent with its correlated genomes. It was annoted 5,094 Genes and from these, 4,885 are coding genes and 172 RNAs genes (Table 1). There are 67 coding sequences (CDS) without proteins (defined as pseudo genes) and also 5 CRISPR arrays. The most part of these coding genes are metabolism related (n=968, 19,82%), followed by energy (n=309, 6,33%), protein processing (n=252, 5,16%) and stress response related genes (n=246, 5,04%).

Table 1. Genome assembly and annotation features to *Serratia nevei* 9rpt1

Assembly features	Parameters	Annotation features	Parameters
Contigs	221	Genes Total	5,094
GC content	59.52%	CDS	4,922
Contig L50	15	Coding genes	4885
Contig N50	108,391	tRNA	82
Genome length	5,302,788	Complete rRNA	6, 60, 7 (5S, 16S, 23S)

The *pst* operon is a such kind of stress response system, important to phosphorus uptake under P_i starvation [13].*S. nevei* 9rpt1 harbor one complete copy of this operon and a second one with only *pst*A and *pst*B annotated. The complete copy is found in the contig 11, and it spans 4552 bp of the complement strand (JAMYWQ010000011.1). The five genes are in the canonical operon gene sequence, *i. e.*, *pst*S (1041 bp), *pst*C (957 bp), *pst*A (891 bp),

*pst*B (777 bp) and *pho*U (735 bp). PstS is a periplasmatic substrate-binding protein, while PstA and PstC are proteins responsible by form the permease channel. PstB is a cytoplasmic ATP-binding protein in its function is hydrolyze ATP to support the active transport of phosphate through the membrane. PhoU is important in the signaling and regulatory process, however the detailed mechanism is still unknown [14].

The complete *pst* operon is very similar to others found in *Serratia* spp., sharing at least 98% of identity with *S. marcescens,* 97% with *S. ureilytica*, *S. surfactantfaciens*, *S. nematodiphila*, among others, and around 90% with *S. liquefaciens*, *S. quinovarans*, *S. rubidaea* and *S. odorifera*. The identity between *S. nevei* 9rpt1 *pst* operon and *E. coli* is around 80% (~96% of Query Coverage) and poor similarity when searching against *Pseudomonas* spp (identity < 80%; Query Coverage < 15%). The phylogenetic tree illustrates these findings, showing in the upper branch the group formed by *S. nevei* and *S. marcescens* and towards the base, the species with less similarity (Fig. 1).

Fig. 1. Phylogenetic analyses applied to the evolutionary history of *Serratia* sp. pst operon. The numbers arranged close to the nodes of common ancestry represent the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test.

In the incomplete operon, *pst*S, *pst*C and *pho*U genes are missing (JAMYWQ010000026.1). As stated early, a similar pattern was reported in *S. marcescens* [4], however in their work *pst*B and the two permeases genes (*pst*A and *pst*C) were found. Even missing PstS and PhoU, the remaining proteins are able to form a viable partial *pst* system. In*S. nevei* 9rpt1 the incomplete *pst* operon is composed by *pst*B, *pst*A and one ABC transporter permease subunit, similar to WP_013814090.1. This findings suggest that if active, the putative permease can replace the function of missing PstC, creating a functional channel able to transport P_i into the cell. The hypothesis of a second functional transport complex is corroborated by the polyphosphate kinase 1 gene (*ppk*1), annotated upstream of the putative ABC transporter permease subunit.

Polyphosphate kinase (EC number 2.7.4.1) is essential in the anabolic pathway of polyphosphate, under favorable conditions, this enzyme catalyzes the polymerization of polyphosphate from ATP [15], a storage of energy and phosphate. Two exopolyphosphatase (EC number 3.6.1.11) were also found in the *S. nevei* 9rpt1 genome. This enzyme is important in the catabolism of polyphosphate when under adverse condition, bacteria is able to mobilize energy and orthophosphate from this biopolymer [16].

4. CONCLUSION

Serratia nevei 9rpt1 has all genes necessary to recover P_i from the environment even in low concentrations. Once inside the cell and under favorable conditions, this strain stores orthophosphate in polyphosphate granules, and when necessary, is able to catabolize the polymer as a source of energy and phosphate.

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