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Short communication

Strain *Serratia* sp. S119: A potential biofertilizer for peanut and maize and a model bacterium to study phosphate solubilization mechanisms

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ABSTRACT

Strain *Serratia* sp. S119 is a peanut native bacterium with high phosphate solubilizing activity that promotes the growth of peanut and maize in the cultivation area of Córdoba in Argentina. The aims of this study were to obtain and analyze the genome sequence of *Serratia* sp. S119 to understand the genetic basis of its beneficial properties on plant growth, and to demonstrate phosphate solubilizing ability in early stages of bacterial growth. Results obtained indicated that soluble P and gluconic acid were detected during exponential growth phase in bacterial supernatant. Analysis of the genome sequence of *Serratia* sp. S119 obtained from this study showed the presence of genes related to several plant growth promoting traits. The genome sequence of this strain is a valuable source of information to study bacterial response to phosphate starvation and to investigate interaction between this bacterium with host plants under nutritional deficient environments.

1. Introduction

Phosphorus (P), next to nitrogen, is the second essential macronutrient required for plant growth. Less than 1% of the total phosphorus (P) in soil is considered available to plants. Plant-available P occurs as soluble, inorganic phosphate ($H_2PO_4^-$/HPO_2^-) in the soil solution at concentrations less than 10 μM (Raghothama and y Karthikeyan, 2005). In leguminous crops, the symbiotic nitrogen (N) fixation requires high amount of P due to the high energy requirement which is mainly contributed through ATP (Jung et al., 2002). Under P sufficient conditions, nodules have a higher P concentration (up to 1.5% of the total plant P) as compared to that of the shoots and roots (Schulze et al., 2006). In the peanut cultivation area of Córdoba (Argentina), maize crop is used in rotation with this legume. In this area, low soil P content has been reported showing critical values for peanut and maize Sainz Rozas et al. (2012).

In nature P exists in a variety of organic and inorganic forms, mainly in insoluble or very poorly soluble forms. Soil microorganisms are involved in a range of processes of soil P transformation and thus influence its subsequent availability to plant roots (Richardson, 2001). Plant growth promoting bacteria (PGPB) are bacteria which directly or indirectly promotes the growth of the plants. They can be found in the rhizosphere, where the effect of roots and its exudates is found, or inside plant tissues (endophytes) (Ditta and Khalid, 2016).

Within PGPB, phosphate solubilizing bacteria (PSB) are of great interest considering low P availability in agricultural soils. PSB release soluble P to plants improving their growth and development. Bacterial mineral phosphate solubilization is a mechanism widely associated with the production of low-molecular-weight organic acids, mainly gluconic and 2-cetogluconic acids (Goldstein, 1995; Kim et al., 1997; Rodríguez et al., 2006). Gluconic acid (gluconate) production is considered the major mechanism of phosphate solubilization by soil bacteria. Gluconate is produced by the direct extracellular oxidation of glucose by glucose dehydrogenase (Gcd) and pyrroloquinoline quinone (PQQ) cofactor codified in a gene cluster (*pqq*A-F) (Choi et al., 2008).

The genus *Serratia* is member of Enterobacteriacea family, and the

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type specie is *Serratia marcescens* (Grimont and Grimont, 2006). *Serratia* genus is widespread in natural environments, and some *Serratia* strains have been associated with nosocomial infections (Mahlen, 2011; Iguchi et al., 2014). Nonetheless, several *Serratia* strains are also reported to promote plant growth by different and diverse mechanisms (synthesis of phytohormones, secretion of exoenzymes, production of siderophores or by induction of systemic resistance) (Selvakumar et al., 2008; Strobel et al., 1999; Jeong et al., 2015; Singh and Jha, 2016; Taurian et al., 2010).

Plant-associated *Serratia* comprise both endophytes and free-living species in the rhizosphere (Khan et al., 2017). Even though several *Serratia* strains have been described as phosphate solubilizers (Perez et al., 2007; Farhat et al., 2013; Glick, 2012; Lavania and Nautiyal, 2013; Sindhu et al., 2014), no native Cordoba strains have been yet characterized. The strain *Serratia* sp. S119 was isolated from peanut root nodules and belongs to a bacterial collection obtained from peanut (*Arachis hypogaea* L.) plants from the producing area of Cordoba, Argentina. *Serratia* sp. S119 exhibits a strong *in vitro* ability to solubilize inorganic and organic phosphates (Taurian et al., 2010; Anzuay et al., 2013, 2017), presents an inhibitory effect on the growth of the fungal peanut pathogen *Sclerotinia sclerotiorum* (Taurian et al., 2010) and promotes the growth of peanut (Taurian et al., 2010, Anzuay et al., 2015; Ludueña et al., 2016) and maize (*Zea mays* L.) plants (Ludueña et al., 2016) under controlled growth conditions. In addition, *Serratia* sp. S119 demonstrated to grow well in plate assays using media supplemented with the herbicides, insecticides and fungicides usually applied in peanut and maize crops (Anzuay et al., 2017). Unlike pathogenic clinical *Serratia* strains which present several antibiotic resistances, *Serratia* sp. S119 presents only resistance to chloramphenicol (30 μg ml^{-1}) (Ludueña et al., 2016).

The fact that other bacteria than rhizobia, like *Serratia* strains, can be isolated from peanut nodules may indicate that the association between legume and other endophytic beneficial bacteria can promote plant's growth even though the mechanisms used by them are yet not well understood (Ibáñez et al., 2009; Ali et al., 2014). The aims of this study were to obtain and analyze the genome sequence of *Serratia* sp. S119 to understand the genetic basis of its beneficial properties on plant growth, and demonstrate phosphate solubilizing ability in early stages of bacterial growth. Genome sequence of *Serratia* sp. S119 is a source of information that will permit to study its interaction with peanut and maize plant considering that both are some of the most relevant crops for Argentinian agriculture. Besides, genome information will allow to deepen our knowledge about the secondary mechanisms of phosphate solubilization used by this bacterium.

2. Materials and methods

Serratia sp. S119 (available in the Deutsche Sammlung von Mikroorganismen und Zelkulturen GmbH, deposit No. DSM 105060) was grown and maintained on Luria-Bertani (LB) agar medium or LB broth at 28 °C.

Total DNA was isolated from *Serratia* sp. S119 using phenolic extraction method described by Ausubel et al. (1995) and re-extracted using DNeasy^{R} Blood and Tissue kit (QIAGEN). The DNA concentration was checked on Nanodrop spectrophotometer (ThermoFisher) and by Qubit Fluorometer (Invitrogen). The sample was diluted to 0.2 ng μl^{-1} concentration.

For whole-genome sequencing, the Illumina MiSeq System (Illumina, Inc.) was used. Libraries were generated using Illumina's Nextera XT V2-kit sequencing preparation kit, PCR clean-up kit (Illumina, Inc) was used to clean the fragments and the library was validated using the Bioanalyzer (Agilent). The quantification of library previously obtained was done by qRT-PCR (Peqlab) performing dilution of the purified library until 10^{-5}. Finally, library sequencing was done at the Loewe Center for Synthetic Microbiology, (Marburg, Germany) using an Illumina MiSeq Diagnostics.

Data obtained from sequencing were *de novo* assembled using SPAdes assembler version 3.5.0 (Nurk et al., 2013). For genome annotation, GenDB platform was used (Meyer et al., 2003). All the bioinformatics procedures were realized at Justus-Liebig-Universität Giessen, in Bioinformatics and Systems Biology lab (Giessen, Germany). An average nucleotide identity (ANI) analysis was performed using all complete genome sequences of the *Serratia* genus available in the Ez-BioCloud database (Yoon et al., 2017 http://www.ezbiocloud.net/eztaxon). Core genome analysis was performed using EDGAR (Blom et al., 2016, http://edgar.computational.bio.uni-giessen.de/cgi-bin/edgar_login.cgi) among multiple *Serratia* species.

For the comparative phylogenetic analysis, the sequences of three core housekeeping loci 16S rRNA, *gyr*B and *rpo*D of different *Serratia* species and *Bradyrhizobium japonicum* (as outgroup) were retrieved from NCBI. A phylogenetic tree was constructed based on the concatenated sequences of the three housekeeping genes using the Maximum Likelihood method in MEGA5 workbench (Tamura et al., 2011). The concensus tree was inferred using 100 bootstrap replicates.

Quantification of soluble phosphate released by *Serratia* sp. S119 in the bacterial supernatant was quantified in NBRIP-BPB broth medium (Mehta and Nautiyal et al., 2001) following Fiske and Subbarow (1925) method. At each incubation time, CFU ml^{-1} by drop plate method (Somasegaran and Hoben, 1994) in LB medium and supernatants' pH of each sample were determined. Detection and quantification of gluconic acid produced by bacteria at 6 and 10 h of growth was performed using the kit D-gluconic acid/D-glucono lactone (K-GATE, Megazyme) with a detection limit of 20 μg ml^{-1}.

3. Results

General characteristics of the complete genome of *Serratia* sp. S119 are summarized in Table 1. The Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession MSFH00000000 and showed an output of 3,083,627 reads. The version described in this paper is version MSFH00000000.1. The complete genome of strain S119 comprises a single circular chromosome of approximately 5,131,899 bp with a 30-fold genome coverage. The assembly comprises 1026 contigs, 55 Scaffolds (N50:332.70 kb) and contains 59.85% of GC (Fig. 1).

Even though bacterial strain S119 was previously identified based on partial 16S rRNA gene sequence (Anzuay et al., 2013), the complete sequence of 16S rRNA, *gyr*B and *rpo*D genes were searched in the genome to realize a phylogenetic analysis using the three core housekeeping genes. The phylogenetic tree of these concatenated gene sequences showed that *Serratia* sp. S119 is closely related to *Serratia marcescens* strains (Fig. 2). Strain S119 clustered with *S. marcescens* SM39, *S. marcescens* WW4 and *S. marcescens* CAV1492, being the former the most phylogenetically closed to S119. In order to confirm this result, an ANI analysis was done by comparing the complete genome sequences of several *Serratia* strains. It was observed that *Serratia marcescens* SM39, *Serratia marcescens* CAV1492, and *Serratia marcescens* B3R3 were the most similar to *Serratia* sp. S119 (Table 2). The first two

Table 1
Genome features of*Serratia* sp. S119.

	Serratia sp. S119
Genome size (bp)	5,131,899
GC content (%)	59.85
N° of CDS	4707
N° of Genes	4361
N° of rRNA	10
N° of tRNA	82
N° of plasmid	0
N° of chromosome	1
Site of isolation	Peanut root nodule