Applied Soil Ecology 126 (2018) 107–112

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Short communication

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

Liliana Mercedes Ludueña[a], Maria Soledad Anzuay[a], Jorge Guillermo Angelini[a], Matthew McIntosh[b], Anke Becker[b], Oliver Rupp[c], Alexander Goesmann[c], Jochen Blom[c], Adriana Fabra[a], Tania Taurian[a,*]

[a] Departamento de Ciencias Naturales, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Agencia Postal 3, 5800 Rio Cuarto, Córdoba, Argentina
[b] Loewe Center for Synthetic Microbiology, Philipps-University Marburg, Hans-Meerwein-Str. 6, 35043, Marburg, Germany
[c] Justus-Liebig-Universität Giessen, Bioinformatics and Systems Biology, Giessen, 35392 Giessen, Germany

ARTICLE INFO

Keywords:
Serratia
Complete genome sequence
Plant growth promoting bacteria

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

* Corresponding author.
E-mail addresses: lluduena@exa.unrc.edu.ar (L.M. Ludueña), manzuay@exa.unrc.edu.ar (M.S. Anzuay), jangelini@exa.unrc.edu.ar (J.G. Angelini), matthew.mcintosh@synmikro.uni-marburg.de (M. McIntosh), anke.becker@synmikro.uni-marburg.de (A. Becker), Oliver.Rupp@computational.bio.uni-giessen.de (O. Rupp), Alexander.Goesmann@Computational.Bio.Uni-Giessen.de (A. Goesmann), Jochen.Blom@computational.bio.uni-giessen.de (J. Blom), afabra@exa.unrc.edu.ar (A. Fabra), ttaurian@exa.unrc.edu.ar (T. Taurian).

https://doi.org/10.1016/j.apsoil.2017.12.024
Received 25 August 2017; Received in revised form 21 December 2017; Accepted 26 December 2017
Available online 22 February 2018
0929-1393/

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

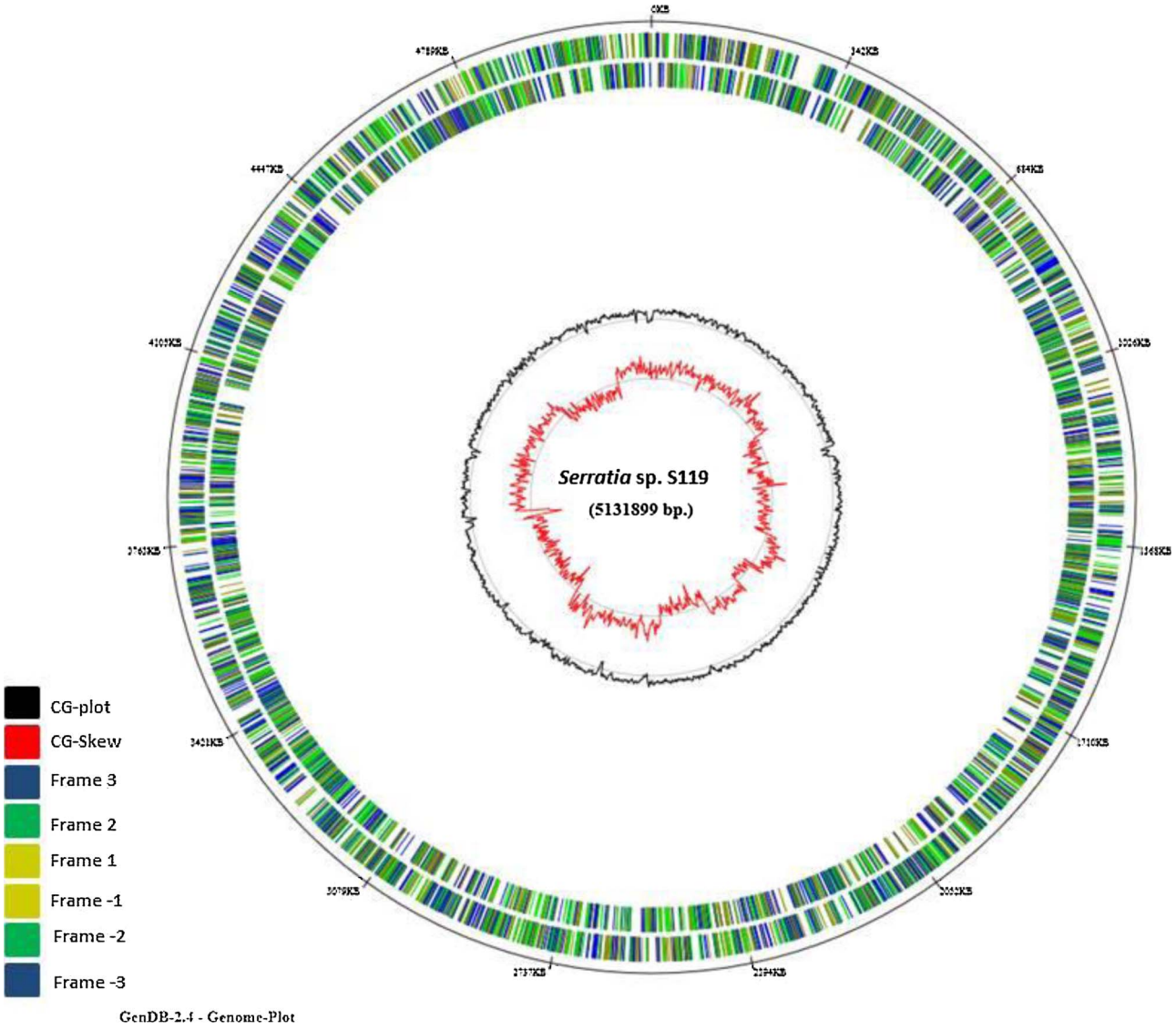

Fig. 1. Graphical circular genomic maps of *Serratia* sp. S119. The red and black circle show GC content (%) and GC skew, respectively. The outer circles show the predicted protein-coding sequences.

strains were obtained from clinical samples (Iguchi et al., 2014) meanwhile B3R3 was obtained from *Zea mays* L. tissues (Wang et al., 2015). These *Serratia* strains (S119, SM39, CAV1492, B3R3) share 3826 CDSs (coding DNA sequences) as shown in the Venn diagram (Fig. 3). *Serratia* sp. S119 shared additional 476, 296 and 110 CDSs with *Serratia marcescens* SM39, *Serratia marcescens* B3R3 and *Serratia marcescens* CAV1492, respectively.

According to previous results, *Serratia* sp. S119 contributes to P availability in soil and benefits the growth of peanut and maize plants (Ludueña et al., 2016; Taurian et al., 2010; Anzuay et al., 2015). This plant growth promotion of *Serratia* sp. S119 has been related to its phosphate solubilization (Ludueña et al., 2016) and phosphate mineralization phenotype (Anzuay et al., 2017). This strain shows maximal concentration of soluble phosphorus released to the supernatant medium during stationary growth phase and this solubilization ability was related to gluconic acid production (Anzuay et al., 2013).

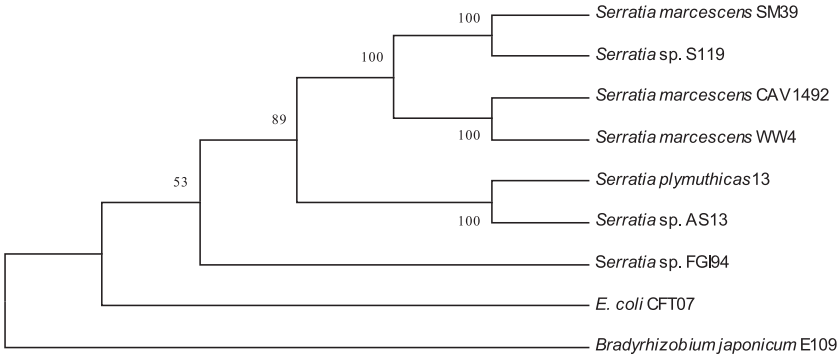

Fig. 2. Phylogenetic tree highlighting the position of *Serratia* sp. S119 with other closely related species within the genus of *Serratia*. The phylogenetic tree was constructed based on concatenated sequences of 16S rRNA, *gyr*B and *rpo*D genes aligned with ClustalW2. The evolutionary history was inferred using the Neighbor-Joining method. Evolutionary analyses were conducted in MEGA5 (Tamura et al., 2011).

Table 2
Phylogenomic overview using average nucleotide identity analysis (ANI) data calculated from whole genome sequences compared to *Serratia* sp. S119 strain by using the online calculator www.ezbiocloud.net/tools/ani.

Bacterial strains	Accession number	Total lenght (bp)	GC (%)	ANI value (%) respect to *Serratia* sp. S119
Serratia sp. S119	MSFH00000000.1	5122074	59.88	100
Serratia marcescens SM39	AP013063.1	5227577	59.82	98.74
Serratia marcescens CAV1492	CP011642.1	5477084	59.08	96.18
Serratia marcescens B3R3	CP013046.2	5471721	59.23	95.34
Serratia marcescens WW4	NC_020211.1	5241455	59.55	95.32
Serratia plymuthica S13	CP006566.1	5467306	56.20	84.55
Serratia sp. FS14	CP005927.1	5249875	56.20	84.50
Serratia sp. AS13	NC_017573.1	5442549	55.96	84.35
Serratia marcescens FGI94	CP003942.1	4858216	58.96	82.96
Serratia fonticola G52	CP013913.1	6100511	53.77	81.17

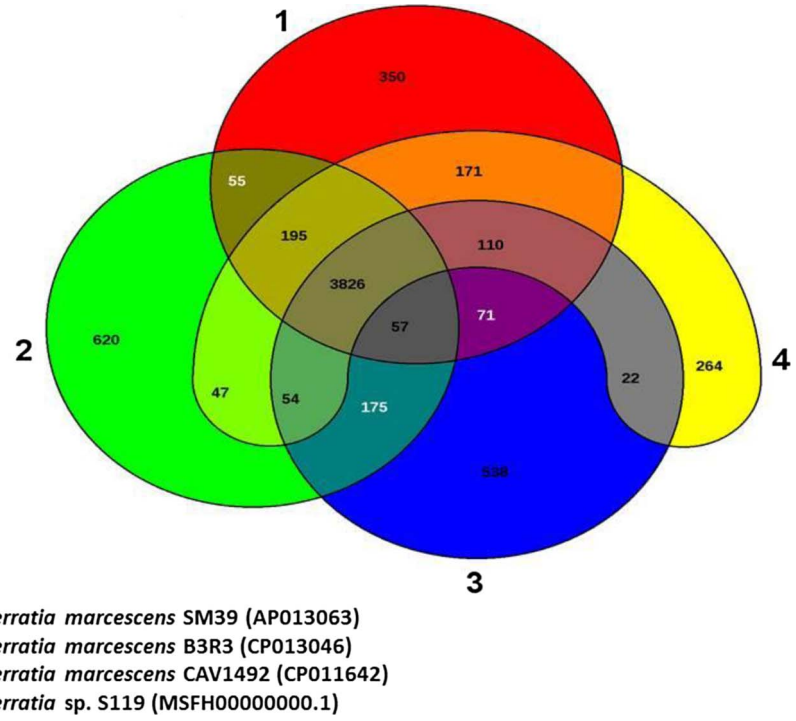

Fig. 3. Venn diagram showing the genes encoded by three *Serratia marcescens* strains and *Serratia* sp. S119. The core genes are those located at the intersection of the four circles.

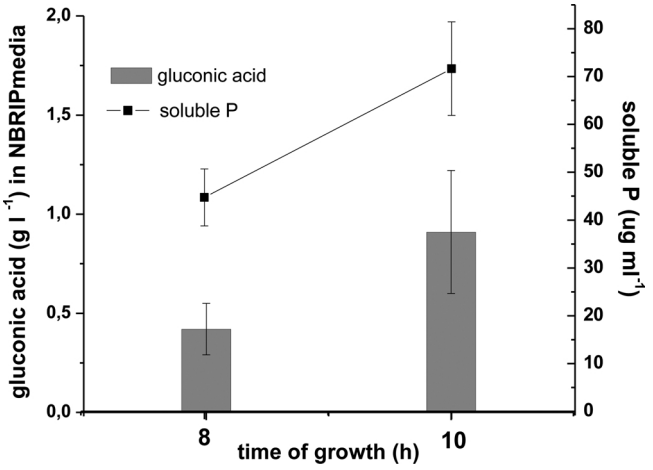

Fig. 4. Gluconic acid production and amounts of P-liberated in NBRIP medium in the peanut associated native bacteria *Serratia* sp. S119 during exponential growth phase (8 and 10 h).

In order to demonstrate phosphate solubilizing ability and gluconic acid production during exponential growth phase, the concentration of this acid and the amount of soluble phosphorus released by this bacterium was determinated at 8 and 10 h of growth in minimal medium NBRIP (Mehta and Nautiyal et al., 2001). Results obtained indicated phosphate solubilization in exponential growth phase by the detection of soluble P released in the bacterial supernatant accompanied by production of gluconic acid (Fig. 4). The increase in this acid concentration was directly correlated with the increase of the phosphorus released into the medium (Fig. 4). Additionally, the pH of medium's supernatant decreased from 3.1 to 2.7 indicating that solubilization of tricalcium phosphate was produced by gluconic acid released.

The genes coding the biosynthesis pathway of gluconic acid; glucose deshidrogenase enzyme and PQQ cofactor, as well as its promoter region were detected in *Serratia* sp. S119's genome sequence (Table 3, Ludueña et al., 2016, 2017). This mechanism was confirmed to be the most important pathway for phosphate solubilization in S119 strain (Ludueña et al., 2016). Nevertheless, it is possible to assume that other secondary mechanisms can be involved in mineral phosphate solubilization of S119 since the PQQ minus mutant presented a significant decrease in gluconic acid production (78%) and in phosphate solubilization (80%) but a null phenotype was not obtained (Ludueña et al.,

Table 3
Accession number and product of the genes involved in the most important plant growth promotion traits present in the genome of *Serratia* sp. S119 strain.

Genename	Accession number	Gene product	Activity
*ipd*C	ONK1668.1	indole-3-pyruvate decarboxylase	synthesis of the phytohormone indole acetic acid (IAA)
*dha*S	ONK20801.1	Indole-3-acetaldehyde dehydrogenase	
*ent*C	ONK16262.1	Isochorismate synthase C	siderophore production
*ent*E	ONK16263.1	Enterobactin synthase subunit E	
*ent*F	ONK16260.1	Enterobactin synthase subunit F	
*ent*B	ONK16264.1	Enterobactin synthase subunit B	
*ent*S	ONK20753.1	Enterobactin exporter	
*exb*D	ONK18797.1	Biolpolymer transporter protein	
*exb*B	ONK19811.1	Biolpolymer transporter protein	
bfr	ONK18112.1	Bacterioferritin	
*bud*A	ONK19677.1	Acetoin decarboxylase	induction of systemic resistance (acetoin synthesis)
*bud*B	ONK19677.1	Acetolactate synthase	
*chi*A	ONK19097.1	Chitinase	chitinase antifungal enzyme
*chi*B	ONK16742.1	Chitinase	
chi	ONK20493.1	Chitinase	
chi	ONK17760.1	Chitinase	
*uxa*A	ONK18862.1	Altronate dehydrolase	plant polymer degradation enzymes
*uxa*C	ONK18863.1	Uronate isomerase	
*bcs*Z	ONK19110.1	Endoglucanase	
–	ONK19677.1	Acetolactate synthase	Acetoin synthesis (volatile compound)
–	ONK18483.1	Acetolactate synthase regulatory	
*bus*A	ONK16701.1	Acetolactate decarboxylase	
*flh*A	ONK20269.1	Flagellar biosynthesis protein	flagella proteins
*flh*B	ONK20268.1	Flagellar biosynthetic protein	
*flh*C	ONK20257.1	Flagellar transcriptional regulator	
*flh*D	ONK20256.1	Flagellar transcriptional regulator	
*flg*B	ONK20276.1	Flagellar basal-body rod protein	
*flg*C	ONK20277.1	Flagellar basal-body rod protein	
*flg*E	ONK20279.1	Flagellar hook protein	
*flg*F	ONK20280.1	Flagellar basal-body rod protein	
*flg*G	ONK20281.1	Flagellar basal-body rod protein	
–	ONK19634.1	Type IV pilin biogenesis protein	type 4 secretion system proteins
*pil*W	ONK17977.1	Type IV pilin assembly protein	
rhs	ONK20221.1	Type IV secretion protein Rhs	
	ONK20850.1	Type IV secretion protein Rhs	
	ONK20852.1	Type IV secretion protein Rhs	
	ONK18185.1	Type IV secretion protein Rhs	
*imp*L	ONK20239.1	Type VI secretion system protein	type 6 secretion system protein
*imp*G	ONK20225.1	Type VI secretion system protein	
*evp*B	ONK20235.1	Type VI secretion protein	
–	ONK20231.1	Type VI secretion protein	
*imp*A	ONK20250.1	Type VI secretion system protein	
–	ONK20241.1	Type VI secretion protein	
tse	ONK20262.1	Methyl-accepting chemotaxis serine transducer	Chemotaxis
tas1	ONK20263.1	Methyl-accepting chemotaxis aspartate transducer	
–	ONK20834.1	Methyl-accepting chemotaxis protein	
–	ONK19053.1	Methyl-accepting chemotaxis protein	
–	ONK17617.1	Methyl-accepting chemotaxis protein	
–	ONK16330.1	Methyl-accepting chemotaxis protein	
cheA	ONK20260.1	Chemotaxis protein	
cheW	ONK20261.1	Chemotaxis protein	
cheR	ONK20264.1	Chemotaxis protein methyltransferase	
cheB	ONK20265.1	Chemotaxis protein response regulator	
cheY	ONK20266.1	Chemotaxis protein	
cheZ	ONK20267.1	Chemotaxis protein. fosfatase	
gcd	ONK20138.1	Glucose dehydrogenase	Gluconic acid (Gluconate) synthesis
*pqq*A	ONK20794.1	Cofactor PQQ biosynthesis protein	
*pqq*B	ONK20795.1	Cofactor PQQ biosynthesis protein	
*pqq*C	ONK20796.1	Cofactor PQQ biosynthesis protein	
*pqq*D	ONK20797.1	Cofactor PQQ biosynthesis protein	
*pqq*E	ONK20798.1	Cofactor PQQ biosynthesis protein	
*pqq*F	ONK20799.1	Cofactor PQQ biosynthesis protein	

2016). The genome sequence of S119 strain constitutes a very useful tool to elucidate the putative secondary mechanisms of phosphate solubilization in the near future.

In addition to its phosphate solubilizing ability, genes related to other plant growth promoting traits like synthesis of plant regulating compounds (IAA), volatile compounds like acetoin, siderophore production, induction of systemic resistance, and production of the antifungal enzyme chitinase were also detected in its genome sequence (Table 3). Genome sequence of *Serratia* sp. S119 contains *ipd*C and *dha*S genes, which encodes indolepyruvate decarboxylase, and indole-3-

acetaldehyde dehydrogenase, respectively. Both gene products are needed for tryptophan dependent IAA synthesis.

On the other hand, chitinase activity, which degrades the fungal wall (and insect exoeskeletons) could explain the inhibition of *Sclerotinia sclerotiorum* growth previously reported (peanut phytopathogen) (Taurian et al., 2010).

As mentioned, *Serratia* sp. S119 is an endophytic strain that colonizes inner tissues of peanut and maize plants (Ludueña et al., 2016). Related to this, genes coding for chemotaxis proteins, plant polymer degradation enzymes, flagella proteins, type 4 and 6 secretion systems proteins were searched and detected in its genome sequence (Table 3).

In this study, it has been determined the genome sequence of *Serratia* sp. S119 and the solubilization of insoluble phosphate by gluconic acid production during exponential growth phase. Additionally, genes related to several plant growth promoting traits as IAA production, acetoin synthesis, siderophore and gluconic acid production were identified. It has been reported that host plants select endophytes that produce IAA (Duca et al., 2014). Considering that this strain contains the genes for IAA synthesis, the growth promotion of peanut and maize observed as a result of S119 inoculation could be explained by the phosphate solubilizing phenotype and the production of IAA.

Finally, the genome sequence of this strain is a valuable source of information to deepen our understanding about bacterial response to phosphate starvation environment, to investigate interaction between this bacterium with host plants, and to study other PGP aspects like the role of IAA production, quorum sensing and motility (features related to endophytic colonization) under nutritional deficient environments.

Acknowledgments

This research was supported by the LOEWE program of the State of Hesse (Germany) and the Max Planck Society. Drs. T. Taurian, M. Anzuay and J. Angelini are members of the Argentinian Scientific Researcher Career-CONICET (National Council of Technological Research). L. Ludueña has a postdoctoral fellowship from CONICET.

References

Ali, S., Duan, J., Charles, T.C., Glick, B.R., 2014. A bioinformatics approach to the determination of genes involved in endophytic behavior in *Burkholderia* spp. J. Theor. Biol. 343, 193–198.

Anzuay, M.S., Frola, O., Angelini, J.G., Ludueña, L.M., Fabra, A., Taurian, T., 2013. Genetic diversity of phosphate-solubilizing peanut (*Arachis hypogaea* L.) associated bacteria and mechanisms involved in this ability. Symbiosis 60, 143–154.

Anzuay, M.S., Ludueña, L.M., Angelini, J.G., Fabra, A., Taurian, T., 2015. Beneficial effects of native phosphate solubilizing bacteria on peanut (*Arachis hypogaea* L) growth and phosphorus acquisition. Symbiosis 66, 89–97.

Anzuay, M.S., Ruiz Ciancio, M.G., Ludueña, L.M., Angelini, J.G., Barros, G., Pastor, N., Taurian, T., 2017. Growth promotion of peanut (*Arachis hypogaea* L.) and maize (*Zea mays* L.) plants by single and mixed cultures of efficient phosphate solubilizing bacteria that are tolerant to abiotic stress and pesticides. Microbiol. Res. 199, 98–109.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., Struhl, K. (Eds.), 1995. Current Protocols in Molecular Biology. Wiley Interscience, New York.

Blom, J., Kreis, J., Spänig, S., Juhre, T., Bertelli, C., Ernst, C., Goesmann, A., 2016. EDGAR 2.0: an enhanced software platform for comparative gene content analyses. Nucleic Acids Res. 44 (W1).

Choi, O., Kim, J., Kim, J.G., Jeong, Y., Moon, J.S., Park, C.S., y Hwang, I., 2008. Pyrroloquinoline quinine is a plant growth promotion factor produced by *Pseudomonas fluorescens* B16. Plant Physiol. 146, 657–668.

Ditta, A., Khalid, A., 2016. Bio-organo-phos: A sustainable approach for managing phosphorus deficiency in agricultural soils. Organic Fertilizers-From Basic Concepts to Applied Outcomes. InTech.

Duca, D., Lorv, J., Patten, C.L., Rose, D., Glick, B.R., 2014. Indole-3-acetic acid in plant-microbe interactions. Antonie Van Leeuwenhoek 106, 85–125. http://dx.doi.org/10.1007/s10482-013-0095-y. PMID: 24445491.

Farhat, M.B., Fourati, A., Chouayekh, H., 2013. Coexpression of the pyrroloquinoline quinone and glucose dehydrogenase genes from *Serratia marcescens* CTM 50650 conferred high mineral phosphate-solubilizing ability to *Escherichia coli*. Appl. Biochem. Biotechnol. 170, 1738–1750.

Fiske, E.H., Subbarow, Y., 1925. The solubilization of phosphate: the action of various organic compounds on dicalcium and tricalcium phosphate. N. Z. J. Sci. Technol. 33, 436–444.

Glick, B.R., 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica 15 2012 Article ID 963401.

Goldstein, A.H., 1995. Recent progress in understanding the molecular genetics and biochemistry of calcium phosphate solubilization by gram negative bacteria. Biol. Agric. Hortic. 12 (2), 185–193.

Grimont, F., Grimont, P.A., 2006. The genus Serratia. The Prokaryotes. Springer, New York, pp. 219–244.

Ibáñez, F., Angelini, J., Taurian, T., Tonelli, M.L., Fabra, A., 2009. Endophytic occupation of peanut root nodules by opportunistic gammaproteobacteria. Syst. Appl. Microbiol. 32 (1), 49–55.

Iguchi, A., Nagaya, Y., Pradel, E., Ooka, T., Ogura, Y., Katsura, K., Sebaihia, M., 2014. Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen. Genome Biol. Evol. 6 (8), 2096–2110.

Jeong, H., Kloepper, J.W., Ryu, C.M., 2015. Genome sequence of rhizobacterium *Serratia marcescens* strain 90–166, which triggers induced systemic resistance and plant growth promotion. Genome Announc. 3 (3) e00667-15.

Jung, S.J., An, K.N., Jin, Y.L., Park, R.D., Kim, K.Y., Shon, B.K., Kim, T.H., 2002. Effect of chitinase-producing *Paenibacillus illinoisensis* KJA-424 on egg hatching of root-knot nematode (Meloidogyne incognita). J. Microbiol. Biotechnol. 12 (6), 865–871.

Khan, A.R., Park, G.S., Asaf, S., Hong, S.J., Jung, B.K., y Shin, J.H., 2017. Complete genome analysis of *Serratia marcescens* RSC-14: a plant growth-promoting bacterium that alleviates cadmium stress in host plants. PloS One 12 (2), e0171534.

Kim, K.Y., Jordan, D., Krishnan, H.B., 1997. *Rahnella aquatilis*, a bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol. Lett. 153, 273–277.

Lavania, M., Nautiyal, C.S., 2013. Solubilization of tricalcium phosphate by temperature and salt tolerant *Serratia marcescens* NBRI1213 isolated from alkaline soils. Afr. J. Microbiol. Res. 7, 4403–4413.

Ludueña, L.M., Anzuay, M.S., Angelini, J.G., Barros, G., Luna, F., Monge, M.P., Fabra, A., Taurian, T., 2016. Role of bacterial pyrroloquinoline quinone in phosphate solubilizing ability and in plant growth promotion on strain *Serratia* sp. S119. Symbiosis 72 (1), 31–43.

Ludueña, L.M., Anzuay, M.S., Magallanes-Noguera, C., Tonelli, M.L., Ibañez, F.J., Angelini, J.G., Fabra, A., McIntosh, M., Taurian, T., 2017. Effects of P limitation and molecules from peanut root exudates on *pqq*E gene expression and *pqq* promoter activity in the phosphate-solubilizing strain *Serratia* sp. S119. Res. Microbiol. 168 (8), 710–721.

Mahlen, S., 2011. *Serratia* infections: from military experiments to current practice. Clin. Microbiol. Rev. 24, 755–791. http://dx.doi.org/10.1128/CMR.00017-11.

Mehta, S., Nautiyal, Ch.Sh, 2001. An efficient method for qualitative screening of phosphate-solubilizing bacteria. Curr. Microbiol. 43, 51–56.

Meyer, F., Goesmann, A., McHardy, A.C., Bartels, D., Bekel, T., Clausen, J., Pühler, A., 2003. GenDB an open source genome annotation system for prokaryote genomes. Nucleic Acids Res. 31, 2187–2195.

Nurk, S., Bankevich, A., Antipov, D., Gurevich, A.A., Korobeynikov, A., Lapidus, A., Stepanauskas, R., 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J. Comput. Biol. 20 (10), 714–737.

Perez, E., Sulbaran, M., Ball, M.M., Yarzabal, L.A., 2007. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol. Biochem. 39, 2905–2914.

Raghothama, K.G., y Karthikeyan, A.S., 2005. Phosphate Acquisition. In Root Physiology: from Gene to Function. Springer, Netherlands, pp. 37–49.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust. J. Plant Physiol. 28, 897–906.

Rodríguez, H., Fraga, R., Gonzalez, T., Bashan, Y., 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287 (1), 15–21.

Sainz Rozas, H., Echeverria, H., Angelini, H., 2012. Fósforo disponible en suelos agrícolas de la región Pampeana y extra Pampeana Argentina. RIA 38 N.º 1.

Singh, R.P., Jha, P.N., 2016. The multifarious PGPR *Serratia marcescens* CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.). PloS One 11 (6), e0155026.

Schulze, J., Temple, G., Temple, S.J., Beschow, H., Vance, C.P., 2006. Nitrogen fixation by white lupin under phosphorus deficiency. Ann. Bot. 98, 731–740.

Selvakumar, G., Mohan, M., Kundu, S., Gupta, A.D., Joshi, P., Nazim, S., Gupta, H.S., 2008. Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (Cucurbita pepo). Lett. Appl. Microbiol. 46 (2), 171–175.

Sindhu, S.S., Phour, M., Choundhary, S.R., Chaudhary, D., 2014. Phosphorus cycling: prospects of using rhizosphere microorganisms for improving phosphorus nutrition of plants. Chapter 11 In: In: Parmar, N., Singh, A. (Eds.), Geomicrobiol. Biochem. Soil Biol. 39. pp. 199–225.

Somasegaran, P., Hoben, H.J., 1994. Handbook for Rhizobia: Methods in Legume–Rhizobium Technology. Section1. pp. 3–6.

Strobel, G., Li, J.Y., Sugawara, F., Koshino, H., Harper, J., Hess, W.M., 1999. Oocydin A, a chlorinated macrocyclic lactone with potent anti-oomycete activity from *Serratia marcescens*. Microbiology 145 (12), 3557–3564.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol (In Press).

Taurian, T., Anzuay, M.S., Angelini, J.G., Tonelli, M.L., Ludueña, L., Pena, D., Ibáñez, F., Fabra, A., 2010. Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329, 421–431.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H.Y., Chun, J., 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA and whole genome assemblies. Int. J. Syst. Evol. Microbiol (in press).

Wang, X.Q., Bi, T., Li, X.D., Zhang, L.Q., Lu, S.E., 2015. First report of corn whorl rot caused by *Serratia marcescens* in China. J. Phytopathol. 163 (11–12), 1059–1063.