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Impact of quorum sensing from native peanut phosphate solubilizing *Serratia* sp. S119 strain on interactions with agronomically important crops

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Abstract

Bacteria communicate with each other using a language based on the use of autoinducer molecules (AI) to collectively control gene expression through the process known as quorum sensing (QS). The *N*-acylhomoserine lactones (AHLs) are the most common AIs known in Gram-negative bacteria. Numerous genes encoding QS systems have been reported in plant beneficial endophytic bacterial genomes although there are fewer studies evaluating the participation of QS in the mechanisms of plant growth promotion. The aim of this study was to describe the genetic background and the types of AHLs produced by a phosphate-solubilizing bacterial strain isolated from the roots of peanut *Serratia* sp. S119, and to evaluate the participation of these AIs in promoting the growth of plants of agronomic importance.

Bioinformatics analysis indicated that the genome of S119 strain harbors genes encoding proteins involved in the production and detection of AHLs. This strain was found to produce the QS molecules C8-HSL, C10-HSL, 3-OH-C8-HSL and 3-OH-C10-HSL with the latter being the most abundant AHL. By overexpressing a heterologous lactonase enzyme in *Serratia* sp. S119, this strain was depleted in the production of all AHLs with the exception of 3-OH-C10-HSL which levels were reduced by 50%. The ability to solubilize phosphate, produce biofilms and promote plant growth was analyzed in the AHL attenuated strain. This strain was able to maintain the ability to solubilize phosphate while biofilm production was significantly reduced. Plant inoculation assays with the QS attenuated strain showed differences responses on growth parameters of the three plants employed.

These results indicate that the AHLs from the phosphate solubilizing *Serratia* sp. S119 strain may not affect its ability to solubilize phosphate. However, they play a role in biofilm formation that is a key trait for bacterial colonization in its interaction with plant species.

Keywords *Serratia* sp. · Quorum sensing · N-acylhomoserine lactone · Peanut · Maize · Soybean

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1 Introduction

Quorum sensing (QS)-mediated cell-cell communication allows populations of bacteria to collectively control gene expression, and thus synchronize group behavior. Several physiological processes in bacteria including bioluminescence, conjugation, symbiosis, virulence and biofilm formation are regulated by QS (Waters and Bassler 2005). In the case of plant growth promoting bacteria (PGPB), QS may be necessary to successfully establish themselves in an ecological niche such as the rhizosphere since they must be able to survive and compete with complex microbial communities. QS is involved in the colonization of the rhizosphere

and rhizoplane of PGPBs, as it plays an important role in root nodulation, protein secretion, antibiotic production, motility and biofilm maturation (Fuqua and Greenberg 2002; Bassler and Losick 2006; Soto et al. 2006). In Gram-negative bacteria, the best-known QS signaling process are based on the use of *N*-acylhomoserine lactones (AHLs), although other signaling chemistries are apparent (Horng et al. 2002). Common features are found in almost all known QS systems in Gram-negative bacteria (Ng and Bassler 2009). AHLs differ in their respective acyl side chains with different strains belonging to the same species having the ability to synthesize different types of AHLs depending on the habitat in which they are found (Eberl 1999). The AHLs QS systems would consist of genes encoding a LuxI AHL signal synthase and a family of LuxR transcriptional regulators which may be present without their *luxI* pair. AHLs binds to their cognate LuxR homologues and in turns activate target gene expression (Parsek and Greenberg 2000).

Some organisms have developed strategies to disrupt QS signaling from other microorganisms. This phenomenon is called quorum quenching (Barrios et al. 2009) and generally leads to the inactivation and /or degradation of AIs (Zhu and Kaufmann 2013). This strategy avoids the sufficient accumulation of AI in the surrounding environment of the microorganisms, preventing the initiation of QS-mediated processes (Medina- Martínez et al. 2007). In the case of the degradation of AHLs, some of the most studied enzymes responsible are the lactonases, that break the lactone ring, and the acylases, that break the acyl side chain (Rashid et al. 2011). N-acylhomoserine lactonase (AiiA) dependent signal degradation is a particularly useful tool to study the impact of quorum sensing in Gram-negative bacteria having AHL regulatory circuits without the need to make mutants in the different AHL synthase genes (Reimman et al. 2002).

The genus *Serratia* belongs to the Enterobacteriaceae. In some strains of the *Serratia* genus the production of prodigiosin, carbapenem, pectate lyase and cellulose, motility and biofilm are controlled by QS systems (Thomson et al. 2000; Horng et al. 2002; Van Houdt et al. 2007b; Morohoshi et al. 2007; Liu et al. 2011; Sakuraoka et al. 2019). A variety of AHLs-type molecules have been described in different strains of *Serratia* spp. (Wei and Lai 2006; Van Houdt et al. 2007a). The phenotypes regulated by AHLs in *Serratia* species are remarkably diverse and of profound biological and ecological importance (Liu et al. 2011). There are however, limited studies evaluating the involvement of QS on direct plant growth promotion mechanisms (Boyer et al. 2008) and the role of AHL-mediated QS systems in endophytic strains of *Serratia* associated with plants (Liu et al. 2011; Kandel et al. 2017) found that many of the studied endophytic bacterial genomes contained pairs of *luxI/LuxR* type genes participating in QS and indicated their importance in the endophytic lifestyle. These QS systems are likely to be involved in communication with the plant root and the subsequent colonization process (Kandel et al. 2017). Thus, the establishment within the rhizosphere, on the root surface and the subsequent infection of the plant tissues could be influenced by QS and with it, the traits associated with these colonization steps such as biofilm formation.

The *Serratia* sp. S119 strain used in this study is a native endophytic bacterial strain isolated from peanut nodule of plants grown in the producing area of Córdoba, Argentina (Taurian et al. 2010). Previous studies demonstrated that this strain stands out for its ability to solubilize significant amounts of insoluble organic and inorganic phosphates in vitro and for promoting the growth of peanut, soybean and maize plants (Anzuay et al. 2013, 2015; Lucero et al. 2021). Toxicity assays demonstrated that this plant growth promoting bacteria does not pose a threat to human health. *Serratia* sp. S119 presents traits associated with endophytic plant colonization such as swimming, swarming and twitching motility, the ability to form biofilms (Lucero et al. 2020) and the production of pectinase and cellulase enzymes (Lucero et al. 2021).

Considering that the role of AHLs in plant-microbe interaction remains poorly understood, this study aims to provide some further insights on the role of AHLs, produced by a phosphate solubilizing bacterial strain isolated from peanut roots, on the promotion of growth from plants of agronomic importance. To address this, we describe the initial characterization of an AHL-mediated QS system in the peanut nodule endophyte *Serratia* sp. S119 using bioinformatics analysis and we identified the nature of the AHLs produced by this organism. Furthermore, considering that S119 strain is an endophytic beneficial plant bacteria, we analyzed the role of AHLs on the interaction with plants. The impact of roots exudates from peanut, maize and soybean on the production of AHLs were also analyzed. Furthermore, the study describes the effect of the exogenous addition of the main AHL produced by *Serratia* sp. S119 on plant growth. Finally, this manuscript shows the impact of attenuating AHL production by the expression of an AHL-degrading lactonase in S119 on its phosphate solubilizing ability, biofilm formation and plant growth promoting properties. These results provide new insights into the importance of QS on plant-microbe interactions and the variations within these.

Table 1 Strains used in the study

Strain	Description	Reference
Serratia sp. S119	Wild type P-solubilizing isolate	Taurian et al. 2010
Serratia sp. S119-pME6863	S119 transformed with the pME6863 vector plasmid	This study
Serratia sp. S119-pME6000	S119 transformed with the pME6000 vector plasmid	This study
E. coli DH5α	F – *recA1 endA1 hsdR17 deoR thi-1 supE44 gyrA96 relA1* D(*lacZYA* ± *argF*) *U169 k*– [*u*80d*lacZ*DM15]	Sambrook et al. 1989
E. coli DH5α-pME6863	DH5 α carries pME6863 plasmid containing cloned gene *aiiA*, Tc^r	Reimmann et al. 2002
E. coli DH5α-pME6000	DH5 α carries pME6000 plasmid containing cloned gene *aiiA*, Tc^r	Reimmann et al. 2002
E. coli HB101/pRK600	HB101 carries Helper plasmid, ColE1, Cm^r	Kessler et al. 1992
Chromobacterium violaceum CV026	Violacein-based biosensor to detect AHL. Smr mini_Tn5 Hg^c*cviI*::*Tn5xy1E* Km^r	McClean et al. 1997
Chromobacterium violaceum VIR 07	ATCC 12,472 derivative; *cviI*::Km^r; Ap^r	Morohoshi et al. 2008
Agrobacterium tumefaciens NT1-pZLR4	*traG-lacZ*-based AHL detection strain	Cha et al. 1998

2 Materials and methods

2.1 Bacterial strains, media and growth conditions

All bacterial strains used in this study are described in Table 1. Bacteria was grown on LB (Tryptone 10 $g.l^{-1}$, yeast extract 5 $g.l^{-1}$, NaCl 5 $g.l^{-1}$, Miller 1972); TY (Tryptone 5 $g.l^{-1}$; yeast extract 3 $g.l^{-1}$; $CaCl_2.6H_2O$ 1.3 $g.l^{-1}$; Beringer 1974); NBRIP (glucose 10 $g.l^{-1}$; $Ca_3(PO_4)_2$ 5 $g.l^{-1}$; $MgCl_2.6H_2O$ 5 $g.l^{-1}$; $MgSO_4.7H_2O$ 0.25 $g.l^{-1}$; KCl 0.2 $g.l^{-1}$; $(NH_4)_2SO_4$ 0.1 $g.l^{-1}$; Mehta and Nautiyal 2001) and NBRIP-Psol (glucose 10 $g.l^{-1}$; KH_2PO_4 3 $g.l^{-1}$; $MgCl_2.6H_2O$ 5 $g.l^{-1}$; $MgSO_4.7H_2O$ 0.25 $g.l^{-1}$; KCl 0.2 $g.l^{-1}$; $(NH_4)_2SO_4$ 0.1 $g.l^{-1}$) media and maintained in 20% glycerol ($v.v^{-1}$) at -80 °C.

The strains with plasmids pME6863 and pME6000 were supplemented with tetracycline (Tc) at a concentration of 25 $\mu g.ml^{-1}$. *Chromobacterium* spp. biosensors were grown in LB medium and supplemented with kanamycin (Km) 100 $\mu g.ml^{-1}$. The biosensor *Agrobacterium tumefaciens* was grown in AB medium (mannitol 2 $g.l^{-1}$; K_2HPO_4 3 $g.l^{-1}$; NaH_2PO_4 1.15 $g.l^{-1}$; NH_4Cl 1 $g.l^{-1}$; $MgSO_4.7H_2O$ 0.3 $g.l^{-1}$; KCl 0.15 $g.l^{-1}$; $CaCl_2$ 0.01 $g.l^{-1}$; $FeSO_4.7H_2O$ 2.5 $mg.l^{-1}$) supplemented with 30 $\mu g.ml^{-1}$ gentamicin (Gm).

2.2 Genome analysis of the AHL quorum sensing-related genes of *Serratia* sp. S119

An *in silico* bioinformatic analysis of QS genes was performed. The genome of *Serratia* sp. S119 (Ludueña et al. 2018) was analysed by alignment using blastn with sequences of AHL QS systems retrieved from the NCBI Genome website (https://www.ncbi.nlm.nih.gov/genome, Zhang et al. 2000; Morgulis et al. 2008) and GenDB data base (Meyer et al. 2003). For this purpose the nucleotide sequences of *lux*I and *lux*R of *Serratia marcescens* SM39 (AP013063.1), *Serratia marcescens* AR-0131 (CP029715.1) and *Serratia marcescens* UMH3 (CP018925.1) were used as probes to search for these genes in the genome of *Serratia* sp S119 strain. The threshold considered for alignment of the sequences corresponded to a score >600 bp since sequences analysed corresponded to ~650 for *lux*I genes and ~600 for *lux*R genes.

The phylogenetic analysis of the amino acid sequence of the LuxI protein from S119 strain in comparison to other *Serratia* strains was performed in line with the study of Sakuraoka et al. (2019). The phylogenetic trees based on LuxI sequences was constructed using the Neighbor-Joining method with the ClustalW of MEGA version 7.0/MEGA 4. Alignments between the nucleotide sequence of LuxI from *Serratia* sp. S119 and the LuxI homologs from other members of *Serratia* were performed, using LuxI from *Allivibrio fischeri* ES114 (AAW87994.1) as outgroup. Confidence in neighbour-joining tree was determined by analysing 1000 bootstrap replicates. The phylogenetic tree based on the whole genome alignments was constructed using REALPHY 1.12 (Bertels et al. 2014).

2.3. Identification of the AHLs produced by *Serratia* sp. S119 and study of the effect of culture medium on their production.

2.2.1 AHL extraction and bioassays

The strain under study was grown in three different culture media in a P limiting minimal media that contains an insoluble P source (NBRIP); the same medium containing soluble P levels (NBRIP-Psol); and rich media (LB). From liquid cultures of the *Serratia* sp. S119 grown in each of the aforementioned media (24 h 28 °C in rotational agitation), a 3 ml aliquot was taken and centrifuged at 7400 g for 5 min. The supernatant (SN) obtained was filtered through a 0.22 µm pore diameter polyethersulfone (PES) membrane. Then 1 ml of SN was taken and mixed with 0.5 ml of acidified ethyl acetate (0.1% ($v.v^{-1}$) acetic acid in ethyl acetate) followed by a stir for 10 min and centrifugation at 1900 g for 3 min to separate the organic and aqueous phases. The organic phase was then extracted with a micropipette and

collected in a new 1.5 ml tube. The extraction with ethyl acetate was repeated twice more on the aqueous phase (SN), and the organic phase collected in the same tube in such a way that a final volume of organic extract of approximately 1.5 ml per ml was obtained. As a last step, the ethyl acetate was evaporated to dryness in a fume hood. Extracts of the culture medium without bacterial inoculum, using the same procedure, were used as a negative control. Samples were extracted in triplicate for each time point/condition. Dried extracted samples were stored at -20 °C.

The initial detection of autoinducers present in the SN and extracts was carried out following the methodology of McClean et al. (1997) with modifications. The biosensors used are described in Table 1. The biosensors tests were carried out in Petri dishes using two different methodologies depending on the biosensor. A bilayer assay was performed using AB medium for *A. tumefaciens* NT1-pZLR4. A lower layer of the 1.5% agar medium was made which, upon setting, was overlayed with 0.7% agar medium to which 10 $\mu l.ml^{-1}$ of the biosensor culture grown to stationary phase and 40 $\mu g.ml^{-1}$ X-Gal were added before plating. Once the upper layer had solidified, holes of approximately 5 mm in diameter were made into which 10 µl of SN or concentrated extract were added.

For both biosensor strains of *C. violaceum*, AHL detection was carried out directly on a plate with 1.5% LB agar medium, in which a streak of the biosensor strain was made and *Serratia* sp. S119 strain was cross-streaked against it or a 5 µl drop of the concentrated extract was added.

2.2.2 Determination of AHLs produced by *Serratia* sp. S119

For the determination of AHLs in bacterial supernatant, Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric (LC-MS/MS) technique was developed (School of Life Sciences, Centre for Biomolecular Sciences, University of Nottingham). *Serratia* sp. S119 was grown in two rich media (LB and TY) and in two minimal media (NBRIP-Psol and NBRIP). The extraction of AHLs molecules was performed in triplicate as described in Sect. 2.3.1. Prior to analysis, each sample was reconstituted in 50 µl of MeOH + 0.1% (v/v) formic acid. The injection volume was 5 µl. Analysis was conducted with the MS operating in MRM (multiple reaction monitoring) mode under positive electrospray (+ ES) conditions, screening the LC eluent for specific AHLs (unsubstituted, 3-oxo and 3-OH AHLs with even chain lengths from C4-C14). Chromatography was achieved using a Shimadzu series 10AD VP LC system. The column oven was maintained at 40 °C. The HPLC Column used was a Phenomenex Gemini C18 column (3.0 µm, 100 × 3.0 mm) with an appropriate guard column. Mobile phase A was 0.1% ($v.v^{-1}$) formic acid in water, and mobile phase B 0.1% ($v.v^{-1}$) formic acid in methanol. The flow rate throughout the chromatographic separation was $450\ \mu l.min^{-1}$. The binary gradient initially began at 10% B for 1.0 min, increased linearly to 50% B over 0.5 min, then to 99% B over 4.0 min. The composition remained at 99% B for 1.5 min, decreased to 10% B over 0.1 min, and stayed at this composition for 2.9 min. Total run time per sample was 10 min. The MS system used was an Applied Biosystems Qtrap 4000 hybrid triple-quadrupole linear ion trap mass spectrometer equipped with an electrospray ionization (ESI) interface. Instrument control, data collection and analysis were conducted using Analyst software. Source parameters were set as curtain gas: 20.0, ion source potential: 5000 V, temperature: 450 °C, nebulizer gas: 20.0, and auxiliary gas: 15.0. AHL Standards used were synthetic standards of C4, C6, C8, C10, C12, C14, 3-oxo-C4, 3-oxo-C6, 3-oxo-C8, 3-oxo-C10, 3-oxo-C12, 3-oxo-C14, 3-OH-C4, 3-OH-C6, 3-OH-C8, 3-OH-C10, 3-OH-C12 and 3-OH-C14 HSLs were synthesised at the University of Nottingham. The RT data and mass characteristics were based on the methodology described by Ortori et al. (2014).

2.4. Effect of root exudates and culture medium on the production of AHLs by *Serratia* sp. S119.

To study the effect of root exudates on the production of AHLs by *Serratia* sp. S119, this strain was grown in the presence of extracts obtained from roots of peanut, maize and soybean plants. The plant root exudates were extracted following the methodology described by Lucero et al. (2020). The analysis was carried out growing the bacteria in the three media used to study AHLs production levels (LB, NBRIP-Psol and NBRIP) supplemented with each RE to a final 1X concentration. The characterization of the AHLs released into de medium was carried out on the supernatants of the liquid cultures of the strain, by quantitative assay performed by LC-MS/MS as described previously.

2.3 Participation of AHLs on biofilm production, phosphate solubilizing capacity and plant-growth-promotion by *Serratia* sp. S119

A lactonase gene was introduced into *Serratia* sp. S119 to attenuate its AHL production with the purpose of analyze the participation of this signal molecules on its biofilm production, phosphate solubilization and plant growth promotion on peanut, maize and soybean plants.

2.3.1 Heterologous expression of the N-acylhomoserine lactonase (AiiA)

A quorum-quenching approach was used to attenuate AHL production in *Serratia* sp. S119. *Escherichia coli*

S17-1-pME6863 carrying the AHL-lactonase *aiiA* gene from the *Bacillus* sp. strain A24 under the control of the constitutive lac promotor was used to mobilize *aiiA* into *Serratia* sp. S119 by conjugation to obtain S119-pME6863 (Reimmann et al. 2002). *Serratia* sp. S119 containing pME6000 was used as a control. Transconjugants were selected on LB plates containing 25 $\mu g.ml^{-1}$ of tetracycline and 40 $\mu g.ml^{-1}$ of rifampicin. Inactivation of the AHLs produced by S119 strain was evaluated by detection with biosensors and further confirmed by LC-MS/MS analysis as described previously.

2.3.2 Biofilm production by *Serratia* sp. S119-pME6863

To evaluate the production of biofilm by *Serratia* sp. S119 strain expressing the *aiiA* lactonase gene, TY and NBRIP culture media were used. The selection of the culture media was made based on those in which the strain presented the highest biofilms production at 24 h of growth (Lucero et al. 2020).

The biofilm formation assay was performed following the methodology described by O'Toole and Kolter (1998). Two hundred µl of a culture ($OD_{620nm} = 0.01$) obtained from a dilution of an overnight culture, were added to each well of 96-well polystyrene microtiter plates. The plates were incubated at 36 °C for 24 h in TY and NBRIP medium. Planktonic cells were gently homogenized and bacterial growth was determined by measuring O.D. at 600 nm. Wells were washed with 200 µl of PBS (phosphate buffered saline) and stained for 15 min with 200 µl of 0.1% crystal violet solution (CV) in 5% ethanol. Each well was then rinsed with water. The retained dye in the adhered cells was resuspended with 200 µl of ethanol: acetone (80:20) solution added to the wells and incubated at room temperature for 30 min. The O.D. at 570 nm of resuspended CV was determined using an Epoch ™ Microplate spectrophotometer. The amount of biofilm produced, based on the number of bacteria contained in each well, was estimated by determining the Biofilm formation Index (BI) from the following formula:

$$BI = O.D._{\cdot 570}/O.D._{\cdot 600}$$

2.3.3 Phosphate solubilizing activity of *Serratia* sp. S119-pME6863 strain

The ability to solubilise insoluble inorganic phosphate by the strain expressing the lactonase gene (S119-pME6863) was analysed in Petri dishes containing NBRIP medium. For these, 10 µl of liquid cultures of *Serratia* sp. S119, S119-pME6000 and S119-pME6863 grown to stationary were added to the plates. Plates were incubated for 72 and 144 h at 28 ° C. The formation of a translucent halo around the colonies indicated the ability to solubilize phosphate. The area of the halo expressed in mm^2 was measured.

2.3.4 Plant growth-promotion by *Serratia* sp. S119-pME6863 and exogenous AHLs on peanut, maize and soybean

The impact on growth promotion of *Serratia* sp. S119-pME6863, the wild type strain and the exogenous application of synthetic AHL was analyzed. Seeds of *Zea mays* ACA470, *Glycine max* NS4309 (Nidera) and *Arachis hypogaea* L. (var. Granoleico), were surface disinfected and pregerminated in Petri dishes containing moist cotton and Whatman N°1 filter paper and incubated at 28 °C in the dark. Pregerminated seeds were transferred to pots of 30 cm diameter and 25 cm high, containing a sterile sand and vermiculite mix (2:1). The pots were supplemented with 0.2% $Ca_3(PO_4)_2$ (Rivas et al. 2007) as the only source of P. Seven days old, seedlings were inoculated with 3 ml of each bacterial culture (~ 10^9 $CFU.ml^{-1}$) in the root crown. Uninoculated plants and plants fertilized with KH_2PO_4 (20 mM), as a source of soluble P, were used as control treatments. Plants were grown in a microcosm with temperature conditions that ranged between 21 and 29 °C and light conditions that corresponded to natural photoperiod of the time of assay. Plants were watered alternately with Hoagland solution without P (Hoagland and Arnon 1950) and water. Experiments were performed with six replicates for each treatment.

To study the effect of AHLs on plant growth, 10 ml of a 100 mM solution of 3-OH-C10-HSL was added on the substrate of uninoculated plants. The addition was performed with a syringe, around the crown of a plant root to guarantee contact with the rhizosphere zone. This application was made weekly to ensure the presence of AHLs molecules throughout the plant growth development. Hoagland nutrient solution (Hoagland and Arnon 1950) was also added to the plants to guarantee their minimal nutritional requirements.

In both bacterial inoculation and AHL application assays, maize plants were harvested at 45 days (V7), soybeans at 50 days (R4) and peanuts at 60 days (R3) post-inoculation (Lucero et al. 2021). The plant growth promoting effect was evaluated by measuring the following parameters: aerial length, aerial and root dry weight, and root volume. Phosphate solubilisation capacity of plant growth substrate was determined at end of the trial using the Das and Debnath (2006) technique. The Fiske and Subbarow (1925) method determined soluble P in supernatants (SN).

2.4 Statistical analysis

Data, previously controlled to comply with the assumptions of normality and homoscedasticity, were analyzed using the software INFOSTAT (Di Rienzo et al. 2018). Comparison of means was conducted using the protected test of Fisher (i.e., LSD), with a significance level of 0.05.

3 Results

3.1 Genome analysis of the AHL quorum sensing-related genes of *Serratia* sp. S119

The bioinformatics analysis indicated that in the genome of *Serratia* sp. S119 strain it was possible to identify genes involved in AHL QS signaling. These included, one synthase gene *luxI*, 16 *luxR*-like genes, one gene related to AHL efflux (*rhtB*) and one putative lactonase gene were found. The AHL synthase gene *luxI* was convergent with one *luxR* gene and other two *luxR*-homologue genes were found in tandem (**Figure S1**). Sequence alignment of each gene found in the genome of *Serratia* sp. S119 with that of the NCBI gene bank indicated that *luxI* and *luxR* (convergent to *luxI*) showed 100% identity with the AHL synthase and the transcriptional regulator/receptor *luxR* genes *of S. marcescens* AR-0131 (Accession number CP029715.1), *S. marcescens* AR-0124 (Accession number CP028946.1), *S. marcescens* UMH3 (Accession number CP018925.1). The *luxI* gene also showed 100% identity with the *luxI* homologue of strain *S. marcescens* BD1b-2wD (Accession number CP075533.1) and the *luxR* gene (convergent to *luxI*) with that of strain *S. marcescens* 1602 (Accession number CP047391.1). Thirteen of the 16 *luxR* genes found in the genome of *Serratia* sp. S119 showed 100% identity with the same genes (based on the genetic context) of *S. marcescens* 1602, *S. marcescens* AR_131, *S. marcescens* AR_124, *S. marcescens* UMH9 (Accession number NZ_CP018923.1) and *Serratia marcescens* 4928STDY738793899.

A phylogenetic analysis of the amino acid sequence of the LuxI protein from S119 strain than other *Serratia* strains was performed considering the study of Sakuraoka et al. (2019). On the basis of the results obtained by these authors which, through the phylogenetic analysis of 25 LuxI sequences of several strains of *Serratia*, divided these LuxI homologs into three classes (I, II and III), the BLAST analysis revealed that the sequence of LuxI of *Serratia* sp. S119 corresponds to Class I **(**Fig. 1**)**. The amino acid sequence of S. *marcescens* BD1-b2WD was included in the analysis because the BLASTN analysis of the nucleotide sequence indicated 100% identity with that of S119 strain. Analysis of the arrangement of the pair *luxI/luxR* in the genome of *Serratia* sp. S119 indicated that it was placed downstream of gene homologs encoding the lipopolysaccharide export system permease of LptFG and the fucose permease FucP (**Figure S2**).

3.2 Identification of the AHLs produced by *Serratia* sp. S119 and effect of the culture medium on their production

To establish whether S119 produce AHLs we initially used the biosensors *C. violaceum* VIR07, *C. violaceum* CV026 and *A. tumefaciens* NT1-pZLR4 (Table 1). The results indicated that *Serratia* sp. S119 was able to produce detectable AHL levels using the *A. tumefaciens* NT1-pZLR4 and *C. violaceum* VIR07 biosensors (Fig. 2 A **and B**). Regarding the data obtained with the biosensor *C. violaceum* CV026, violacein production (purple colour) was only observed when concentrated extracts of the supernatant were used (Fig. 2 C).

The type of AHL molecules and the relative levels of each was determined by LC-MS/MS. *Serratia* sp. S119 produced four different types of AHL molecules: C8-HSL, C10-HSL, 3-OH-C8-HSL and 3-OH-C10-HSL, being the last the one produced in greater proportion (Fig. 3).

Although for all AHLs there was a tendency to produce slightly higher production of AHLs in minimal media, this was only statistically significant for the two hydroxyl substituted AHL molecules. The enhancement observed in AHL production on minimal media was independent of the source of P used (Fig. 3B).

3.3 Effect of root exudates and culture medium on the production of AHLs by *Serratia* sp. S119

A differential effect of RE was observed on the production of some AHLs by S119 strain. In the case of C8-HSL in LB medium, the addition of REs decreased the production of these molecules to non-detectable levels by the LC-MSMS equipment used. However, in NBRIP and NBRIP-Psol media, RE did not decrease the production of this AHL and, in the case of maize RE, there was an increase in the production of these molecules regardless of the phosphate source used (Fig. 4A). The concentration of C10-HSL in LB medium decreased with the addition of the three RE. The same trend, although without statistically significant difference, was observed in NBRIP-Psol medium. In NBRIP medium, peanut RE increased the production of these molecules (Fig. 4B). Similarly, 3-OH-C8-HSL was not detected when S119 grew in LB medium with the addition of RE from soybeans and maize. In the minimal media, the addition of RE did not produce a significant effect on AHL production (Fig. 4C). Regarding the 3-OH-C10-HSL, which

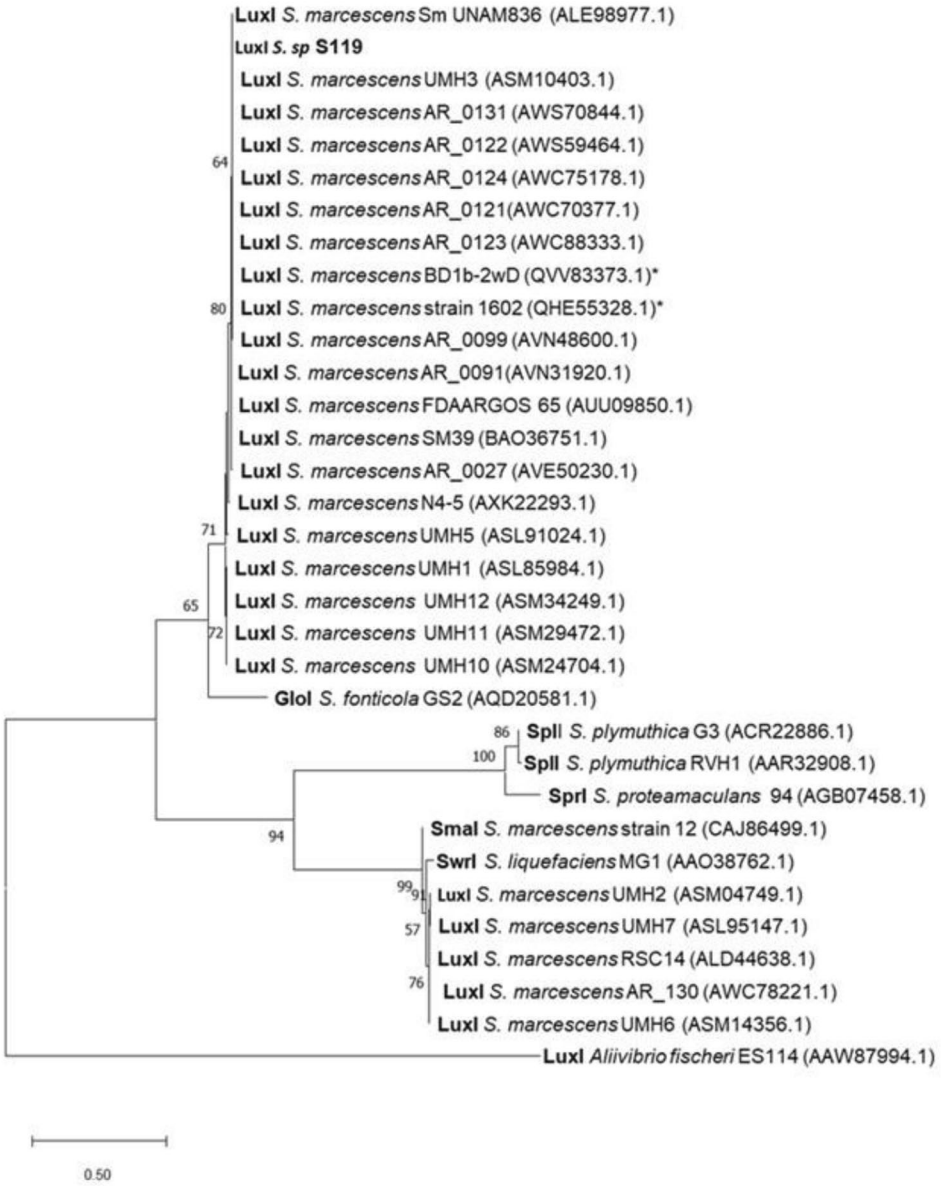

Fig. 1 Phylogenetic tree based on amino acid sequences of LuxI homologs from the genus *Serratia*. The phylogenetic tree was inferred using the Neighbor-Joining method. The optimal tree is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the JTT matrix-based method (Jones et al. 1992) and are in the units of the number of amino acid substitutions per site. The rate variation among sites was modelled with a gamma distribution (shape parameter = 1.36). This analysis involved 34 amino acid sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018)

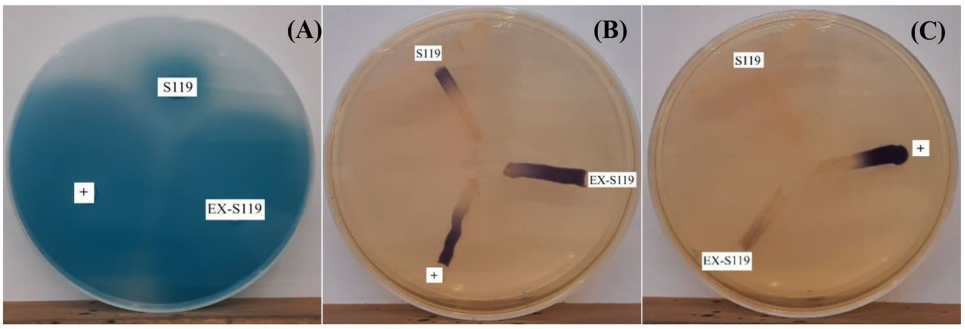

Fig. 2 Production of AHLs-like molecules using the biosensor *A. tumefaciens* NT1-pZLR4 (A); *C. violaceum* VIR07 (B) and *C. violaceum* CV026 (C). References: **S119**: *Serratia* sp. S119; **EX-S119**: 500X Extract of *Serratia* sp. S119; **+**: positive control, C8-AHL standard molecule

were produced in higher concentration by S119 strain, the addition of RE, into both LB and NBRIP media, did not show a significant difference in AHLs levels (Fig. 4D).

With regard to the effect of the culture medium for each of the RE treatments, in the case of C8-HSL, maize RE showed to induce the greatest production in the minimal media by S119 strain, while peanut RE produced this effect

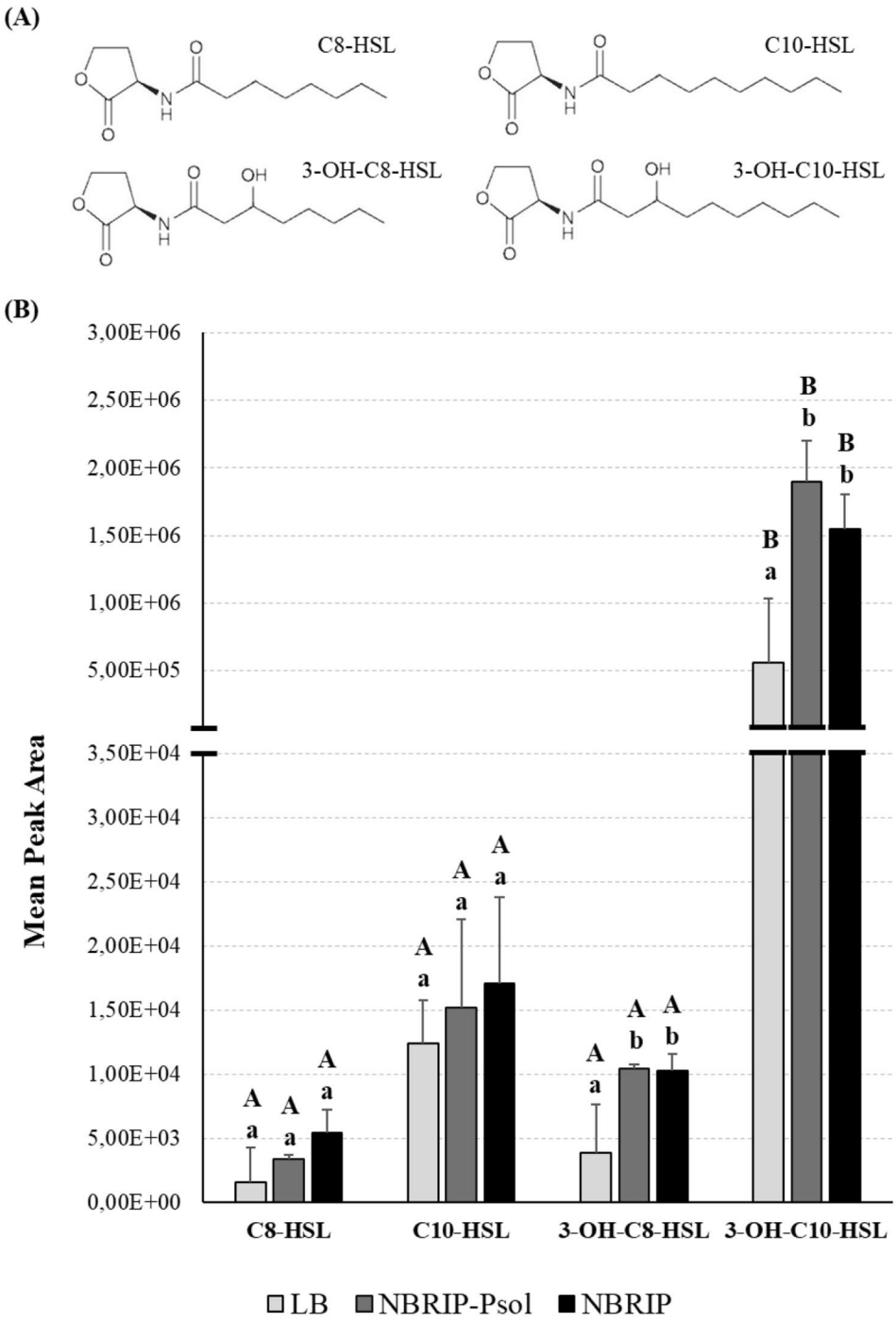

Fig. 3 Types of AHLs molecules synthesized (A) and their production on rich and minimal media (B) by the *Serratia* sp. S119 strain. Data represent mean ± S.E. (n = 3). Different capital letters above indicate significant differences ($p < 0.05$) between AHLs for the same culture medium. Lower case letters below indicate significant differences ($p < 0.05$) between culture media for the same AHLs

in NBRIP medium (Fig. 4 A). Moreover, in the case of C10-HSL, the addition of REs showed the greatest production in NBRIP medium by the bacterial strain (Fig. 4B). The 3-OH-C8-HSL and 3-OH-C10-HSL significantly increased their levels in the two minimal media used than LB rich medium, irrespective of the addition of RE (Fig. 4 C and **D**). For these last two AHLs, it was also possible to observe that the treatments with soybean and maize RE reached the highest values when S119 grew in the minimum medium with soluble P (NBRIP-Psol) (Fig. 4 C and **D**).

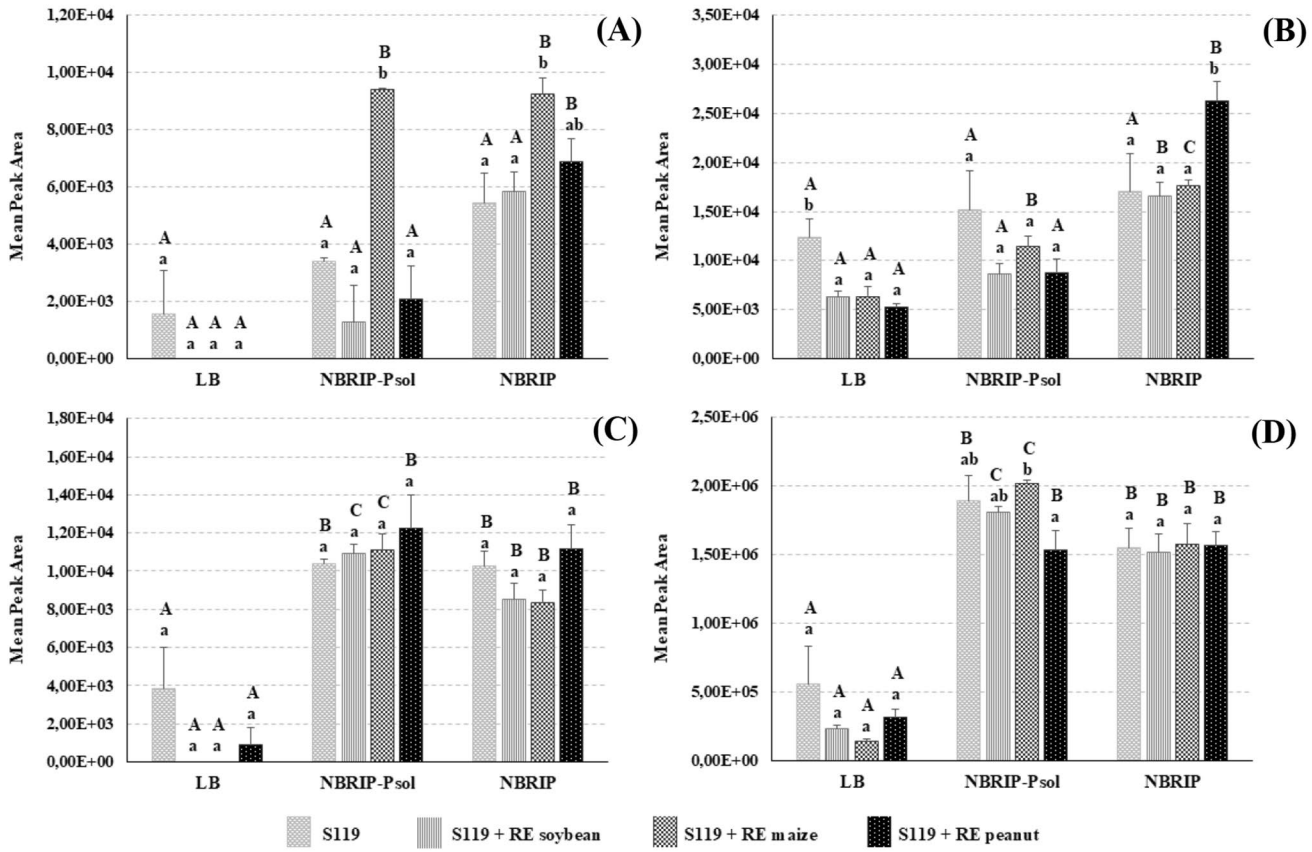

Fig. 4 Determination of the production of molecules C8-HSL (A); C10-HSL (B); 3-OH-C8-HSL (C) and 3-OH-C10-HSL (D) by *Serratia* sp. S119 grown in different culture media with addition of root exudates of soybean (RE soybean), maize (RE maize) and peanuts (RE peanut). Data represent mean ± S.E. (n = 3). Different capital letters above indicate significant differences ($p < 0.05$) between culture media for the same treatment (RE). Lower case letters below indicate significant differences ($p < 0.05$) between treatments in the same culture medium

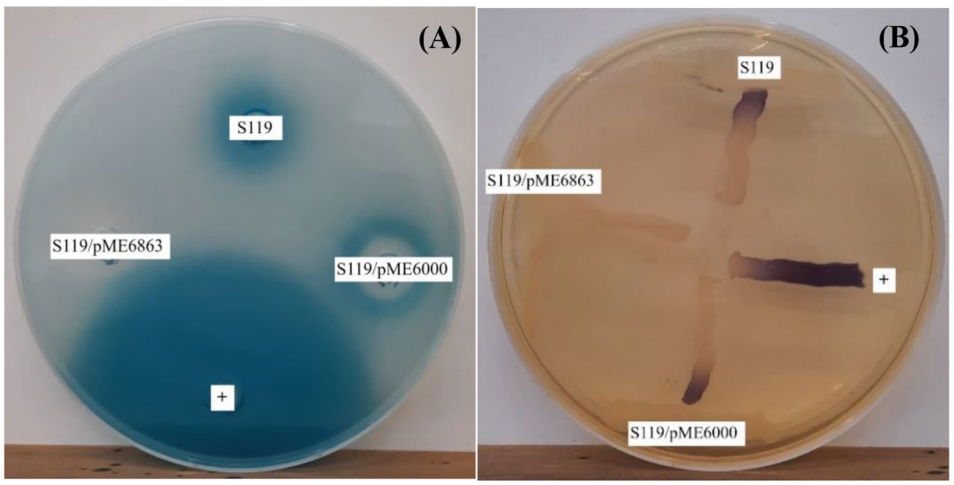

Fig. 5 Impact of AiiA on AHLs production by *Serratia* sp. strain using the biosensors *A. tumefaciens* NT1-pZLR4 (A) and *C. violaceum* VIR07 (B). References: **S119**: *Serratia* sp. S119; **S119-pME6863**: *Serratia* sp. S119-pME6863; **S119-pME6000**: *Serratia* sp. S119-pME6000; **+** : positive control, C8-HSL standard molecule

3.4 Impact of the expression of an exogenous lactonase on the AHL production by *Serratia* sp. S119

Biosensor assays using *A. tumefaciens* NT1-pZLR4 and *C. violaceum* VIR07 indicated no detectable AHL activity by induction of pigment production, when S119-pME6863 strain was exposed to both biosensors (Fig. 5). In contrast,

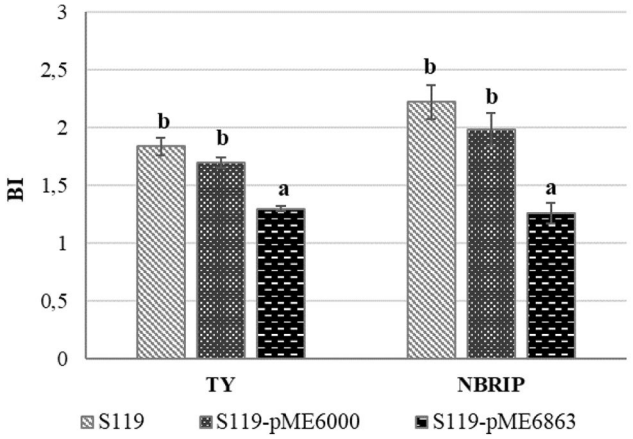

Fig. 6 Biofilm formation index (BI) in TY and NBRIP medium. Data represent mean ± S.E. (n = 24). Different letters indicate significant differences ($p < 0.05$) between treatments in a culture medium. Treatments: **S119**: *Serratia* sp. S119 (wild type); **S119-pME6000**: *Serratia* sp. S119-pME6000; **S119-pME6863**: *Serratia* sp. S119-pME6863

Table 2 Area of the phosphate solubilization halo in solid NBRIP-PBP medium of Serratia sp. S119-pME6863, S119-pME6000 and wild type strain

Strain	Area (mm^2)	
	72 h	144 h
Serratia sp. S119	75.00 ± 3.87 ; **a**	141.56 ± 16.12 ; **a**
Serratia sp. S119-pME6000	67.74 ± 4.73 ; **a**	144.90 ± 11.15 ; **a**
Serratia sp. S119-pME6863	65.87 ± 4.19 ; **a**	130.18 ± 13.39 ; **a**

Data represent mean ± S.E. (n=8). Different letters indicate statistically significant difference ($p < 0.05$) between strains for each of the times studied

biosensor activation was observed by the control *Serratia* sp. S119 WT and S119-pME6000 stains (Fig. 5A and B).

LC-MS/MS analysis on the supernatant from *Serratia* sp. S119-pME6863 detected only production of 3-OH-C10-HSL. The concentration of the AHL detected showed a decrease of 50% compared to the control strain harboring pME6000 (without *aiiA* gene) and the wild type strain. This indicates that expression of *aiiA* leads to a significant attenuation on AHL production in *Serratia* sp. S119 and hence *Serratia* sp. S119-pME6863 could be used to study the impact of AHL production on a number of biological traits.

3.5 Participation of AHLs on biofilm production, phosphate solubilizing capacity and plant-growth-promotion by *Serratia* sp. S119

3.5.1 Impact of AHL attenuation on biofilm production by *Serratia* sp. S119-pME6863

In TY and NBRIP culture media, it was possible to observe that the reduction in AHLs production led to a significant decrease in biofilm production (Fig. 6).

3.5.2 Effect of AHL reduction on phosphate solubilizing capacity by *Serratia* sp. S119-pME6863 strain

The results obtained of qualitative analyzes indicated no significant differences between area of halo formation by *Serratia* sp. S119-pME6863 than that obtained for the wild type and S119-pME6000 strains at both 72 and 144 h (Table 2).

3.5.3 Impact of *aiiA* expression on the plant growth-promotion by *Serratia* sp. S119 on peanut, maize and soybean

Inoculation of *Serratia sp*. S119-*pME6863* on peanut, maize and soybean plants did not show a significant impact on almost all growth parameters analyzed (Fig. 7). It was possible to observe a decrease in the aerial dry weight of soybean plants of *Serratia* sp. S119-pME6863 strain than its control *Serratia* sp. S119-pME6000 strain (Fig. 7 A). On the contrary, for peanut plants there was an increase in aerial dry weight when they were inoculated with S119-pME6863 strain compared to that inoculated with S119-pME6000 strain (Fig. 7 A).

3.5.4 Effect of the addition of exogenous synthetic 3-OH-C10-HSL on the growth of peanut, maize and soybean plants

The addition of this AHL showed to produce a decrease in the root volume of the plants (Fig. 8C). This effect was accentuated in maize and soybean plants that presented lower volume and dry weight of root than fertilized plants (Fig. 8B and C). Furthermore, a decrease in aerial dry weight was observed in soybean plants supplement with the AHL molecules (Fig. 8A).

4 Discussion

Recent studies evaluated the participation of quorum sensing (QS) on the direct mechanisms of plant growth promotion (Hanif et al. 2020; Jung et al. 2020; Kandel et al. 2017)

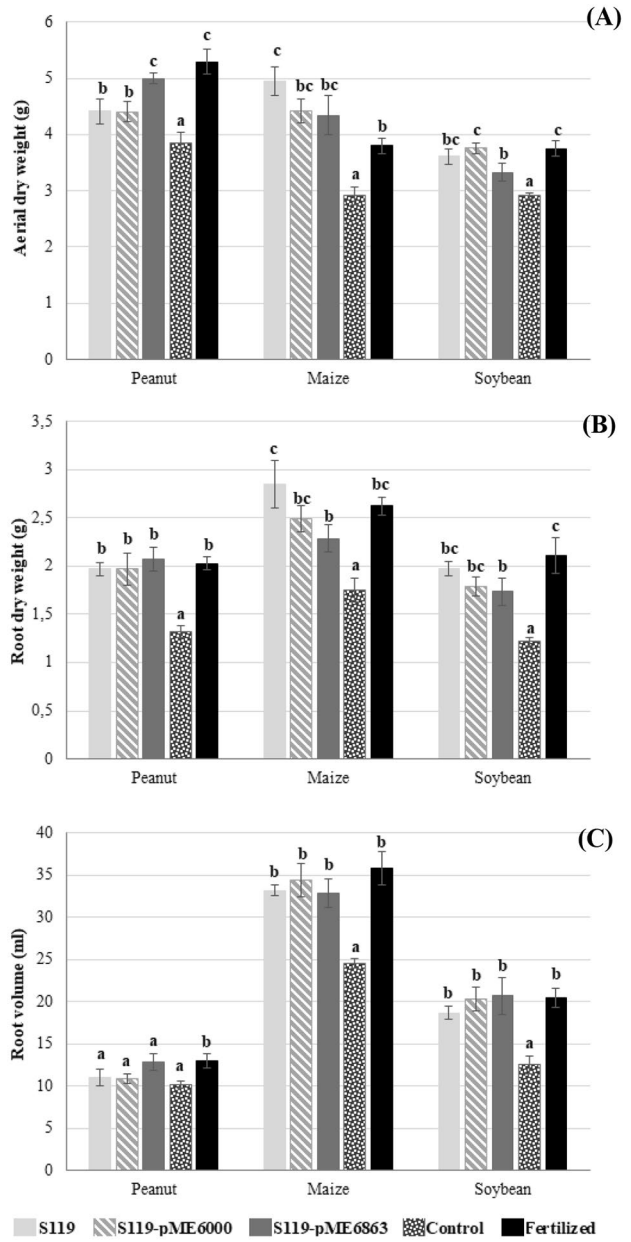

Fig. 7 Aerial dry weight (A); root dry weight (B) and root volume (C) of peanut, maize and soybean plants inoculated with ***Serratia*sp. S119**; **S119-pME6000 and S119-pME6863. Control**: Plants not inoculated and not fertilized with phosphorus; **Fertilized**: Phosphorus fertilized plants (solution 20mM KH_2PO_4). Data represent mean ± S.E. (n = 6). Different letters indicate statistically significant differences ($p < 0.05$) between treatments within the same plant species

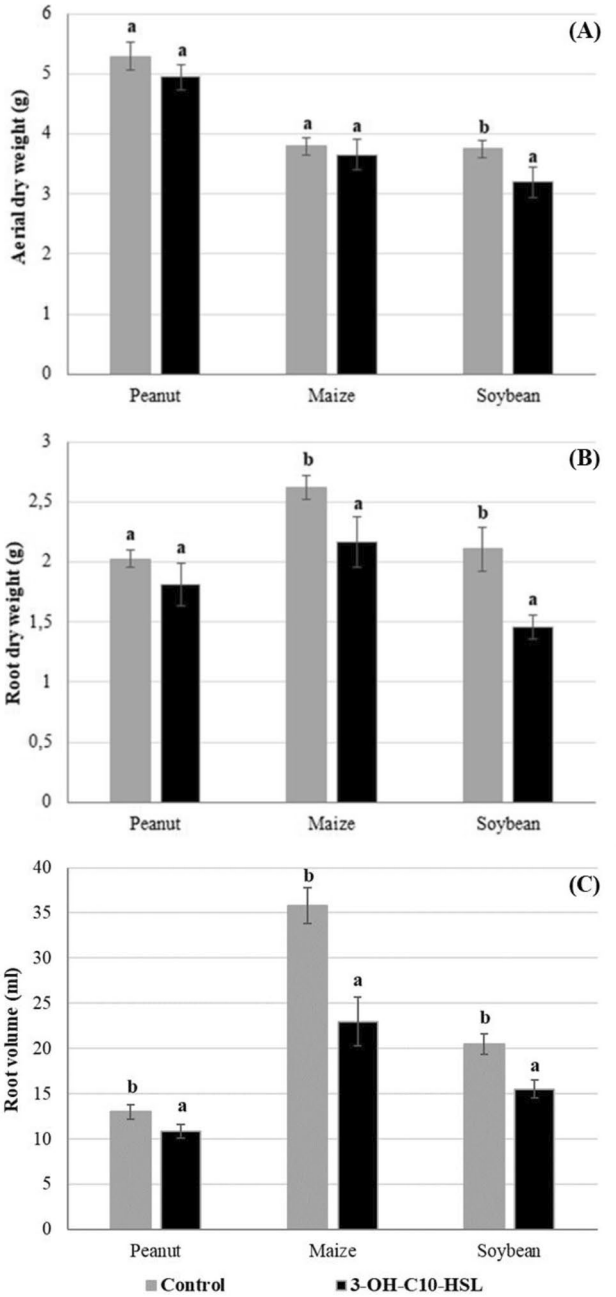

Fig. 8 Impact of exogenous addition of 3-OH-C10-HSL on plant growth. Aerial dry weight (A); root dry weight (B) and root volume (C) of peanut, maize and soybean plants supplemented with 3-OH-C10-HSL. **Control**: plants without AHL addition: Data represent mean ± SE (n = 6). Different letters indicate statistically significant differences ($P < 0.05$) between treatments within the same plant species

found that many of the endophytic genomes studied contained pairs of *luxR/luxI* genes and pointed to their importance in the endophytic lifestyle. In the genome of *Serratia* sp. S119 genes associated of AHLs were found. LuxR and LuxI-like proteins have been studied in many different bacterial species and in many cases, they are adjacent (Abisado et al. 2018). However, some species, including *Salmonella enterica* and *Escherichia coli*, have *luxR* homologues not linked to a *luxI*-type gene (Ahmer 2004). These are commonly referred to as "LuxR solos", as they can detect AHLs and other substrates, but cannot produce their own QS signal (Patel et al. 2013). In *Serratia* spp, at least six different *luxR/I* systems have been described, which include *spsI/R*

and *splI/R* from *S. plymuthica* G3, *sprI/R* from *S. proteamaculans*, *swrI/R* from *S. marcescens* MG-1, *spnI/R* from *S. marcescens* SS-1 and *smaI/R* from *Serratia* sp. ATCC39006 (Wei and Lai 2006; Liu et al. 2011). In the genome of *Serratia* sp. S119, only one LuxI-homologue was found while 16 genes of LuxR proteins were detected. One of them was contiguous to *lux*I gene and in a convergent arrangement.

In relation to the phylogenetic analysis of LuxI homologue and the location of the *luxI* and *luxR*-type genes, the results of this study are in line with what was reported by Sakurakoa et al. (2019). Based on the phylogenetic analysis of the amino acid sequences of several LuxI proteins of *Serratia* strains, these authors grouped them into three classes designated as Class I, II and III. They described that in general, those strains that presented Class I Lux I produced C6 and C8-HSL, those of Class II produced C4 and C6-HSL and those of ClassIII produced C6 and oxo-C6 HSL. In this regard, the phylogenetic analysis of the sequence of LuxI protein of *Serratia* sp. S119 indicated that it belongs to Class I group. In addition, and similarly to the strains of this group, C8-HSL was found to be produced by S119 strain. Moreover, these authors described that in the genome of those strains that encode LuxI of Class I, upstream of the *luxI* and *luxR* genes it was found the *lptF* and *lptG* genes (lipopolysaccharide export system permease) and downstream the *fucP* gene (fucose permease) as it was found in the genome of *Serratia* sp. S119. The analysis of the strains that are part of the same LuxI group (Class I) of *Serratia* sp. S119 showed that some of them also share 99–100% identity with the nucleotide sequence of other genes of this strain (Lucero et al. 2020).

Different types of AHLs molecules produced by bacteria belonging to the genus *Serratia* have been described (Harris et al. 2004). The AHL-type molecules most commonly reported in strains of *Serratia* spp. are *N*-hexanoyl-L-homoserine lactone (C6-HSL), *N*-butanoyl-L-homoserine lactone (C4-HSL) and *N*-3-oxo-hexanoyl-L-homoserine lactone (3-O-C6-HSL) (Van Houdt et al. 2007b). The AHLs produced by S119 strain are not the more conventional 4 and 6 carbon atoms found in *Serratia* genus. It is important to highlight that the 8 and 10 carbons AHLs produced by *Serratia* sp. S119 are less frequently described. In addition, a novel AHL 3-OH-C10-HSL was identified for the first time in this species. This variability could be because in some cases genes of the LuxI/LuxR type have been found in a transposon encoding a mobile genetic element, which can increase its dissemination through horizontal gene transfer (Wei et al. 2006). In addition, the presence of several *lux*R not contiguous to another *lux*I genes in the genome of *Serratia* sp. S119 suggests that probably horizontal gene transfer of them have occurred. This phenomenon would play an important role in the distribution of QS genes between bacterial species (Lerat and Moran 2004).

The plant-bacteria interaction is mainly regulated by the host plant, with root exudates (RE) being the main players in this regulation (Altaf et al. 2017). QS communication can occur between different species, and even between organisms from different kingdoms (Cámara et al. 2002; Barriuso et al. 2008; Barriuso 2017). The addition of RE of peanut, maize and soybean on the culture of *Serratia* sp. S119 generated different responses in the concentrations of the four AHLs produced by this strain. This effect was variable depending on the type of molecule and the culture medium in which the bacteria grew.

The presence of higher levels of AHLs in the rhizosphere compared to non-rhizospheric soil, demonstrates its importance in root colonization (Jha et al. 2018). In some PGPBs, beneficial activities are controlled by these small diffusible signaling molecules that regulate gene expression in response to bacterial population density and interactions with plants (Jung et al. 2017). A recent study indicated that in an *ahlR* mutant of *Pseudomonas savastanoi* pv *glycinea*, resulting in a non-functional QS system, the in vitro phosphate solubilizing capacity was lost (Degrassi et al. 2019). These results suggest that phosphate solubilization would be regulated by QS. Nevertheless, in the present study, the phosphate solubilizing ability of *Serratia* sp. S119 containing pME6863 was not affected. This could be explained by the fact that the remaining levels of 3-OH-C10-HSL produced by this strain would be sufficient to maintain its phosphate solubilizing activity. On the contrary, the biofilm formation capacity of S119 strain expressing the exogenous lactonase was decreased. QS allow bacteria to assess the size and status of their population, and to coordinate the changes in gene expression necessary for the first steps in biofilm formation (Fatima et al. 2010; Angus and Hirsch 2013; Ansari et al. 2017). In the case of the *Serratia* sp. S119 strain, the decrease in the rate of biofilm formation observed when the AiiA enzyme was expressed heterologously, would indicate the importance of AHLs in biofilm formation.

In addition to bacterial communication, a second function for AHL molecules related to the interaction with the plant has been described (Hartmann and Schikora 2012; Schenk et al. 2012; Schikora et al. 2016; Hartmann et al. 2021). The inoculation of the peanut, maize and soybean plants with the lactonase expressing strain showed differential effects on their growth. In general, it was not possible to detect a common behavior pattern in the evaluated growth parameters. The responses of plants to a particular AHL molecule are highly specific and depend on the length of the acyl chain (Schikora et al. 2011; Liu et al. 2012; Schenk et al. 2012, 2014). In their study, von Rad et al. (2008), found

that both C6-HSL and C4-HSL promoted root growth in *Arabidopsis*, while AHL with longer carbon chains had an opposite effect. Other authors also observed that some AHL molecules were able to significantly improve root growth or plant biomass, while others negatively influenced plant growth (Shrestha et al. 2020). These latter studies are consistent with those reported in the present study, where the 3-OH-C10-HSL decreased the parameters associated with the roots in the three plants studied. Furthermore, in soybean plants, a decrease in aerial dry weight was observed. Other studies have shown that 3-O-C10-HSL, stimulated the formation of adventitious roots of *Vigna radiata* seedlings (Bai et al. 2012). In general, the previous reports discussed above suggest that plant responses to AHLs depend on the structure and concentration of AHLs and the recipient plant species (Alabid et al. 2020).

5 Conclusion

Serratia sp. S119 has an active AHL-mediated QS signaling mechanism. In particular, the characterization of AHL produced by this strain indicated that it produces molecules not commonly observed in other *Serratia* strains. Plant root exudates exert a differential effect on the production of AHLs by *Serratia* sp. S119, which in turn, is also influenced by the culture medium used. The AHLs molecules produced by S119 strain may be involved in the formation of biofilms, while the phosphate solubilizing capacity did not show dependence on the concentration of AHLs. Regarding the effect of these bacterial molecules on plant growth, it would be possible to suggest a variable phenotype depending on the plant.

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Declarations

Competing interests The authors have no financial or proprietary interests in any material discussed in this article.

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