Received: 22 June 2023 | Accepted: 22 January 2024

DOI: 10.1111/1751-7915.14420

MICROBIAL BIOTECHNOLOGY Applied Microbiology International

RESEARCH ARTICLE

Potential of the quorum-quenching and plant-growth promoting halotolerant *Bacillus toyonensis* AA1EC1 as biocontrol agent

Amalia Roca[1,2] | **Mónica Cabeo**[1] | **Carlos Enguidanos**[1] | **Fernando Martínez-Checa**[1,2] | **Inmaculada Sampedro**[1,2] | **Inmaculada Llamas**[1,2]

[1]Department of Microbiology, Faculty of Pharmacy, University of Granada, Granada, Spain

[2]Institute of Biotechnology, Biomedical Research Center (CIBM), University of Granada, Granada, Spain

Correspondence
Amalia Roca and Fernando Martinez-Checa, Department of Microbiology, Faculty of Pharmacy, Campus de la Cartuja s/n, Granada, Spain.
Email: amaliaroca@ugr.es and fmcheca@ugr.es

Funding information
Consejería de Universidad, Investigación e Innovación de la Junta de Andalucía and "ERDF A way of making Europe", Grant/Award Number: B-AGR-222-UGR20; Spanish Ministry for Science and Innovation/Agencia Estatal de Investigación, Grant/Award Number: PID2019-106704RB-I00; ERDF A way of making Europe, Grant/Award Number: RYC2019-026481-I

Abstract

The use of fertilizers and pesticides to control plant diseases is widespread in intensive farming causing adverse effects together with the development of antimicrobial resistance pathogens. As the virulence of many Gram-negative phytopathogens is controlled by N-acyl-homoserine lactones (AHLs), the enzymatic disruption of this type of quorum-sensing (QS) signal molecules, mechanism known as quorum quenching (QQ), has been proposed as a promising alternative antivirulence therapy. In this study, a novel strain of *Bacillus toyonensis* isolated from the halophyte plant *Arthrocaulon* sp. exhibited numerous traits associated with plant growth promotion (PGP) and degraded a broad range of AHLs. Three lactonases and an acylase enzymes were identified in the bacterial genome and verified in vitro. The AHL-degrading activity of strain AA1EC1 significantly attenuated the virulence of relevant phytopathogens causing reduction of soft rot symptoms on potato and carrots. In vivo assays showed that strain AA1EC1 significantly increased plant length, stem width, root and aerial dry weights and total weight of tomato and protected plants against *Pseudomonas syringae* pv. tomato. To our knowledge, this is the first report to demonstrate PGP and QQ activities in the species *B. toyonensis* that make this strain as a promising phytostimulant and biocontrol agent.

INTRODUCTION

Diseases caused by plant pathogens constitute one of the main causes that affect the yield worldwide and produce important economic losses (O'Brien, 2017). The most predominant plant pathogenic bacteria are members of the genera *Pseudomonas*, *Ralstonia*, *Erwinia*, *Agrobacterium*, *Pectobacterium* and *Dickeya* (Nazarov et al., 2020).

The wide and excessive use of chemical compounds (fungicides, antibiotics and fertilizers) to control infectious plant diseases has become an important cause of soil and water pollution and other environmental problems, including the development of antimicrobial resistance pathogens (Almasri & Kaluarachchi, 2004). Consequently, this dramatic situation implies that new eco-friendly strategies that combine plant growth promotion and plant protection from pathogenic bacteria without the risk of resistance development are required. These two biological functions have been demonstrated in plant growth-promoting bacteria (PGPB) that are successfully used as biofertilizers and biocontrol agents in agriculture acting through direct and/or indirect mechanisms (Richardson et al., 2009; Vejan et al., 2016).

Direct and indirect mechanisms include biological nitrogen fixation, production of phytohormones such as auxins, cytokinins and gibberellins, solubilization of minerals such as phosphorus and iron through the production of siderophores, induction of systemic resistance, biocontrol properties of the bacteria that confer the ability to inhibit the growth of pathogens such as the capacity of the bacteria to produce antibiotics and toxins, their capability to synthesize extracellular enzymes to hydrolyse the cell wall, chelation of available Fe in the rhizosphere, and competition for niches within the rhizosphere (Olanrewaju et al., 2017; van Loon, 2007; Zahir et al., 2003).

Plant pathogenic bacteria cause plant infection and tissue damage through multiple mechanisms, including the production of toxins, hydrolytic enzymes, alteration of phytohormone levels, among others. As these virulence factors are regulated in many bacteria by a cell-concentration-dependent mechanism known as quorum sensing (QS) (Papenfort & Bassler, 2016), its interference has been proposed as a promising antivirulence therapy. The two main mechanisms of QS interference described to date are the production of quorum sensing inhibitors (QSI) and the enzymatic degradation of signal molecules, known as quorum quenching (QQ). The aim of this novel approach is disarming rather than killing or inhibiting the growth of the pathogen. Since it does not affect essential bacterial genes, it is believed to impose less selective pressure to develop resistances when compared to conventional treatments based on the use of pesticides chemical and antibiotics (Koul et al., 2016; Munguia & Nizet, 2017).

QS system in Gram-negative bacteria is mediated by the diffusion and recognition of signal molecules such as N-acylhomoserine lactones (AHLs) (Fuqua et al., 1994) that have been reported to regulate the expression of many important virulence-associated phenotypes in pathogens such as *Pectobacterium carotovorum* (Põllumaa et al., 2012), *P. atrosepticum* (Smadja et al., 2004), *Dickeya solani* (Crépin et al., 2012) and *Pseudomonas syringae* (Quiñones et al., 2005). To date, one of the most studied mechanisms of QS disruption is the enzymatic degradation of AHLs signal molecules (Uroz et al., 2009). There are described three main types of enzymes that degrade or modify AHLs, including lactonases (lactone hydrolysis), acylases (amide hydrolysis) and oxidoreductases (oxidoreduction) (Fetzner, 2015). Nowadays, many QQ enzymes have been reported, mainly produced by bacteria isolated from marine environments, and only a few have been identified in plant-associated bacteria (Torres et al., 2019). The expression of AHL-degrading enzymes in agricultural pathogens (Dong et al., 2000) or the cultivation of these pathogens in the presence of AHL-degrading bacteria have caused the reduction of enzymatic activity as well as the virulence of many plant pathogenic bacteria, producing promising results (Fan et al., 2020; Faure & Dessaux, 2007; Helman & Chernin, 2015; Reina et al., 2019; Rodríguez et al., 2020; Uroz et al., 2003; Vega et al., 2020; Zhang et al., 2020, 2021).

The genus *Bacillus* is a well-known group of PGPB with a remarkable production of endospores that are resistant to stress conditions (Borriss, 2011). To date, many species of *Bacillus* such as *B. subtilis*, *B. thuringiensis*, *B. amyloliquefaciens*, *B. pumilus*, *B. megaterium*, *B. firmus and*, *B. velezensis* are extensively used in commercial agricultural bioformulations (Borriss, 2011; Gotor-Vila et al., 2019; Jiang et al., 2018; Parnell et al., 2016; Rabbee et al., 2019). In this regard, among the active substances approved or pending approval in the EU as possible components of pesticide products (more than 500 substances), about 100 are bacteria, fungi or viruses that act as insecticides, acaricides, nematicides, fungicides, bactericides, elicitors or disinfectants (Roca & Matilla, 2023). Among these active substances based on microorganisms, about 30 are bacteria belonging to the *Bacillus* genus. In respect of the ability to produce QQ enzymes, some *Bacillus* species have described to synthetize AHL-lactonases (Ayyappan & Bhaskaran, 2022; Dong et al., 2002). Indeed, the first AHL-degrading enzyme, named AiiA, was identified in a *Bacillus* sp. 240B1 and its effect on the attenuation of virulence of bacterial pathogens such as *Erwinia carotovorum* (Dong et al., 2000), *Burkholderia glumae* (Cho et al., 2007), *Dickeya chrysantyemi* (Hosseinzadeh et al., 2013) has been demonstrated. As far as our knowledge is concerned, scare information is available related to *Bacillus* strains with both, PGP and QQ activities. In the present study, we demonstrated the ability to promote plant growth as well as the AHL-degrading activity of the halotolerant *Bacillus* sp. strain AA1EC1, a novel strain isolated from the halophyte plant *Arthrocaulon* sp. We discussed its potential use as a biocontrol agent against plant pathogenic bacteria as well as its phytostimulant characteristics in tomato plants.

EXPERIMENTAL PROCEDURES

Bacterial strains and culture conditions

Strain AA1E1C1 is an endophytic bacterium isolated from the aerial part of the *Arthrocaulon* sp. plant taken from El Saladar del Margen (Cúllar, Granada; 37°38′43.6″ N, 2°35′59.0″ W). The AHL-producing phytopathogens used for in vitro and in vivo assays were *Pectobacterium carotovorum* subsp. *carotovorum* CECT 225^T, *P. atrosepticum* CECT 314^T, *Dickeya solani* IPO 2222^T and *Pseudomonas syringae* pv. tomato DC3000. The biosensor strains used were *Chromobacterium subtsugae* CV026

(Harrison & Soby, 2020), formerly *C. violaceum* CV026 (McClean et al., 1997), *C. violaceum* VIR07 (Morohoshi et al., 2008) and *Agrobacterium tumefaciens* NTL4 (pZLR4) (Shaw et al., 1997). All strains were grown in Luria-Bertani (LB) medium. In the case of biosensor NTL4, it was grown in LB medium supplemented with 2.5 mM $CaCl_2 \times 2H_2O$ and 2.5 mM $MgSO_4 \times 7H_2O$ (LB–MC) or AB medium (composition per litre: 3 g K_2HPO_4, 1 g $Na_2H_2PO_4$, 1 g NH_4Cl, 0.3 g $MgSO_4 \times 7H_2O$, 0.15 g KCl, 0.01 g $CaCl_2$, 0.0025 g $FeSO_4$ $7H_2O$ and 5 g glucose; Chilton et al., 1974). When necessary, kanamycin (Km) and gentamicin (Gm) were used in final concentrations of 50 μg mL^{-1}. All strains were grown at 28°C and at 120 rpm in a rotary shaker.

Characterization of strain AA1EC1

Salinity tolerance and optimal growth conditions of strain AA1EC1 were determined in LB agar medium supplemented with 0.5; 2.5; 5; 7.5; 10 and 20% (w/v) NaCl. Other phenotypic characteristics were evaluated: amylase (Barrow & Feltham, 1993), ß-glucosidase (Gong et al., 2012), caseinase (Barrow & Feltham, 1993), gelatinase (Tindall et al., 2007), cellulase (Tasse et al., 2010), DNAase (Jeffries et al., 1957), chitinase, lecithinase (Larpent & Larpent-Gourgaud, 1975), hemolysine (Columbia blood agar plates, Difco), phytase (Hosseinkhani & Hosseinkhani, 2009), xylanase (Tasse et al., 2010), acid and alkaline phosphatase (Baird-Parker, 1963; Pikovskaya, 1948) hydrolysis of Tween 20 and Tween 80 (Sierra, 1957), siderophore production (Alexander & Zuberer, 1991) and Indole-3-acetic acid production (Naik et al., 2008).

DNA extraction, purification, and sequencing

Genomic DNA was extracted using the X-DNA purification kit (Xtrem Biotech, Granada, Spain) from an overnight culture of strain AA1EC1 in LB medium. 16S rRNA gene was amplified by PCR using the universal bacterial primers 16F27 and 16R1488 (Lane, 1991). The PCR product was purified and cloned into the pGEM-T vector (Promega) and then sequenced by a direct sequencing using an ABI prism DyeTerminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer) and an ABI prism 377 sequencer (Perkin-Elmer) according to the manufacturer's instructions. Additionally, the sequence of 16 rRNA gene was obtained from the genome sequence. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence is OQ443196. Genome of strain AA1EC1 was sequenced by using the Illumina MiSeq methodology (PE 150 × 2).

Phylogenetic analysis based on 16S rRNA gene sequence comparison

Phylogenetic analyses based on the 16S rRNA gene were conducted as described previously (Castro et al., 2018; Martínez-Checa et al., 2005). The identification of phylogenetic neighbours and the calculation of the pairwise 16S rRNA gene sequence similarities was carried out by using the EzBiocloud www.ezbiocloud.net (Yoon, Ha, Kwon, et al., 2017) and by using the BLASTN (Altschul et al., 1997) program against the GenBank/EMBL/DDBJ database containing type strains with validly published prokaryotic names and representatives of uncultured phylotypes. The phylogenetic tree of strain AA1EC1 was constructed using the Maximum Likelihood method, using the Tamura-Nei model (Felsenstein, 1985; Tamura & Nei, 1993) and performing the evolutionary analyses in MEGA11 (Tamura et al., 2021).

Genomic analyses

The genome of strain AA1EC1 was sequenced using Illumina sequencing platform at the installations of NOVOGENE (Cambridge, UK), with 2 × 150-bp paired-end reads. Illumina reads of strain AA1EC1 were trimmed using a combination of software tools implemented in the BBMap project (Bushnell, 2014), and de novo assembled using SPADES v3.11.1 (Bankevich et al., 2012). CheckM v1.0.18 (Parks et al., 2015) and Quast v5.0.2 (Gurevich et al., 2013) were used for assembly quality checked.

Genome of strain AA1EC1 was annotated using RASTtk – v1.073 (Aziz et al., 2008; Brettin et al., 2015; Overbeek et al., 2014) and gene coding proteins were analysed using Artemis (Carver et al., 2012), a free genome browser and annotation tool of Sanger Institute, that allows visualization of sequence features, next generation data and the results of analyses within the context of the sequence, and also its six-frame translation.

Average Nucleotide Identity (ANI) and in silico DNA–DNA hybridization values were calculated using the OrthoANI-usearch (OrthoANIu) software (Yoon, Ha, Lim, et al., 2017) and Genome-to-Genome Distance Calculator (GGDC) of DSMZ website (Meier-Kolthoff et al., 2013) and recommended formula 2 (Identities/HSP length) (Auch et al., 2010).

Quorum-quenching activity: Range of AHLs and localization of the enzyme

The capacity of strain AA1EC1 to degrade synthetic AHLs was tested using well diffusion agar-plate assays (Romero et al., 2011; Torres et al., 2013). Briefly, AHLs were added to 500 μL of a 24 h culture of strain

AA1EC1 in a final concentration of 10 μM. Cell-free LB medium supplemented with the same concentration of AHLs was incubated as a negative control. After 24 h of incubation at 28°C and at 120 rpm in a rotary shaker, 100 μL aliquots of the culture supernatant were placed in wells on LB agar plates previously seeded with the biosensors CV026 or VIR07 and on AB agar plates supplemented with 80 μg mL^{-1} of 5-bromo-4-chloro-3-indolyl-ß-D-galactopyranoside (X gal) seeded with the biosensor NTL4 to check for the development of purple or blue colour around each well. The AHL degradation was confirmed by high-performance liquid chromatography-multiple reaction monitoring (HPLC-MRM) (Torres et al., 2013).

The synthetic AHLs (Sigma-Aldrich, Saint Louis, USA) used were as follows: C4-HSL (N-butyryl-DL-homoserine lactone), C6-HSL (N-hexanoyl-DL-homoserine lactone), C8-HSL (N-octanoyl-DL-homoserine lactone), C10-HSL (N-decanoyl-DL-homoserine lactone), 3-OH-C10-HSL (N-3-hydroxydecanoyl-DL-homoserine lactone), C12-HSL (N-dodecanoyl-DL-homoserine lactone) and 3-O-C12-HSL (N-3-oxo-dodecanoyl-DL-homoserine lactone).

To identify the cellular localization of QQ activity, the previous described AHL-degradation assay was performed with the supernatant and crude cellular extract (CCE) fractions from a 24 h culture of strain AA1EC1 against C6-HSL, C10-HSL and C12-HSL. Previously, both fractions were filtered through a 0.22 μm-pore membrane filter (Romero et al., 2011).

Identification of genes encoding quorum quenching activity

In silico search for genes encoding QQ enzymes in the genome of strain AA1EC1 was carried out by using the UNIPROT Database (https://www.uniprot.org).

Each potential candidate was selected and used to designed specific primers: "Quorum-quenching lactonase YtnP" (MDD9261378.1) (Lact1-FWD: 5′-ATGGAAGAATTACGAATTGG-3′ and Lact1-REV: 5′-TTAAGCTTCTATACTCGTTTTTC-3′); "N-acyl-homoserine lactonase" (MDD9262952.1) (Lact2-FWD: 5′-ATGACGGTAAAGAAGCTTTA-3′ and Lact2-REV: 5′-CTATATATATTCCGGGAAC-3′); "Metallo-beta-lactamase" (MDD9259744.1) (Lact3-FWD: 5′-ATGAAGGGTATACGAAGTA-3′ and Lact3-REV: 5′-TTACTTCAATAATGGCATG-3′); and "Penicillin-acylase" (MDD9262231.1) (Acyl-FWD: 5′- ATGGAAGTTGTTATAAAGAAG-3′ and Acyl-REV: 5′: TCACTTCGGCTGTAAAGTTA-3′).

PCR fragments were amplified from the extracted DNA using Q5 HF DNA polymerase (New England BioLabs) and the corresponding primers and cloned into the pGEM-T Easy Vector (Promega) following the manufacture's conditions. The plasmid constructions were transformed into *Escherichia coli* DH5α and QQ activities were tested in LB supplemented with IPTG and C10-HSL as described above.

To examine whether the observed QQ activity occurs through the degradation of the AHLs by a lactonase, an assay based on lactone ring closure in acidic conditions was performed (Yates et al., 2002). Briefly, C10-HSL (10 μM) was added to 5 mL of a 24 h culture. Cell-free LB medium supplemented with the same concentration of AHLs was incubated as a negative control. After 24 h incubation at 28°C, one aliquot of 500 μL was acidified to pH 2.0 with HCl 1 N and incubated for 24 h. The remaining AHLs in each condition were extracted (pH 7 and pH 2) and measured by well diffusion agar-plate method (Torres et al., 2013).

Antagonism assays

The antagonist activity of strain AA1EC1 against the plant pathogenic bacteria used in this study was evaluated using the well diffusion method (Frikha-Gargouri et al., 2017). Briefly, an overlay of each pathogen was prepared on LB agar plates and 100 μL aliquots of the supernatant (filtered through a 0.22 μm-pore membrane filter) from a 5-day culture of strain AA1EC1 was placed in the wells previously done. After 48 h of incubation at 28°C, plates were examined for growth inhibition halos surrounding the wells. The same approach was used to assess the antagonistic activity caused by the hydrolytic enzymes excreted by the AA1EC1 strain. In this case, the wells were filled with a 100 μL aliquot of a culture of the strain grown 24 h at 28°C and 120 rpm. Assays were conducted in triplicate.

Co-culture assays to evaluate the interference of QS system of phytopathogens

Co-culture assays of strain AA1EC1 and the phytopathogens were carried out according to previous studies (Torres et al., 2017). Briefly, 24 h cultures of each pathogen (10^9 CFU mL^{-1}) were co-cultured with strain AA1EC1 (10^9 CFU mL^{-1}) at a ratio of 1:100 in LB medium and incubated for 24 h at 28°C and at 120 rpm in a rotary shaker. Each bacterium was monocultured under similar conditions as controls. The remaining AHLs from each co-culture and monoculture were detected using the well diffusion agar-plate method previously described, using *A. tumefaciens* NTL4 (pZLR4) and *C. subtsugae* CV026 as bioindicators. In the case of *P. syringae* pv. tomato DC3000, 300 μL-aliquots were necessary to detect AHLs. Finally, the abundance of each bacterium in the co-cultures was determined by serial dilutions and plate counts. For some pathogens, specific media contained different sugars and the pH

indicator bromothymol blue (0.03 g L^{-1}) was used to distinguish the colonies of the strain AA1EC1 from the pathogens. The media used were tryptic soy agar (TSA) supplemented with 1% (w/v) mannitol for *D. solani*; TSA supplemented with 1% (w/v) glucose for *P. atrosepticum* and TSA supplemented with 1% (w/v) lactose for *P. carotovorum* subsp. *carotovorum*. In the case of *P. syringae* pv. tomato DC3000 co-cultures, plate counts were performed on King B medium, which allowed identification of pathogen colonies by the emission of fluorescence upon exposure of plates to UV light.

The interference of AHL degradation on the virulence-associated cellular functions controlled by QS in the phytopathogens was evaluated in co-cultures. Monocultures were used as controls. Thus, 10 μL of each culture was spotted on different media to test the following phenotypic characteristics and enzymatic activities: acid phosphatase (Pikovskaya, 1948), cellulase (Tasse et al., 2010), phytase (Hosseinkhani & Hosseinkhani, 2009), Indole-3-acetic acid production (Naik et al., 2008), ß-glucosidase (Gong et al., 2012), amylase (Barrow & Feltham, 1993), siderophore production (Alexander & Zuberer, 1991) and xylanase (Tasse et al., 2010). Each assay was repeated three times. For the estimation of enzyme activity efficiency, the hydrolysis index for cellulase and xylanase activities (Florencio et al., 2012); phosphate solubilization index for phosphatase and phytase activities (Tariq et al., 2022) and siderophore index (Eshaghi et al., 2019; Tamariz-Angeles et al., 2021) were calculated. In all cases, the activity index was calculated using the following equation: Solubilization index (SI) = diameter of hydrolysis zone/diameter of colony. In addition, in order to determine the capacity of the strains to produce IAA, IAA quantification (μg mL^{-1}) was performed following the protocol developed by Khan et al. (2014).

In vivo virulence assays

The ability of strain AA1EC1 to interfere with soft rot caused by *D. solani* IPO 2222^T and *P. atrosepticum* CECT 314^T was tested on potato slices while the infection produced by *P. carotovorum* subsp. *carotovorum* CECT 225^T was tested on potato and carrot slices (Garge & Nerurkar, 2016; Torres et al., 2017). Briefly, surface-sterilized, and sliced potato tuber (*Solanum tuberosum*) and carrots (*Daucus carota*) were inoculated with AA1EC1-phytopathogen co-cultures and incubated at 28°C for 48 h. Monocultures and sterilized distilled water were used as controls. Maceration zones were visually detected, and the spatial extent of the damage was calculated using ImageJ software (Schneider et al., 2012). Assays were conducted in triplicate using 5 samples in each replica.

The effect of QQ activity of strain AA1EC1 on *P. syringae* pv. tomato DC3000 virulence was tested in tomato plants according to the technique described by Yan et al. (2008). Thus, tomato seeds were surface-sterilized and sown in pots as described below in the section plant growth promotion assays. After seeds sowing (50 seeds per pot), the pots were kept in an indoor greenhouse during a long-day photoperiod (16:8 h, light:dark) at 25°C and watered with 50 mL sterile distilled water twice a week. After 3 weeks, the pots were exposed to 100% humidity for 24 h to induce stomatal opening and were then sprayed with a 5 mL *P. syringae* pv. tomato DC3000 suspension (10^9 CFU mL^{-1}), or the co-culture of AA1EC1 with *P. syringae* (ratio 100:1). A suspension of strain AA1EC1 (10^9 CFU mL^{-1}) was used to determine if the strain exerted any detrimental effect on tomato plant leaves. For the negative control treatment, seedlings were sprayed with sterile distilled water. Relative humidity was maintained at 100% for a further 24 h to facilitate pathogen infection. One week after inoculation, seedlings were photographed and affected (necrotic and chlorotic leaves, dead leaves) and unaffected shoots were counted.

The interference with the expression of QS-regulated functions in *P. syringae* pv. tomato DC3000 was also evaluated by heterologous expression of a QQ enzyme of strain AA1EC1. The N-acyl-homoserine lactonase gene (MDD9262952.1) was cloned in pME6010 obtaining the construction named as pME6010::*BtAiiA* (for *Bacillus toyonensis* with high homology to AiiA). pME6010::*BtAiiA* and the empty plasmid pME6010 (Tc^R) (Heeb et al., 2000), a broad-host-range plasmid that was used as negative control, were transferred into the pathogen by electroporation. *P. syringae* pv. tomato DC3000 contained pME6010::*BtAiiA* transformants were confirmed by PCR using as the template the plasmid DNA that was extracted by the alkaline lysis protocol (Morelle, 1989) and the specific primers described above. To determine AHL production in each transformants, AHL molecules were extracted from 5-mL of the wild type and transformants containing pME6010 and pME6010::*BtAiiA* (Marketon & González, 2002). The entire crude extracts were added to 5-mm sterile disks placed onto AB-Xgal medium in which the biosensor *A. tumefaciens* NTL4 (pZLR4) was previously spreaded. The plates were incubated at 28°C to allow the development of blue colour surrounding the paper disks. To analyse the effect of *BtAiiA* upon some of the virulence factors regulated by QS in *P. syringae* pv. tomato DC3000, 10 μL of overnight cultures of the wild-type strain and the transformants carrying the plasmids pME6010 and pME6010::*BtAiiA* were spotted on different enzymatic media described above and solubilization indexes were calculated.

The ability of *BtAiiA* to interfere with the virulence of the pathogenic strain was also evaluated in tomato plants. For this purpose, commercial tomato plants of the variety gordo rama of 1 month old (48 plants; 12 plants per treatment) were exposed to 100% humidity

for 24 h to induce stomatal opening and were then sprayed with a 25 mL *P. syringae* pv. tomato DC3000, *P. syringae* pv. tomato DC3000 pME6010::*BtAiiA* or *P. syringae* pv. tomato DC3000 pME6010 suspensions (10^9 CFU mL^{-1}). For the negative control treatment, plants were sprayed with sterile distilled water. Relative humidity was maintained at 100% for a further 24 h to facilitate pathogen infection. One week after inoculation, affected leaves (necrotic and chlorotic leaves) and healthy leaves were counted. Subsequently, a random sample of leaves was taken from different plants of each treatment and the quantification of the chlorophyll present in the leaves was carried out. For this purpose, the leaves were pulverized using a mortar and the addition of liquid N_2. Three subsamples of 0.1 g were taken from each pool of leaves and incubated at 4°C in the presence of 2 mL of 80% (v/v) acetone for 24 h. After incubation, the samples were centrifuged for 10 min at 13,550 *g*, the supernatant was collected, and the absorbance was measured at λ = 663.2 nm and λ = 646.8 nm. The absorbance data were used to determine the concentration of chlorophyll a (Ca), chlorophyll b (Cb) and total chlorophyll (Ct) using the equations developed by Lichtenthaler (1987).

The equations used for the quantification of chlorophyll in μg g^{-1} fresh tissue were as follows:

$$\mathrm{Ca} = \frac{(12.25 \times A663.2 - 2.79 \times A646.8) \times Vf}{W}$$

$$\mathrm{Cb} = \frac{(21.5 \times A646.8 - 5.1 \times A663.2) \times Vf}{W}$$

$$\mathrm{Ct} = \frac{(7.15 \times A663.2 + 18.71 \times A646.8) \times Vf}{W}$$

where A663.2 is the absorbance value at λ = 663.2 nm; A646.8 is the absorbance value at λ = 646.8 nm; *Vf* is the final volume of the sample (mL) and *W* is the weight of the sample in g.

In vitro tomato leaves infection assay

An in vitro assay with tomato leaves was used to evaluate the possible loss of virulence of *P. syringae* pv. tomato in co-culture with strain AA1EC1. For this purpose, leaves from 50-day-old tomato plants with similar morphology and size were placed on a layer of glass beads in special Petri dishes (Star™Dish, 90 mm diameter × 25 mm height) with 15 mL of sterile distilled water and left at room temperature for 24 h to induce stomatal opening. After this time, leaves were immersed in 25 mL of *P. syringae* pv. tomato DC3000 suspension (10^9 CFU mL^{-1}), AA1EC1 co-culture with *P. syringae* suspension (10^9:10^7 CFU mL^{-1}), AA1EC1 strain suspension (10^9 CFU mL^{-1}), or distilled water. One and two weeks after inoculation, leaves were photographed and affected (necrotic and chlorotic leaves) and unaffected areas were visually detected. The total area of the leaves, areas affected by chlorosis and by necrosis were measured with the ImageJ software (Schneider et al., 2012) and then the percentage of severity of the disease (Disease severity (%)) was calculated according to Nutter et al. (2006), applying the following equation: D.S. (%) = (A/B) × 100, where A refers to the diseased leaf area and B refers to the total leaf area.

Plant growth promotion assays

The plant growth promoting capacity was evaluated on strain AA1EC1 in plant growth promotion assays on tomato seedlings (*Solanum lycopersicum* Roma VF variety). To this end, tomato seeds were surface-sterilized (Molan et al., 2010) with 2.5% (v/v) sodium hypochlorite for 15 min, followed by 7% (v/v) hydrogen peroxide for 15 min. Seeds were washed four times in sterile distilled water and left to soak in sterile distilled water for 16 h at 4°C in the dark. Then, 40 sterilized seeds were sown in seedling trays (40 cells, volume of each cell 130 mL) containing a mixture of vermiculite: perlite 3:1 (v/v) sterilized (3 cycles at 121°C and at 1 atm for 20 min). When the seedlings reached a size of 5 cm (3 weeks after sowing), inoculations with strain AA1EC1 were started. Each seedling was irrigated twice a week with 250 μL of suspension of 10^9 CFU mL^{-1} (centrifuged culture and cell pellet resuspended in distilled water) and 250 μL of sterile distilled water (control). Inoculations were carried out for 1 month. Seedling trays were kept in an indoor greenhouse during a long-day photoperiod (16:8 h, light:dark) at 25°C for 4 weeks. The plants were then harvested and root and shoot length and dry weight were measured. To determine the dry weight, the plant material was oven-dried at 40°C for 48 h.

To evaluate the colonizing effectiveness of the strain, root samples were collected from the plants at the end of the assay, weighed and surface disinfected. For disinfection, tomato roots were washed with tap water to remove attached substrate particles. Subsequently, the roots were immersed in 70% (v/v) ethanol for 1 min, washed with 2% (v/v) sodium hypochlorite solution for 3 min, immersed in 70% (v/v) ethanol for 30 s, rinsed with 70% (v/v) ethanol for 30 s and finally washed 3 times with sterile distilled water. Triplicate portions of roots were cut aseptically, macerated, and homogenized with a mortar and pestle and resuspended in 10 mL of saline solution (NaCl 0.85%). Finally, the CFU g^{-1} were counted by serial dilution and subsequent seeding in TSA supplemented with 1% (w/v) mannitol and the pH indicator phenol red (0.03 g L^{-1}). This culture medium allows

easy detection of the AA1EC1 strain by the macroscopic appearance of the colony and the change in coloration of the medium caused by the change in pH.

Statistical analysis

The results were statistically evaluated using GraphPad Prism version 8.0.2. for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com. The normality of the data was evaluated using the D'Agostino and Pearson, Shapiro–Wilk and Kolmogorov–Smirnov tests. When the data were normal, they were analysed by 1-way ANOVA using Tukey's test for comparison of means. When the data did not show normality, a Kruskal–Wallis analysis was performed using the Dunn test as a post-hoc analysis. In case only two samples were compared, the statistical analysis was conducted by unpaired *T*-test or unpaired *T*-test with Welch's correction. In all cases, the confidence interval was fixed at 95%.

Nucleotide sequence accession number

The complete genome sequence of *B. toyonensis* AA1EC1 is available in GenBank/EMBL/DDBJ under Accession Number JARADC010000000.1 (Bioproject accession PRJNA935526).

RESULTS

Characterization of strain AA1EC1

Strain AA1EC1 was isolated from the inner tissue of the aerial part of the halophyte plant *Arthrocaulon* sp. It was a motile, Gram-positive, halotolerant rod that was capable of growing in a wide range of NaCl [0.5 to 2.5% (w/v)]. AA1EC1 produced siderophores, indole-3-acetic acid (IAA) as well as the enzymes amylase, alkaline phosphatase and phytase, protease, β-glucosidase, hemolysin (beta hemolysis). It also hydrolysed DNA, lecithin, gelatine, and Tween 80 but not Tween 20. Additionally, it did not synthesize the enzymes acid phosphatase, cellulase, xylanase nor chitinase (Figure S1).

Based on the 16S rRNA gene sequence analysis, strain AA1EC1 belongs to the genus *Bacillus*, sharing 100% 16S rRNA gene sequence similarity with *Bacillus toyonensis* and *B. mobilis*, followed by *B. pacificus* (99.93%) and *B. luti* (99.79%) (Figure S2).

The draft genome sequence of strain AA1EC1 was obtained and compared with the ones from the closest phylogenetic species, *B. toyonensis* NCIMB 14858^T and *B. mobilis* MCCC 1A05942^T and with other members of the genus *Bacillus* with available genomes (Table S1).

The draft genome of strain AA1EC1 was de novo assembled into a total of 90 contigs, with a N50 value of 131,220 bp and a sequencing depth of 309X. This genome sequence is in accordance with the minimal standards for the use of genome data for the taxonomy of prokaryotes (Chun et al., 2018). The G+C content of strain AA1EC1 was 34.92 mol%. The genome size was 5,895,347 bp, with a number of predicted protein of 5896; those values were within the range of the genomes of the genus *Bacillus*. Genomic characteristics are detailed in Table S1. Besides, the 16S rRNA gene sequence of strain AA1EC1 obtained from the draft genome sequence was identical to that from the PCR, verifying the authenticity of this genome.

To elucidate whether strain AA1EC1 may constitute a new species within the genus *Bacillus*, or nevertheless belongs to a known species, the genome-based sequence similarity analysis (Average Nucleotide Identity (ANI) and in silico DNA–DNA Hybridization (DDH)) between the AA1EC1 strain and the members of this genus was estimated (Table S1). For species delineation, the proposed and accepted limits for ANI and DDH are 95–96% and 70%, respectively (Goris et al., 2007; Meier-Kolthoff et al., 2013; Richter & Rosselló-Móra, 2009). The ANI and digital DDH (dDDH) values between strain AA1EC1 and *B. toyonensis* NCIMB 14858^T and *B. mobilis* MCCC 1A05942^T, the closest phylogenetic neighbours, were 99.2% and 92.2% and 91.1% and 42.2%, respectively. In addition, the ANI and DDH estimations of strain AA1EC1 in comparison to the other members of the genus *Bacillus* with available genomes, were in all cases below the established cut-off values.

Based on phylogenetic analysis and the results of ANI and DDH support the conclusion that the AA1EC1 strain belongs to the species *B. toyonensis*.

Characterization of the AHL degradation activity of strain AA1EC1

The ability of strain AA1EC1 to degrade a wide range of synthetic AHLs (C4-HSL, C6-HSL, C8-HSL, C10-HSL, 3-OH-C10-HSL, C12-HSL, 3-oxo-C12-HSL) was tested. A well diffusion agar-plate assay using the corresponding biosensors *A. tumefaciens* NTL4 (pZLR4), *C. subtsugae* CV026 and *C. violaceum* VIR07 indicated that strain AA1EC1 was able to completely degrade all the AHLs tested (Figure S3a). To confirm the QQ activity, an assay against C10-HSL was quantified by HPLC-MRM (Figure S3c).

To assess the cellular localization of the QQ enzymes, supernatant (SN) and crude cellular extract (CCE) from strain AA1EC1 were obtained and AHL degradation activity was tested in both fractions against C6-HSL, C10-HSL and C12-HSL. Well diffusion agar-plate assays with the corresponding biosensors CV026, VIR07 and NTL4 indicated that QQ activity was detected mainly in the supernatant and only a slight activity was seen in CCE, indicating that the enzymes were secreted (Figure S3b).

To identify the genes encoding for AHL-degradation activity in strain AA1EC1, the automatic annotation of the whole genome by RAST was mined by using Artemis tool. As a result, four potential proteins automatically annotated as "Quorum-quenching lactonase YtnP" (Protein No. MDD9261378.1), "N-acyl-homoserine lactonase" (Protein No. MDD9262952.1), "Metallo-beta-lactamase superfamily" (Protein No. MDD9259744.1) and "Penicillin acylase" (Protein No. MDD9262231.1) were selected (Table 1). The corresponding genes 926138.1 (855 bp), 9262952.1 (752 bp), 9259744.1 (903 bp) and 9262231.1 (2,391 bp) were cloned in the pGEM-T vector and transformed into *Escherichia coli* DH5α. Then, the AHL-degrading activity of the four DNA constructs was confirmed by well diffusion agar-plate assays against C10-HSL. All of them degraded C10-HSL to a greater or lesser extent, being the most efficient protein MDD9262952.1 (Figure S4). To confirm the enzymatic activity of each QQ putative protein, the lactone ring closure assay was carried out by acidification of the supernatant at pH 2 with HCl. The AHL signal was significantly recovered for the 3 lactonases (Proteins No. MDD9261378.1; MDD9262952.1 and MDD9259744.1), as evidenced by activation of the NTL4 biosensor (Figure S4).

Interference of bacterial phytopathogen AHL-QS systems and impact on associated phenotypes by strain AA1EC1

Since the strain AA1EC1 showed a QQ activity against a wide broad of synthetic AHLs, we evaluated whether it had the capacity to degrade the AHLs produced by *P. carotovorum* subsp. *carovovorum* CECT 225^T, *P. atrosepticum* CECT 314^T, *D. solani* IPO 2222^T and *P. syringae* pv. tomato DC3000 and consequently, the effect on the production of virulence factors controlled by QS. Firstly, an antagonist experiment was performed to discard any inhibitory effect of strain AA1EC1 against each phytopathogen. The results obtained confirmed that neither the supernatant nor the strain culture inhibited the growth of the pathogens tested and that therefore AA1EC1 did not exert antagonistic activity against these phytopathogens (Figure S5). Thus, each pathogen was co-cultured with strain AA1EC1 in a ratio 1:100 for 24 h. The concentration of each strain was maintained through the experiment (10^7:10^9 CFU mL^{-1}). Then, the remaining AHL were detected by well diffusion agar-plate assay and the corresponding biosensor strains according to the type of AHLs produced by each phytopathogen. As shown in Figure 1, no AHLs were detected the biosensors in the co-cultures with *D. solani* and *P. syringae* pv. tomato, indicating a total AHL degradation (Figure 1C,D). The co-cultures with *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum* activated the biosensor NTL4 at lower extension while AHLs were not detected by the biosensor CV026, indicating a partial degradation of AHLs (Figure 1A,B).

The same co-cultures were used to evaluate the effect of AHL degradation produced by strain AA1EC1 on QS-associated phenotypes of each phytopathogen. Some phenotypes of bacterial pathogens were reduced or inhibited when co-cultured with strain AA1EC1 (Figure S6). Indole-3-acetic acid (IAA) and siderophores production was reduced in all of pathogens in co-culture with strain AA1EC1 (Figure S6e). The production of acid phosphatase, cellulase and

TABLE 1 Homologues to quorum quenching putative proteins identified in the genome of *Bacillus toyonensis* AA1EC1.

Homologues	Identity (%)
Protein No. MDD9261378.1 "Quorum-quenching lactonase YtnP"	
MBL fold metallo-hydrolase [*Bacillus*] (WP_016109226.1)	100
MBL fold metallo-hydrolase [*Bacillus toyonensis*] (WP_098059277.1)	99.3
MBL fold metallo-hydrolase [*Bacillus cereus* group] (WP_098000241.1)	99.3
Protein No. MDD9262952.1 "N-acyl-homoserine lactonase"	
N-acyl homoserine lactonase family protein [*Bacillus*] (WP_000216616.1)	100
N-acyl homoserine lactonase family protein [*Bacillus toyonensis*] (WP_061530505.1)	99.6
N-acyl homoserine lactonase family protein [*Bacillus cereus group* sp. N11](WP_199634274.1)	99.2
Protein No. MDD9259744.1 "Metallo-beta-lactamase superfamily"	
ComEC/Rec2 family competence protein [*Bacillus*] (WP_000676376.1)	100
ComEC/Rec2 family competence protein [*Bacillus toyonensis*] (WP_098628128.1)	99.7
Metallo-beta-lactamase [*Bacillus cereus* Rock3-28] (EEL32943.1)	99.3
Protein No. MDD9262231.1 "Penicillin acylase"	
Penicillin acylase family protein [*Bacillus*] (WP_016108791.1)	100
Penicillin acylase family protein [*Bacillus thuringiensis*] (QPW47882.1)	99.6
Penicillin acylase family protein [*Bacillus toyonensis*] (WP_215595821.1)	99.4

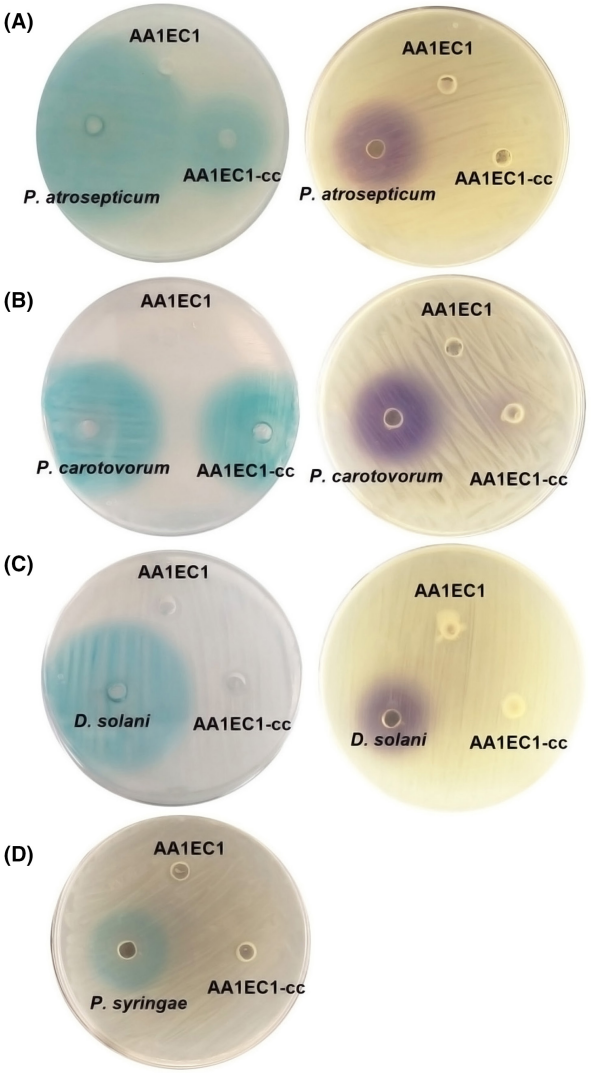


FIGURE 1 Detection of AHLs in the culture and co-cultures of strain AA1EC1 and the pathogens *Pectobacterium atrosepticum* (A), *P. carotovorum* subsp. *carotovorum* (B), *Dickeya solani* (C) and *Pseudomonas syringae* pv. tomato DC3000 (D). *Agrobacterium tumefaciens* NTL4 (pZLR4) (right) and *Chromobacterium subtsugae* CV026 (left) were used as biosensor strains to detect AHLs. Biosensor *C. subtsugae* CV026 is not efficient in detecting AHLs produced by *P. syringae* pv tomato DC3000 and the assay with this biosensor has been omitted for this phytopathogen.

xylanase in *D. solani* were found to be inhibited in the presence of strain AA1EC1 and the production of phytase was reduced (Figure S6c,e). Co-culture of *P. atrosepticum* with AA1EC1 exhibited an inhibition of acid phosphatase activity and a reduction in amylase activity (Figure S6a,e). Acid phosphatase, and phytase production in *P. syringae* pv. tomato (Figure S6d,e) as well as in *P. carotovorum* subsp. *carotovorum* (Figure S6b,e) was reduced in co-culture with AA1EC1. Interference by QQ activity of other phenotypes of the pathogens could not be tested since the strain AA1EC1 showed high activity in other enzymatic activities analysed (Figure S1).

Interference of bacterial phytopathogen virulence by strain AA1EC1

The impact of AHL degradation on the virulence of the phytopathogens studied was evaluated by assays in potato tubers and carrot slices. Co-cultures of strain AA1EC1 with *D. solani* and *P. atrosepticum* prepared as described above were inoculated on the surface of the potato slices. As it is shown in Figure 2, strain AA1EC1 drastically reduced the virulence capacity of *D. solani* and *P. atrosepticum* to cause soft rot since no maceration zone was produced in each case (0% maceration) while a maceration zone of 22.5 ± 3.3% and 15.86 ± 4.1% was originated in potatoes by each pathogen in monoculture respectively (Figure 2A). With respect to *P. carotovorum* subsp. *carotovorum*, a reduction in virulence was observed in the potato slice assay when treated with the co-culture and resulted in a maceration area of 8.51 ± 1.2% while the pathogen in monoculture caused a maceration of 28.1 ± 3.8% (Figure 2A). Nevertheless, no soft rot symptoms (0% maceration) were observed in carrot slices inoculated with *P. carotovorum* subsp. *carotovorum*-AA1EC1 co-culture as compared to the 11.31 ± 2.6% maceration produced by the pathogen in monoculture (Figure 2B). In all assays performed, strain AA1EC1 did not produce soft rot symptoms on potato or carrot slices.

Plant growth-promoting and QQ activities of strain AA1EC1 against *Pseudomonas syringae* pv. tomato DC3000

Plant growth promoting assays

The growth promotion efficacy of strain AA1EC1 was evaluated on tomato seedlings. Seedlings of 5 cm in size from previously sterilized seeds were irrigated with strain cultures at a frequency of two irrigations per week. After 1 month, seedlings (20 plants from each treatment) were collected and shoot length, root length, stem width and dry weights of root, shoot and total weight of the seedling were measured.

As shown in Figure 3, treatment with the strain AA1EC1 resulted in a remarkable increase in all plant parameters measured except root length. They showed significant increases with respect to control plants in shoot length (Figure 3B) and stem width (Figure 3C) of 31% and 57%, respectively. In dry weight determinations, significant differences were also observed with respect to the control, representing increases of 88% in root weight (Figure 3D), 99% in shoot weight (Figure 3E) and 98% in total plant weight (Figure 3F). The persistence of the strain in the roots was analysed at the end of the experiment by counting the number of colonies per gram of root in the internal tissue, revealing

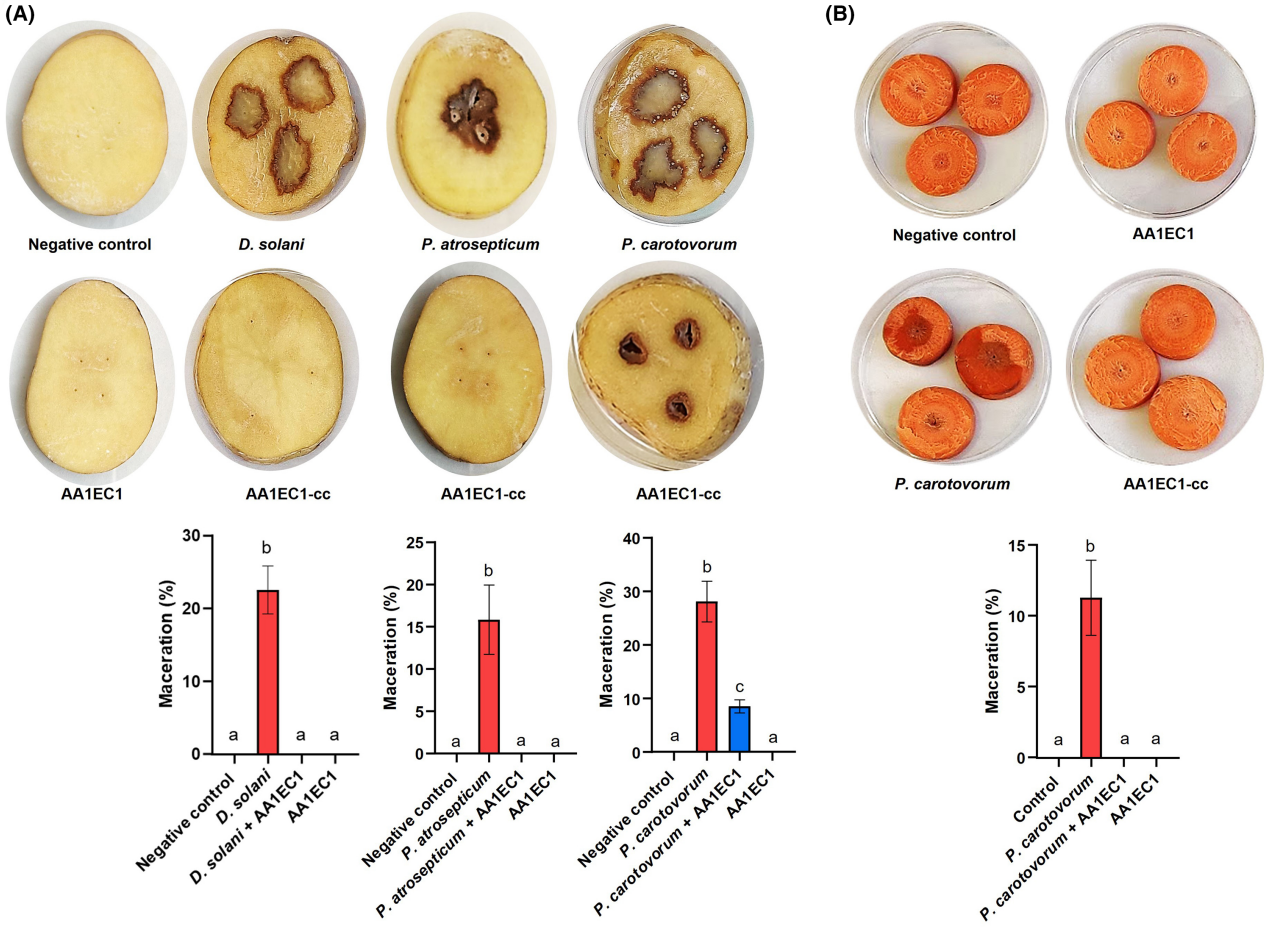


FIGURE 2 Virulence assay in potato tuber and carrot slices. Virulence and maceration of cultures and co-cultures of strain AA1EC1 and the different pathogens (*Dickeya solani*, *Pectobacterium atrosepticum*, *P. carotovorum* subsp. *carotovorum)* on the surface of potato (A) and carrot (B) slices after 48 h of incubation. Sterile water was used as a negative control. Top: Representative images of pathogen maceration damage to tissues caused by pathogens and co-cultures of pathogens on potato and carrot slices. Bottom: Percentage of maceration in potato and carrot treated with the cultures and co-cultures of the pathogens and strain AA1EC1. Data are means ± SD of three independent experiments conducted on 5 slices of fruit (potato, $n = 5$) or 6 slices of fruit (carrot, $n = 6$). Different letters above the bars indicate statistically significative differences at $p \leq 0.001$ by Kruskal–Wallis test and Dunn's post-hoc test. Error bars correspond to the standard deviation of the samples.

that AA1EC1 colonized the internal tissue of the roots with a concentration of $2.5\ 10^4 \pm 34$ CFU g^{-1} root.

QQ activities of strain AA1EC1 against *Pseudomonas syringae* pv. tomato DC3000

In vivo tomato plants infection assay

The effect of AHL degradation on the virulence of *P. syringae* pv tomato DC3000 was evaluated by in vivo assays on tomato seedlings Roma variety. Experiments to test QQ activity were performed with tomato because is one of the most important vegetable crops in the world, is a model plant for research of abiotic and biotic stress and is closely related to many commercially important plants belongs to the family *Solanaceae*. Three-week-old seedlings from previously sterilized seeds were sprayed inoculated with co-cultures of *P. syringae* pv. tomato and strain AA1EC1, prepared as previously described. One week after inoculation, seedlings were photographed and affected (necrotic and chlorotic leaves, dead leaves) and unaffected shoots were counted.

Application of the co-culture of AA1EC1-*P. syringae* pv. tomato to tomato plants revealed that the strain AA1EC1 exerted excellent biocontrol properties, suggesting its potential formulation as a biocontrol agent. As shown in Figure 4, plants treated with the co-culture exhibited less damage symptoms than those infected only with the pathogen (Figure 4A,B). Tomato plants treated with the co-culture showed an increase in the percentage of healthy leaves of 42% compared to plants inoculated with *P. syringae* pv. tomato (Figure 4C). Furthermore, marked differences were observed with respect to the number of dead and necrotic/chlorotic leaves, which decreased by 20.1% and 22%, respectively, compared to plants treated with the pathogen alone, indicating a lower incidence of pathogen virulence (Figure 4C).

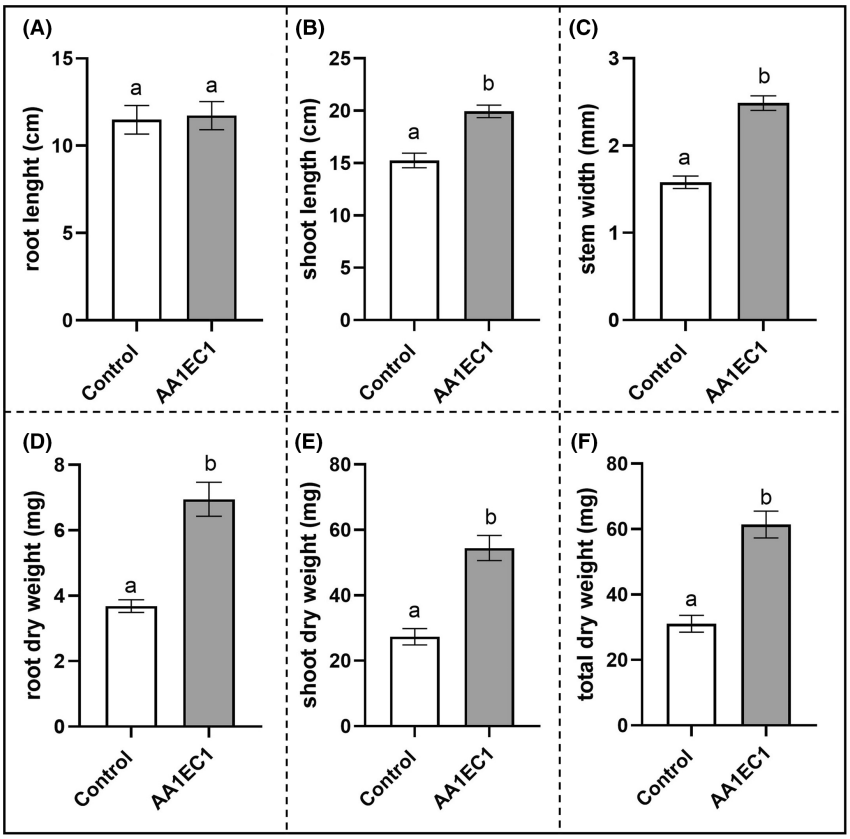


FIGURE 3 Effects on tomato seedlings after treated with strain AA1EC1 in plant growth-promoting assays. Values are expressed as mean ± SE of the mean (SEM). Different letters above the bars indicate statistically significative differences between treatments at $p \leq 0.0001$ by unpaired *T*-test or unpaired *T*-test with Welch's correction. Error bars correspond to the standard error of the mean.

Further investigation regarding the effect of QQ of strain AA1EC1 on the virulence of *P. syringae* pv. tomato DC3000 was carried out. The N-acyl-homoserine lactonase gene (MDD9262952.1) of strain AA1EC1 that showed high homology to the lactonase AiiA was expressed into the pathogenic strain. As a result, *P. syringae* pv. tomato DC3000 pME6010::*BtAiiA* did not activate the biosensor NTL4 (Figure 5A) and siderophore and IAA production and acid phosphatase and phytase activities were reduced with respect to the wild-type strain (Figure 5B). Measurements of phosphate solubilization index and siderophore index revealed that the DC3000 pME6010::*BtAiiA* strain showed a statistically significant reduction relative to the wild-type strain, exhibiting SI reductions of 14%, 11%, and 19% for acid phosphatase activity (Figure 5B-1), phytase activity (Figure 5B-2), and siderophore production (Figure 5B-3), respectively. IAA production was also affected, as the strain was able to produce 35% less IAA than the wild-type strain (Figure 5B-4).

BtAiiA expression in the pathogen also attenuated the symptoms of infection in tomato plants, reducing the percentage of affected leaves (necrotic or chlorotic) to 14%, which represented a reduction of 50% compared to the damage caused by the wild-type strain (27.7% necrotic or chlorotic leaves) (Figure 5C). Leaf chlorophyll quantification showed that plants infected with the strain had chlorophyll a, chlorophyll b and total chlorophyll concentrations similar to those of control plants and in some cases (Cb) significantly higher than those observed in plants treated with the wild-type strain (Figure 5D).

In vitro tomato leaves infection assay

A similar assay to the previous one was carried out by inoculating the co-culture of the strain AA1EC1 with *P. syringae* pv. tomato on leaves with similar morphology and size collected from 50-day-old plants. At 7 days after infection, the leaves treated with the co-culture showed lower percentages of disease severity than those observed in the leaves treated with the pathogen in monoculture, with reductions of 38%, 25.3% and 12.7% in the percentages of total damage, chlorosis damage and necrosis damage, respectively (Figure S7). Similar results were recorded 14 days after infection, with a greater difference in leaves affected by necrosis. Leaves treated with co-culture of AA1EC1 and *P. syringae* showed reductions in the percentages disease severity of 22.7% and 13.1%, in the affected areas by necrosis and chlorosis respectively, which resulted in a reduction of the percentage of the total damage on the leaves of the 35.8% (Figure S7).

DISCUSSION

Current agriculture is based on the use of chemical fertilizers, antibiotics, and pesticides to increase crop productivity, promote plant growth and inhibit growth and damage caused by phytopathogenic microorganisms

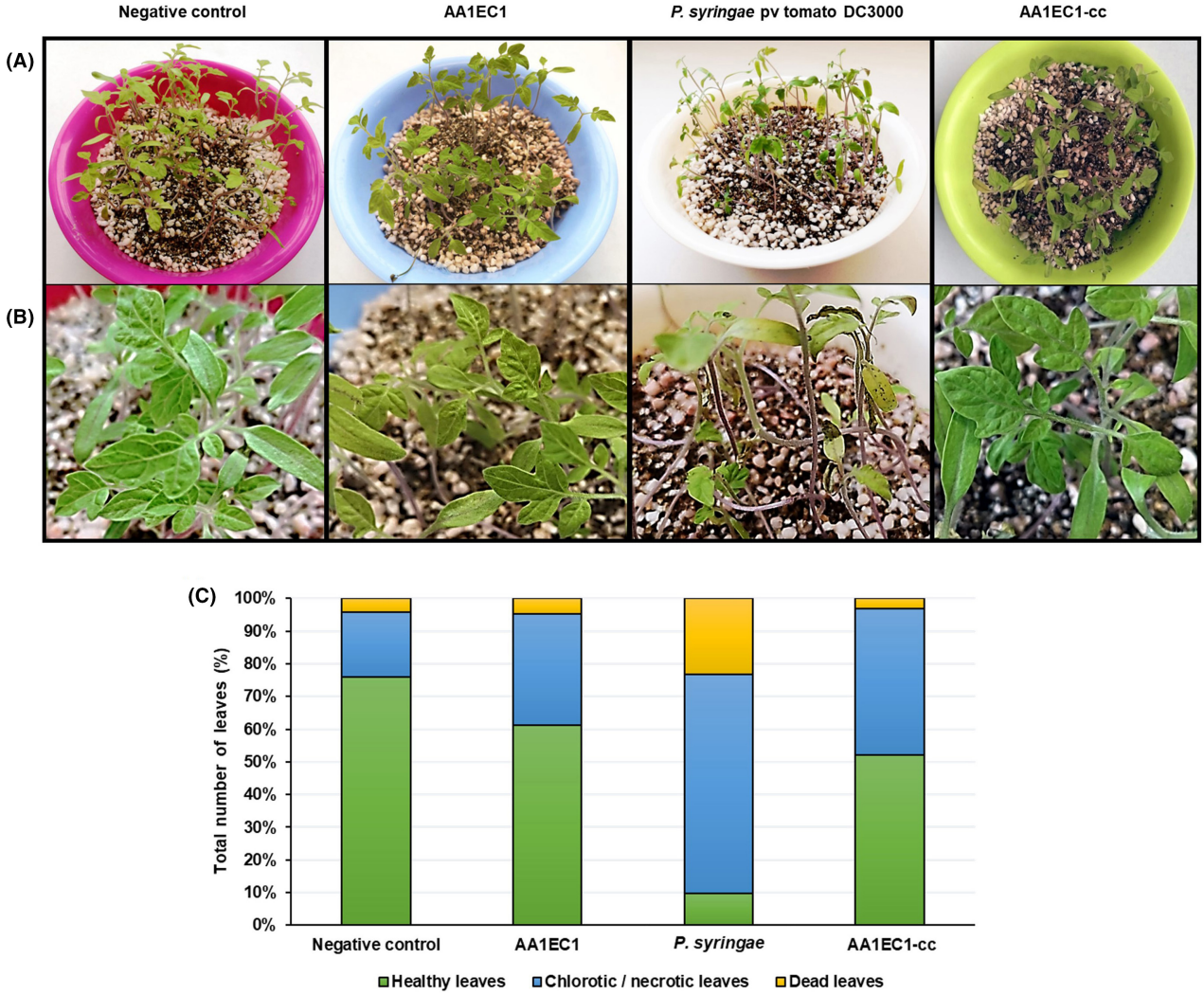
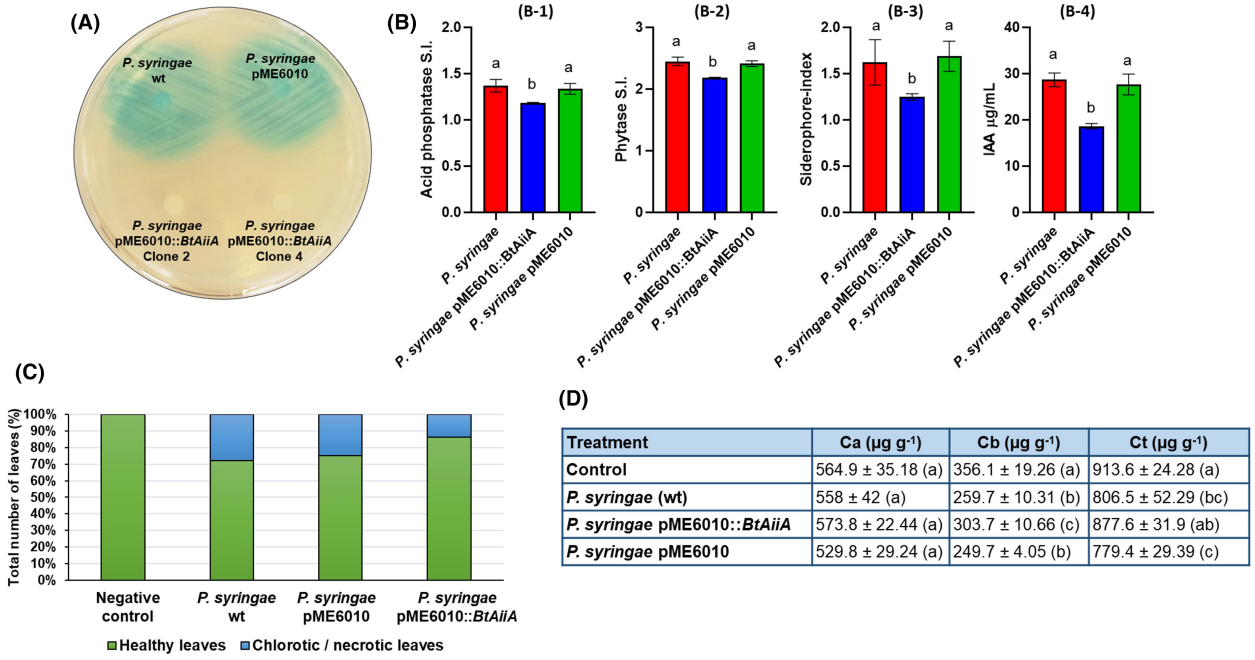


FIGURE 4 Infection assay in tomato plants (*Solanum licopersicum* var Roma) treated with cultures and co-cultures of *Pseudomonas syringae* pv. tomato DC3000 and *Bacillus toyonensis* AA1EC1. (A) Tomato plants sprayed with sterile water (negative control), strain AA1EC1, *P. syringae* pv. tomato DC3000 and *P. syringae* pv. tomato DC3000 + strain AA1EC1 (AA1EC1-cc). (B) Photographs showing the healthy and necrotic symptoms of the leaves in each case. (C) Total percentage of healthy, necrotic, and chlorotic and dead leaves after each treatment.

(Boro et al., 2022). On the other hand, these chemical compounds, lead to environmental pollution, degrade soil quality, cause problems for human health and affect the ecological balance by disturbing the natural microflora and food chains (De Silva et al., 2019). To overcome these problems, the use of microorganisms as biocontrol agents and biofertilizers as an alternative to the use of chemical fertilizers and pesticides is currently proposed as a sustainable strategy to ensure the productivity and health of crops (Luo et al., 2022; Sharma, 2020). Among the microorganisms used as PGP bacteria or as biocontrol agents, species included in the genera *Pseudomonas*, *Azotobacter*, *Bacillus*, *Spirillium*, *Microbacterium*, *Mesorhizobium*, *Flavobacterium*, *Achromobacter*, etc. are highlighted (Glick, 1995; Ma et al., 2009; Narożna et al., 2015). There are several studies that demonstrate the great potential of some of the species of the *Bacillus* genus, such as *B. subtillis*, *B. amyloliquefaciens*, *B. velezensis*, *B. thuringiensis*, etc. (Boro et al., 2022; Luo et al., 2022) as PGP bacteria. An example is represented by several strains of *B. amyloliquefaciens* species, which shows many traits that promote plant growth and protect them against pathogens (Luo et al., 2022).

In the present work, we have identified a halotolerant strain belonging to the genus *Bacillus*, an endophyte of the halophyte plant *Arthrocaulon* sp. The sequencing and analysis of the genome of the AA1EC1 strain, as well as the results obtained from phylogenetic analyses based on ANI and dDHH, have allowed us to assert that this strain belongs to the *B. toyonensis* species.

The first strain described of this species was *B. toyonensis* BCT-7112 (NCIMB 14858), which was initially identified as *B. cereus* var *toyoi*. This strain has been used for more than 30 years as the active ingredient of the preparation TOYOCERIN®, an additive used in



Treatment	Ca (µg g^{-1})	Cb (µg g^{-1})	Ct (µg g^{-1})
Control	564.9 ± 35.18 (a)	356.1 ± 19.26 (a)	913.6 ± 24.28 (a)
P. syringae (wt)	558 ± 42 (a)	259.7 ± 10.31 (b)	806.5 ± 52.29 (bc)
P. syringae pME6010::*BtAiiA*	573.8 ± 22.44 (a)	303.7 ± 10.66 (c)	877.6 ± 31.9 (ab)
P. syringae pME6010	529.8 ± 29.24 (a)	249.7 ± 4.05 (b)	779.4 ± 29.39 (c)

FIGURE 5 Effect of the expression of BtAiiA on *Pseudomonas syringae* pv. tomato DC3000. (A) Well-diffusion agar-plate assay to detect AHL production of wild type strain and its transformants (pME6010 and pME::BtAiiA) using the biosensor *Agrobacterium tumefaciens* NLT4 (pZRL4). 20 µL of each bacterial culture extracts were placed in each sterile disk. (B) Phenotypes affected by the AHL degradation produced by BtAiiA, solubilization index for phosphatase and phytase activities, siderophore index and IAA quantification (µg mL^{-1}) of wild type strain and its transformants. Data are means ± SD of three independent experiments. Different letters above the bars indicate statistically significative differences at $p \leq 0.01$ by Kruskal–Wallis test and Dunn's post-hoc test. Error bars correspond to the standard deviation of the samples. (C) Total percentage of healthy and necrotic-chlorotic leaves after treatment tomato plants (*Solanum licopersicum* var gordo rama) with cultures of *Pseudomonas syringae* pv. tomato DC3000 and its transformants. (D) Chlorophyll quantification (µg g^{-1} fresh tissue) in the leaves of tomato treated with cultures of *Pseudomonas syringae* pv. tomato DC3000 and its transformants. Ca, chlorophyll a; Cb, chlorophyll b; Ct, total chlorophyll. Data are means ± SD of three independent samples. Different letters located in the same column of the table indicate statistically significative differences at $p \leq 0.01$ by Kruskal–Wallis test and Dunn's post-hoc test.

animal nutrition (e.g., swine, poultry, cattle, rabbits, and aquaculture) (Jiménez et al., 2013). Among the strains described within the *B. toyonensis* species, there are few studies showing the ability of these microorganisms to promote plant growth or their biocontrol potential. The initial characterization performed in our laboratory allowed us to identify several characteristics of strain AA1EC1 associated with its capability to promote plant growth such as its ability to solubilize inorganic and organic phosphate (alkaline phosphatase activity and phytase activity), produce IAA and siderophores, as well as the enzymes amylase, protease, and β-glucosidase. The strain AA1EC1 also hydrolyses DNA, lecithin, gelatine, and Tween 80. Some of these properties have been described in strains of *B. toyonensis* species (COPE52, Bt04, MG430287) that have shown the ability to promote the growth of blueberry and tomato plants under salt stress (strain COPE52, Contreras-Pérez et al., 2019; Rojas-Solis et al., 2020); promote maize growth and enhance its root development under aluminium toxicity (strain Bt04, Zerrouk et al., 2020) and promote *Brassica juncea* growth (strain MG430287, Jinal et al., 2019).

The main phenotypic difference between strain AA1EC1 and other strains of this species is its ability to solubilize organic phosphate by the production of phytase and alkaline phosphatase enzymes. These activities can provide an improvement in plant nutrition by making available to the plant the insoluble forms of phosphate present in the soil. Taken together, the biochemical activities observed in strain AA1EC1 allow us to affirm that this strain exhibits many features to exert a positive effect on plant growth. To test whether the inoculation of the strain had plant growth promoting effect, we carried out plant growth promotion assays on tomato plants. Our results revealed that the inoculation of the plants with strain AA1EC1 resulted in significant increases over control plants in shoot length and width of 31% and 57%, respectively. In dry weight determinations, significant differences respect to the control were observed, representing increases of 88% in root weight, 99% in shoot weight, and 98% in total plant weight (Figure 3).

Most plant pathogenic bacteria such as *P. carotovorum*, *A. tumefaciens*, *Pantoea stewartii*, *Ralstonia solanacearum*, *P. syringae*, *P. aeruginosa* and *Xanthomonas campestris* are Gram-negative bacteria and produce N-acylhomoserine lactones (AHLs) as signal molecules to control genes related to pathogenesis and

colonization of host surfaces. Traits regulated under QS in plant pathogenic bacteria include production of EPS, hydrolytic enzymes, antibiotics, siderophores, motility, biofilm formation, and epiphytic fitness (Ansari & Ahmad, 2018; von Bodman et al., 2003). Therefore, mechanisms based on enzymatic degradation of AHLs by lactonases, acylases and oxidoreductases are proposed as a novel strategy to combat diseases caused by plant pathogenic bacteria. *Bacillus* species are among the most promising bacteria producing AHL-lactonases. Recent studies by Noor et al. (2022) have revealed that AiiA lactonase is common among strains of *B. subtilis* species showing a high degree of sequence identity.

The results obtained in the present work using specific biosensor strains as well as analysis by HPLC-MRM have revealed that strain AA1EC1 presents a high efficiency in AHLs degradation, since this strain was able to degrade all the synthetic AHLs analysed. To identify potential enzymes involved in such enzymatic degradation we performed a bioinformatic analysis of the AA1EC1 genome looking for potential enzymes with AHLs degrading activity. We have identified 4 proteins that present high percentages of identity with respect to enzymes with AHLs degrading activity, 3 proteins with potential lactonase activity "Quorum-quenching lactonase YtnP" (Protein No. MDD9261378.1), "N-acyl-homoserine lactonase" (Protein No. MDD9262952.1), "Metallo-beta-lactamase superfamily" (Protein No. MDD9259744.1) and one with acylase activity "Penicillin acylase" (Protein No. MDD9262231.1). The expression of these 4 genes in *E. coli* corroborated the degradation of AHLs and the type of activity detected in silico, being the most efficient enzyme the one annotated as "N-acyl-homoserine lactonase" (Protein No. MDD9262952.1) (Figure S4). It should be noted that the sequence of the protein MDD9262952.1 showed a high identity with respect to that annotated as AiiA-like protein of *B. thuringiensis* serovar *kyushuensis* (98.54% identity) and *B. toyonensis* BAC3151 (100% identity). Lopes et al. (2017) identified in silico this protein in *B. toyonensis* BAC3151; however, there are no studies to date that have verified the QQ activity of this protein in this species. The alignment between the AiiA-like proteins of strains BAC3151 and AA1EC1 presented a high percentage of similarity, and we could observe that both enzymes presented the motif ^{106}HXDH-H^{169}-D^{191}-Y^{194} necessary for the enzyme activity (Dong et al., 2000, 2002; Lu et al., 2006) (data not shown).

Relative to the other two lactonases identified in the AA1EC1 genome, "Quorum-quenching lactonase YtnP" (Protein No. MDD9261378.1), and "Metallo-beta-lactamase" (Protein No. MDD9259744. 1), the annotated protein MDD9261378.1, showed a high degree of similarity with other proteins described as lactonase in other *Bacillus* species such as *B. subtilis* strain 168, *B. licheniformis* T-1 and *B. paralicheniformis* ZP1. Recently Peng et al. (2021) have revealed that the YtnP protein of *B. licheniformis* strain T-1 causes a reduction in virulence of *Aeromonas hydrophila* ATCC 7966 in *Carassius auratus* both in vitro and in vivo. On the other hand, recent studies carried out by Djokic et al. (2022) showed that this enzyme in *B. paralicheniformis* ZP1 is highly effective in reducing the virulence of *P. aeruginosa* PAO-1 in zebrafish. To our knowledge, this is the first time that this enzyme has been described in the species *B. toyonensis*.

The third lactonase of strain AA1EC1, "Metallo-beta-lactamase" (Protein No. MDD9259744.1) showed high similarity to the ComEC/Rec2 protein from *B. toyonensis* BCT-7112 and an enzyme with metallo-beta-lactamase activity from *B. cereus* Rock3-28.

The acylase detected in the AA1EC1 genome, "Penicillin acylase" (Protein no. MDD9262231.1), showed high similarity to other penicillin acylases annotated in *B. thuringiensis* and *B. toyonensis* species. Although previous studies have reported that penicillin acylases are able to degrade AHL in other bacteria, for example, PaPVA enzyme from *P. atrosepticum*, AtPVA from *A. tumefaciens* (Sunder et al., 2017), PvdQ and QuiP from *P. aeruginosa* PAO-1, and enzymes with acylase activity identified in *Stenotrophomonas maltophilia* M9-54 (Reina et al., 2019), *Pseudomonas segetis* P6 (Rodríguez et al., 2020) and *Peribacillus castrilensis* N3 (Rodríguez et al., 2022), this activity had not previously been identified in *B. toyonensis* species.

The array of enzymes with QQ activity detected in AA1EC1 suggests that this strain has a potential capacity to reduce the QS-virulence traits of Gram-negative pathogenic bacteria. Thus, we evaluated the efficiency of *B. toyonensis strain* AA1EC1 to degrade the AHL produced by the pathogenic bacteria *D. solani* IPO 2222^T, *P. atrosepticum* CECT 314^T, *P. carotovorum* subsp. *carotovorum* CECT 225^T, and *P. syringae* pv. tomato DC3000 in co-cultures. Total AHL degradation was shown in *D. solani* and *P. syringae* pv. tomato, whereas partial degradation of AHLs produced by *P. atrosepticum* and *P. carotovorum* was observed, showing high activity against the short and medium-chain AHLs.

To confirm the effect of AHL-degrading activity of strain AA1EC1 against the virulence of different plant pathogens, in vitro and in vivo assays were carried out against different phytobacteria that cause great economic losses in agronomically relevant crops. Our studies have shown that the strain AA1EC1 significantly attenuated the virulence of the pathogens analysed, causing reduction of disease symptoms compared those caused by the pathogens alone in the plants and fruits analysed. A slight decrease in the percentage of healthy tomato leaves after AA1EC1 inoculation was observed in in vivo assays compared to uninoculated control plants, while no such impact was observed in in vitro assays. This effect will be the subject of future research, but it may be attributed to the production of

any of the secondary metabolites or hydrolytic enzymes that AA1EC1 has the genetic potential to synthesize.

Similar results have been reported in the literature, describing plant-associated bacteria such as *P. segetis* P6 and *Staphylococcus equorum* EN21 capable of decreasing the virulence of these pathogens by QQ mechanisms involving enzymatic degradation of AHLs produced by these phytopathogens (Rodríguez et al., 2020; Vega et al., 2020).

On the other hand, AA1EC1 strain did not exert any antagonistic activity against the pathogens and caused a reduction in several virulence factors that are controlled by QS mechanisms. In *D. solani*, it is widely described that the main virulence factors involved in the development of disease symptoms are plant cell wall degrading enzymes (PCWDE). This group of proteins includes pectinases, cellulases and proteases, whose combined action leads to maceration of plant tissues (Reverchon & Nasser, 2013). Co-culture of strain AA1EC1 with *D. solani* strikingly affected the cellulase and xylanase activities of the pathogen, as these activities were totally inhibited. Our results agree with those observed by Singh et al. (2021) in which they observed an inhibition of the activities of different PCWDEs enzymes of *P. carotovorum* such as polygalacturonase, pectinase, protease and cellulase when exposed to extracts of the QQ bacterium *Bacillus* sp. OA10.

Importantly, another effect observed was the reduction of siderophore synthesis and IAA production in all pathogens. Siderophores play a very important role among the competition mechanisms of plant-associated microorganisms since these molecules help to capture iron found in insoluble form in soil and thus favour its competition for the niche (Pizarro-Tobias et al., 2020; Roca et al., 2013). On the other hand, it is important to note that recent studies describe IAA as a global signal molecule. The ability to synthesize IAA is widely distributed in plant-associated bacteria (Duca & Glick, 2020) and there are numerous studies demonstrating the key role of IAA as a signal molecule in bacteria regulating stress resistance, antibiotic biosynthesis and virulence factors, nutrient transport and bacterial catabolism, among other processes (Cassan et al., 2021; Djami-Tchatchou et al., 2022; Duca et al., 2014; Duca & Glick, 2020; Kunkel & Johnson, 2021; Laird et al., 2020; Rico-Jiménez et al., 2022).

Another phenotypic activity reduction was observed in the phytase activity of the pathogens *D. solani*, *P carotovorum*, and *P. syrinage*. Chatterjee et al. (2003) reported that a mutant of *X. oryzae* pv. *oryzae* defective in the *phyA* gene coding for a phytase showed reduced virulence in rice plants. According to these authors, this loss of virulence could be due, at least in part, to the inability of the pathogen to utilize host phytic acid as a source of phosphate. This mechanism could also occur in *D. solani*, *P carotovorum* and *P. syrinage* pv. tomato DC3000 analysed in the present work.

CONCLUSIONS

In this study, AA1EC1, a novel strain of *B. toyonensis* isolated from the halophyte plant *Arthrocaulon* sp. was successfully evaluated as a potential biocontrol agent based on its plant growth-promoting and quorum-quenching activities. Several lactonases and an acylase enzymes capable of degrading AHLs are identified in the species *B. toyonensis*. In vitro and in vivo experiments have demonstrated that strain AA1EC1 attenuated the QS-mediated virulence factors of agriculture relevant phytopathogens through the enzymatic degradation of AHLs. To our knowledge, this is the first study in which PGP and QQ activities has been reported in the species *B. toyonensis*.

AUTHOR CONTRIBUTIONS

Amalia Roca: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Mónica Cabeo:** Investigation (equal). **Carlos Enguidanos:** Investigation (equal). **Fernando Martínez-Checa:** Conceptualization (equal); investigation (equal); methodology (equal); software (equal). **Inmaculada Sampedro:** Conceptualization (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); visualization (equal). **Inmaculada Llamas:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGEMENTS

This research was funded by Spanish Ministry for Science and Innovation/Agencia Estatal de Investigación 10.13039/501100011033 and by ESF Investing in your future (Grant: RYC2019- 026481-I) to A.R. F.M.-C., I.S. and I.L. were supported by Spanish Ministry for Science and Innovation/Agencia Estatal de Investigación 10.13039/501100011033 (Grant: PID2019-106704RB-100), by Consejería de Universidad, Investigación e Innovación de la Junta de Andalucía and ERDF A way of making Europe (Grant: B-AGR-222-UGR20).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ORCID

Amalia Roca https://orcid.org/0000-0003-2332-3112
Fernando Martínez-Checa https://orcid.org/0000-0003-2852-5071
Inmaculada Sampedro https://orcid.org/0000-0003-0528-3954
Inmaculada Llamas https://orcid.org/0000-0001-6333-5033

REFERENCES

Alexander, D. & Zuberer, D. (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology and Fertility of Soils*, 12, 39–45.

Almasri, M.N. & Kaluarachchi, J.J. (2004) Implications of on-ground nitrogen loading and soil transformations on ground water quality Management1. *JAWRA Journal of the American Water Resources Association*, 40, 165–186.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402.

Ansari, F.A. & Ahmad, I. (2018) Biofilm development, plant growth promoting traits and rhizosphere colonization by *Pseudomonas entomophila* FAP1: a promising PGPR. *Advances in Microbiology*, 8, 235–251.

Auch, A.F., von Jan, M., Klenk, H.-P. & Göker, M. (2010) Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Standards in Genomic Sciences*, 2, 117–134.

Ayyappan, S.V. & Bhaskaran, K. (2022) Extracellular lactonase-mediated quorum quenching by a novel *Bacillus velezensis*. *FEMS Microbiology Letters*, 369, fnac095.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A. et al. (2008) The RAST server: rapid annotations using subsystems technology. *BMC Genomics*, 9, 75.

Baird-Parker, A.C. (1963) A classification of micrococci and staphylococci based on physiological and biochemical tests. *Microbiology*, 30, 409–427.

Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S. et al. (2012) SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, 19, 455–477.

Barrow, G.I. & Feltham, R.K.A. (Eds.). (1993) *Cowan and Steel's manual for the identification of medical bacteria*, 3rd edition. Cambridge: Cambridge University Press.

Boro, M., Sannyasi, S., Chettri, D. & Verma, A.K. (2022) Microorganisms in biological control strategies to manage microbial plant pathogens: a review. *Archives of Microbiology*, 204, 666.

Borriss, R. (2011) Use of plant-associated bacillus strains as biofertilizers and biocontrol agents in agriculture. In: Maheshwari, D.K. (Ed.) *Bacteria in agrobiology: plant growth responses*. Berlin, Heidelberg: Springer, pp. 41–76.

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J. et al. (2015) RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. *Scientific Reports*, 5, 8365.

Bushnell, B. (2014) BBMap: a fast, accurate, splice-aware aligner.

Carver, T., Harris, S.R., Berriman, M., Parkhill, J. & McQuillan, J.A. (2012) Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. *Bioinformatics*, 28, 464–469.

Cassan, F.D., Coniglio, A., Amavizca, E., Maroniche, G., Cascales, E., Bashan, Y. et al. (2021) The *Azospirillum brasilense* type VI secretion system promotes cell aggregation, biocontrol protection against phytopathogens and attachment to the microalgae *Chlorella sorokiniana*. *Environmental Microbiology*, 23, 6257–6274.

Castro, D.J., Cerezo, I., Sampedro, I. & Martínez-Checa, F. (2018) *Roseovarius ramblicola* sp. nov., a moderately halophilic bacterium isolated from saline soil in Spain. *International Journal of Systematic and Evolutionary Microbiology*, 68, 1851–1856.

Chatterjee, S., Sankaranarayanan, R. & Sonti, R.V. (2003) PhyA, a secreted protein of *Xanthomonas oryzae* pv. oryzae, is required for optimum virulence and growth on Phytic acid as a sole phosphate source. *IS-MPMI*, 16, 973–982.

Chilton, M.-D., Currier, T.C., Farrand, S.K., Bendich, A.J., Gordon, M.P. & Nester, E.W. (1974) *Agrobacterium tumefaciens* DNA and PS8 bacteriophage DNA not detected in crown gall tumors. *Proceedings of the National Academy of Sciences*, 71, 3672–3676.

Cho, H.-S., Park, S.-Y., Ryu, C.-M., Kim, J.F., Kim, J.-G. & Park, S.-H. (2007) Interference of quorum sensing and virulence of the rice pathogen *Burkholderia glumae* by an engineered endophytic bacterium. *FEMS Microbiology Ecology*, 60, 14–23.

Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S. et al. (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 68, 461–466.

Contreras-Pérez, M., Hernández-Salmerón, J., Rojas-Solís, D., Rocha-Granados, C., Orozco-Mosqueda, M.C., Parra-Cota, F.I. et al. (2019) Draft genome analysis of the endophyte, *Bacillus toyonensis* COPE52, a blueberry (*Vaccinium* spp. var. *Biloxi*) growth-promoting bacterium. *3 Biotech*, 9, 370.

Crépin, A., Beury-Cirou, A., Barbey, C., Farmer, C., Hélias, V., Burini, J.-F. et al. (2012) N-acyl homoserine lactones in diverse *Pectobacterium* and *Dickeya* plant pathogens: diversity, abundance, and involvement in virulence. *Sensors*, 12, 3484–3497.

De Silva, N.I., Brooks, S., Lumyong, S. & Hyde, K.D. (2019) Use of endophytes as biocontrol agents. *Fungal Biology Reviews*, 33, 133–148.

Djami-Tchatchou, A.-T., Li, Z.A., Stodghill, P., Filiatrault, M.J. & Kunkel, B.N. (2022) Identification of indole-3-acetic acid-regulated genes in *Pseudomonas syringae* pv. tomato strain DC3000. *Journal of Bacteriology*, 204, e00380-21.

Djokic, L., Stankovic, N., Galic, I., Moric, I., Radakovic, N., Šegan, S. et al. (2022) Novel quorum quenching YtnP lactonase from *Bacillus paralicheniformis* reduces *Pseudomonas aeruginosa* virulence and increases antibiotic efficacy in vivo. *Frontiers in Microbiology*, 13, 906312.

Dong, Y.-H., Gusti, A.R., Zhang, Q., Xu, J.-L. & Zhang, L.-H. (2002) Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus* species. *Applied and Environmental Microbiology*, 68, 1754–1759.

Dong, Y.-H., Xu, J.-L., Li, X.-Z. & Zhang, L.-H. (2000) AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proceedings of the National Academy of Sciences*, 97, 3526–3531.

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.

Duca, D.R. & Glick, B.R. (2020) Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. *Applied Microbiology and Biotechnology*, 104, 8607–8619.

Eshaghi, E., Nosrati, R., Owlia, P., Malboobi, M.A., Ghaseminejad, P. & Ganjali, M.R. (2019) Zinc solubilization characteristics of efficient siderophore-producing soil bacteria. *Iran Journal of Microbiology*, 11, 419–430.

Fan, X., Ye, T., Li, Q., Bhatt, P., Zhang, L. & Chen, S. (2020) Potential of a quorum quenching bacteria isolate *Ochrobactrum*

intermedium D-2 against soft rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum*. *Frontiers in Microbiology*, 11, 898.
Faure, D. & Dessaux, Y. (2007) Quorum sensing as a target for developing control strategies for the plant pathogen *Pectobacterium*. *European Journal of Plant Pathology*, 119, 353–365.
Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39, 783–791.
Fetzner, S. (2015) Quorum quenching enzymes. *Journal of Biotechnology*, 201, 2–14.
Florencio, C., Couri, S. & Farinas, C.S. (2012) Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by *Trichoderma* strains. *Enzyme Research*, 2012, e793708.
Frikha-Gargouri, O., Ben Abdallah, D., Bhar, I. & Tounsi, S. (2017) Antibiosis and *bmyB* gene presence as prevalent traits for the selection of efficient *Bacillus* biocontrol agents against crown gall disease. *Frontiers in Plant Science*, 8, 1363.
Fuqua, W.C., Winans, S.C. & Greenberg, E.P. (1994) Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *Journal of Bacteriology*, 176, 269–275.
Garge, S.S. & Nerurkar, A.S. (2016) Attenuation of quorum sensing regulated virulence of *Pectobacterium carotovorum* subsp. carotovorum through an AHL lactonase produced by *Lysinibacillus* sp. Gs50. *PLoS One*, 11, e0167344.
Glick, B. (1995) The enhancement of plant growth by free-living bacteria. *Canadian Journal of Microbiology*, 41, 109–117.
Gong, X., Gruninger, R.J., Qi, M., Paterson, L., Forster, R.J., Teather, R.M. et al. (2012) Cloning and identification of novel hydrolase genes from a dairy cow rumen metagenomic library and characterization of a cellulase gene. *BMC Research Notes*, 5, 566.
Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P. & Tiedje, J.M. (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *International Journal of Systematic and Evolutionary Microbiology*, 57, 81–91.
Gotor-Vila, A., Usall, J., Torres, R., Solsona, C. & Teixidó, N. (2019) Enhanced shelf-life of the formulated biocontrol agent *Bacillus amyloliquefaciens* CPA-8 combining diverse packaging strategies and storage conditions. *International Journal of Food Microbiology*, 290, 205–213.
Gurevich, A., Saveliev, V., Vyahhi, N. & Tesler, G. (2013) QUAST: quality assessment tool for genome assemblies. *Bioinformatics*, 29, 1072–1075.
Harrison, A.M. & Soby, S.D. (2020) Reclassification of *Chromobacterium violaceum* ATCC 31532 and its quorum biosensor mutant CV026 to *Chromobacterium subtsugae*. *AMB Express*, 10, 202.
Heeb, S., Itoh, Y., Nishijyo, T., Schnider, U., Keel, C., Wade, J. et al. (2000) Small, stable shuttle vectors based on the minimal pVS1 replicon for use in gram-negative, plant-associated bacteria. *Molecular Plant-Microbe Interactions*, 13, 232–237.
Helman, Y. & Chernin, L. (2015) Silencing the mob: disrupting quorum sensing as a means to fight plant disease. *Molecular Plant Pathology*, 16, 316–329.
Hosseinkhani, B. & Hosseinkhani, G. (2009) Analysis of phytase producing bacteria (*Pseudomonas* sp.) from poultry faeces and optimization of this enzyme production. *African Journal of Biotechnology*, 8, 4229–4232.
Hosseinzadeh, S., Shams-bakhsh, M. & Yamchi, A. (2013) A comparative study on effect of two different *aiiA* genes on pathogenicity factors of *Dickeya chrysanthemi* pv. *chrysanthemi*. *Archives of Phytopathology and Plant Protection*, 46, 1468–1479.
Jeffries, C.D., Holtman, D.F. & Guse, D.G. (1957) Rapid method for determining the activity of microorganisms on nucleic acids. *Journal of Bacteriology*, 73, 590–591.
Jiang, C.-H., Liao, M.-J., Wang, H.-K., Zheng, M.-Z., Xu, J.-J. & Guo, J.-H. (2018) *Bacillus velezensis*, a potential and efficient biocontrol agent in control of pepper gray mold caused by *Botrytis cinerea*. *Biological Control*, 126, 147–157.
Jiménez, G., Blanch, A.R., Tamames, J. & Rosselló-Mora, R. (2013) Complete genome sequence of *Bacillus toyonensis* BCT-7112T, the active ingredient of the feed additive preparation Toyocerin. *Genome Announcements*, 1, e01080-13.
Jinal, H.N., Gopi, K., Prittesh, P., Kartik, V.P. & Amaresan, N. (2019) Phytoextraction of iron from contaminated soils by inoculation of iron-tolerant plant growth-promoting bacteria in *Brassica juncea* L. Czern. *Environmental Science and Pollution Research*, 26, 32815–32823.
Khan, A.L., Waqas, M., Kang, S.-M., Al-Harrasi, A., Hussain, J., Al-Rawahi, A. et al. (2014) Bacterial endophyte *Sphingomonas* sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *Journal of Microbiology*, 52, 689–695.
Koul, S., Prakash, J., Mishra, A. & Kalia, V.C. (2016) Potential emergence of multi-quorum sensing inhibitor resistant (MQSIR) bacteria. *Indian Journal of Microbiology*, 56, 1–18.
Kunkel, B.N. & Johnson, J.M.B. (2021) Auxin plays multiple roles during plant–pathogen interactions. *Cold Spring Harbor Perspectives in Biology*, 13, a040022.
Laird, T.S., Flores, N. & Leveau, J.H.J. (2020) Bacterial catabolism of indole-3-acetic acid. *Applied Microbiology and Biotechnology*, 104, 9535–9550.
Lane, D. J. (1991) 16S/23S rRNA Sequencing. In *Nucleic Acid Techniques in Bacterial Systematic*. Stackebrandt, E., and Goodfellow, M. (eds). New York: John Wiley and Sons, pp. 115–175.
Larpent, J.-P. & Larpent-Gourgaud, M. (1975) Mémento technique de microbiologie: la cellule bactérienne, métabolisme, systématique, bactéries utiles, milieux de culture et réactifs, Techniques documentation.
Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: *Methods in enzymology. Plant cell membranes*. Cambridge, MA, USA: Academic Press, pp. 350–382.
Lopes, R., Cerdeira, L., Tavares, G.S., Ruiz, J.C., Blom, J., Horácio, E.C.A. et al. (2017) Genome analysis reveals insights of the endophytic *Bacillus toyonensis* BAC3151 as a potentially novel agent for biocontrol of plant pathogens. *World Journal of Microbiology and Biotechnology*, 33, 185.
Lu, X., Yuan, Y., Xue, X.-L., Zhang, G.-P. & Zhou, S.-N. (2006) Identification of the critical role of Tyr-194 in the catalytic activity of a novel N-acyl-Homoserine lactonase from marine *Bacillus cereus* strain Y2. *Current Microbiology*, 53, 346–350.
Luo, L., Zhao, C., Wang, E., Raza, A. & Yin, C. (2022) *Bacillus amyloliquefaciens* as an excellent agent for biofertilizer and biocontrol in agriculture: an overview for its mechanisms. *Microbiological Research*, 259, 127016.
Ma, Y., Rajkumar, M. & Freitas, H. (2009) Inoculation of plant growth promoting bacterium *Achromobacter xylosoxidans* strain Ax10 for the improvement of copper phytoextraction by *Brassica juncea*. *Journal of Environmental Management*, 90, 831–837.
Marketon, M.M. & González, J.E. (2002) Identification of two quorum-sensing systems in *Sinorhizobium meliloti*. *Journal of Bacteriology*, 184, 3466–3475.
Martínez-Checa, F., Quesada, E., Martínez-Cánovas, M.J., Llamas, I. & Béjar, V. (2005) *Palleronia marisminoris* gen. Nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium belonging to the 'Alphaproteobacteria', isolated from a saline soil. *International Journal of Systematic and Evolutionary Microbiology*, 55, 2525–2530.
McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M. et al. (1997) Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology*, 143, 3703–3711.

Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.-P. & Göker, M. (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*, 14, 60.

Molan, Y.Y., Ibrahim, Y.E. & Al-Masrahi, A.A. (2010) Identification in Saudi Arabia of *Pseudomonas corrugata*, the tomato pith necrosis pathogen, and assessment of cultivar resistance and seed treatment. *Journal of Plant Pathology*, 92, 213–218.

Morelle, G. (1989) Plasmid extraction procedure on miniprep scale. *Focus (BRL, USA)* 11: 7–8.

Morohoshi, T., Kato, M., Fukamachi, K., Kato, N. & Ikeda, T. (2008) N-Acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC 12472. *FEMS Microbiology Letters*, 279, 124–130.

Munguia, J. & Nizet, V. (2017) Pharmacological targeting of the host–pathogen interaction: alternatives to classical antibiotics to combat drug-resistant superbugs. *Trends in Pharmacological Sciences*, 38, 473–488.

Naik, P.R., Sahoo, N., Goswami, D., Ayyadurai, N. & Sakthivel, N. (2008) Genetic and functional diversity among fluorescent pseudomonads isolated from the rhizosphere of Banana. *Microbial Ecology*, 56, 492–504.

Narożna, D., Pudełko, K., Króliczak, J., Golińska, B., Sugawara, M., Mądrzak, C.J. et al. (2015) Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. *Applied and Environmental Microbiology*, 81, 5552–5559.

Nazarov, P.A., Baleev, D.N., Ivanova, M.I., Sokolova, L.M. & Karakozova, M.V. (2020) Infectious plant diseases: etiology, current status, problems and prospects in plant protection. *Acta Naturae*, 12, 46–59.

Noor, A.O., Almasri, D.M., Basyony, A.F., Albohy, A., Almutairi, L.S., Alhammadi, S.S. et al. (2022) Biodiversity of N-acyl homoserine lactonase (*aiiA*) gene from *Bacillus subtilis*. *Microbial Pathogenesis*, 166, 105543.

Nutter, F.W., Esker, P.D. & Netto, R.A.C. (2006) Disease assessment concepts and the advancements made in improving the accuracy and precision of plant disease data. *European Journal of Plant Pathology*, 115, 95–103.

O'Brien, P. A. (2017) Biological control of plant diseases. *Australasian Plant Pathol.* 46: 293–304.

Olanrewaju, O.S., Glick, B.R. & Babalola, O.O. (2017) Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology*, 33, 197.

Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T. et al. (2014) The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Research*, 42, D206–D214.

Papenfort, K. & Bassler, B.L. (2016) Quorum sensing signal–response systems in gram-negative bacteria. *Nature Reviews. Microbiology*, 14, 576–588.

Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P. & Tyson, G.W. (2015) CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Research*, 25, 1043–1055.

Parnell, J.J., Berka, R., Young, H.A., Sturino, J.M., Kang, Y., Barnhart, D.M. et al. (2016) From the lab to the farm: an industrial perspective of plant beneficial microorganisms. *Frontiers in Plant Science*, 7, 1110.

Peng, M., Tong, W., Zhao, Z., Xiao, L., Wang, Z., Liu, X. et al. (2021) Attenuation of *Aeromonas hydrophila* infection in *Carassius auratus* by YtnP, a N-acyl Homoserine lactonase from *Bacillus licheniformis* T-1. *Antibiotics*, 10, 631.

Pikovskaya, R.I. (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, 17, 362–370.

Pizarro-Tobias, P., Ramos, J.-L., Duque, E. & Roca, A. (2020) Plant growth-stimulating rhizobacteria capable of producing *L*-amino acids. *Environmental Microbiology Reports*, 12, 667–671.

Põllumaa, L., Alamäe, T. & Mäe, A. (2012) Quorum sensing and expression of virulence in pectobacteria. *Sensors*, 12, 3327–3349.

Quiñones, B., Dulla, G. & Lindow, S.E. (2005) Quorum sensing regulates exopolysaccharide production, motility, and virulence in *Pseudomonas syringae*. *MPMI*, 18, 682–693.

Rabbee, M.F., Ali, M.S., Choi, J., Hwang, B.S., Jeong, S.C. & Baek, K. (2019) *Bacillus velezensis*: a valuable member of bioactive molecules within plant microbiomes. *Molecules*, 24, 1046.

Reina, J.C., Torres, M. & Llamas, I. (2019) *Stenotrophomonas maltophilia* AHL-degrading strains isolated from marine invertebrate microbiota attenuate the virulence of *Pectobacterium carotovorum* and *Vibrio coralliilyticus*. *Marine Biotechnology*, 21, 276–290.

Reverchon, S. & Nasser, W. (2013) *Dickeya* ecology, environment sensing and regulation of virulence programme. *Environmental Microbiology Reports*, 5, 622–636.

Richardson, A., Barea, J., Mcneill, A. & Prigent-Combaret, C. (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant and Soil*, 321, 305–339.

Richter, M. & Rosselló-Móra, R. (2009) Shifting the genomic gold standard for the prokaryotic species definition. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 19126–19131.

Rico-Jiménez, M., Roca, A., Krell, T. & Matilla, M.A. (2022) A bacterial chemoreceptor that mediates chemotaxis to two different plant hormones. *Environmental Microbiology*, 24, 3580–3597.

Roca, A. & Matilla, M.A. (2023) Microbial antibiotics take the lead in the fight against plant pathogens. *Microbial Biotechnology*, 16, 28–33.

Roca, A., Pizarro-Tobías, P., Udaondo, Z., Fernández, M., Matilla, M.A., Molina-Henares, M.A. et al. (2013) Analysis of the plant growth-promoting properties encoded by the genome of the rhizobacterium *Pseudomonas putida* BIRD-1. *Environmental Microbiology*, 15, 780–794.

Rodríguez, M., Reina, J.C., Sampedro, I., Llamas, I. & Martínez-Checa, F. (2022) *Peribacillus castrilensis* sp. nov.: a plant-growth-promoting and biocontrol species isolated from a river otter in Castril, Granada, southern Spain. *Frontiers in Plant Science*, 13, 896728.

Rodríguez, M., Torres, M., Blanco, L., Béjar, V., Sampedro, I. & Llamas, I. (2020) Plant growth-promoting activity and quorum quenching-mediated biocontrol of bacterial phytopathogens by *Pseudomonas segetis* strain P6. *Scientific Reports*, 10, 4121.

Rojas-Solis, D., Vences-Guzmán, M.A., Sohlenkamp, C. & Santoyo, G. (2020) *Bacillus toyonensis* COPE52 modifies lipid and fatty acid composition, exhibits antifungal activity, and stimulates growth of tomato plants under saline conditions. *Current Microbiology*, 77, 2735–2744.

Romero, M., Martin-Cuadrado, A.-B., Roca-Rivada, A., Cabello, A.M. & Otero, A. (2011) Quorum quenching in cultivable bacteria from dense marine coastal microbial communities. *FEMS Microbiology Ecology*, 75, 205–217.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012) NIH image to ImageJ: 25 years of image analysis. *Nature Methods*, 9, 671–675.

Sharma, V. (2020) Plant growth promoting rhizobacteria: potential for sustainable agriculture. *Microorganisms*, 11, 1088.

Shaw, P.D., Ping, G., Daly, S.L., Cha, C., Cronan, J.E., Rinehart, K.L. et al. (1997) Detecting and characterizing N-acyl-homoserine lactone signal molecules by thin-layer chromatography. *Proceedings of the National Academy of Sciences*, 94, 6036–6041.

Sierra, G. (1957) A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence

of the contact between cells and fatty substrates. *Antonie Van Leeuwenhoek*, 23, 15–22.
Singh, A.A., Singh, A.K. & Nerurkar, A. (2021) Disrupting the quorum sensing mediated virulence in soft rot causing *Pectobacterium carotovorum* by marine sponge associated *Bacillus* sp. OA10. *World Journal of Microbiology and Biotechnology*, 37, 5.
Smadja, B., Latour, X., Faure, D., Chevalier, S., Dessaux, Y. & Orange, N. (2004) Involvement of N-acylhomoserine lactones throughout plant infection by *Erwinia carotovora* subsp. *atroseptica* (*Pectobacterium atrosepticum*). *MPMI*, 17, 1269–1278.
Sunder, A.V., Utari, P.D., Ramasamy, S., van Merkerk, R., Quax, W. & Pundle, A. (2017) Penicillin V acylases from gram-negative bacteria degrade N-acylhomoserine lactones and attenuate virulence in *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*, 101, 2383–2395.
Tamariz-Angeles, C., Huamán, G.D., Palacios-Robles, E., Olivera-Gonzales, P. & Castañeda-Barreto, A. (2021) Characterization of siderophore-producing microorganisms associated to plants from high-Andean heavy metal polluted soil from Callejón de Huaylas (Ancash, Perú). *Microbiological Research*, 250, 126811.
Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10, 512–526.
Tamura, K., Stecher, G. & Kumar, S. (2021) MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38, 3022–3027.
Tariq, M.R., Shaheen, F., Mustafa, S., Ali, S., Fatima, A., Shafiq, M. et al. (2022) Phosphate solubilizing microorganisms isolated from medicinal plants improve growth of mint. *PeerJ*, 10, e13782.
Tasse, L., Bercovici, J., Pizzut-Serin, S., Robe, P., Tap, J., Klopp, C. et al. (2010) Functional metagenomics to mine the human gut microbiome for dietary fiber catabolic enzymes. *Genome Research*, 20, 1605–1612.
Tindall, B.J., Sikorski, J., Smibert, R.A. & Krieg, N.R. (2007) Phenotypic characterization and the principles of comparative systematics. In: *Methods for general and molecular microbiology*. Washington, DC, USA: John Wiley & Sons, Ltd, pp. 330–393.
Torres, M., Dessaux, Y. & Llamas, I. (2019) Saline environments as a source of potential quorum sensing disruptors to control bacterial infections: a review. *Marine Drugs*, 17, 191.
Torres, M., Romero, M., Prado, S., Dubert, J., Tahrioui, A., Otero, A. et al. (2013) N-acylhomoserine lactone-degrading bacteria isolated from hatchery bivalve larval cultures. *Microbiological Research*, 168, 547–554.
Torres, M., Uroz, S., Salto, R., Fauchery, L., Quesada, E. & Llamas, I. (2017) HqiA, a novel quorum-quenching enzyme which expands the AHL lactonase family. *Scientific Reports*, 7, 943.
Uroz, S., D'Angelo-Picard, C., Carlier, A., Elasri, M., Sicot, C., Petit, A. et al. (2003) Novel bacteria degrading N-acylhomoserine lactones and their use as quenchers of quorum-sensing-regulated functions of plant-pathogenic bacteria. *Microbiology*, 149, 1981–1989.
Uroz, S., Dessaux, Y. & Oger, P. (2009) Quorum sensing and quorum quenching: the Yin and Yang of bacterial communication. *Chembiochem*, 10, 205–216.
van Loon, L.C. (2007) Plant responses to plant growth-promoting rhizobacteria. *European Journal of Plant Pathology*, 119, 243–254.
Vega, C., Rodríguez, M., Llamas, I., Béjar, V. & Sampedro, I. (2020) Silencing of Phytopathogen communication by the halotolerant PGPR *Staphylococcus equorum* strain EN21. *Microorganisms*, 8, 42.
Vejan, P., Abdullah, R., Khadiran, T., Ismail, S. & Nasrulhaq Boyce, A. (2016) Role of plant growth promoting Rhizobacteria in agricultural sustainability—a review. *Molecules*, 21, 573.
von Bodman, S.B., Bauer, W.D. & Coplin, D.L. (2003) Quorum sensing in plant-pathogenic bacteria. *Annual Review of Phytopathology*, 41, 455–482.
Yan, S., Liu, H., Mohr, T.J., Jenrette, J., Chiodini, R., Zaccardelli, M. et al. (2008) Role of recombination in the evolution of the model plant pathogen *Pseudomonas syringae* pv. tomato DC3000, a very atypical tomato strain. *Applied and Environmental Microbiology*, 74, 3171–3181.
Yates, E.A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S.R., Sockett, R.E. et al. (2002) N-Acylhomoserine lactones undergo Lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infection and Immunity*, 70, 5635–5646.
Yoon, S.-H., Ha, S.-M., Kwon, S., Lim, J., Kim, Y., Seo, H. et al. (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *International Journal of Systematic and Evolutionary Microbiology*, 67, 1613–1617.
Yoon, S.-H., Ha, S.-M., Lim, J., Kwon, S. & Chun, J. (2017) A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek*, 110, 1281–1286.
Zahir, Z.A., Arshad, M. & Frankenberger, W.T. (2003) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. *Advances in Agronomy*, 81, 97–168.
Zerrouk, I.Z., Rahmoune, B., Auer, S., Rößler, S., Lin, T., Baluska, F. et al. (2020) Growth and aluminum tolerance of maize roots mediated by auxin- and cytokinin-producing bacillus toyonensis requires polar auxin transport. *Environmental and Experimental Botany*, 176, 104064.
Zhang, W., Fan, X., Li, J., Ye, T., Mishra, S., Zhang, L. et al. (2021) Exploration of the quorum-quenching mechanism in *Pseudomonas nitroreducens* W-7 and its potential to attenuate the virulence of *Dickeya zeae* EC1. *Frontiers in Microbiology*, 12, 694161.
Zhang, W., Luo, Q., Zhang, Y., Fan, X., Ye, T., Mishra, S. et al. (2020) Quorum quenching in a novel *Acinetobacter* sp. XN-10 bacterial strain against *Pectobacterium carotovorum* subsp. *carotovorum*. *Microorganisms*, 8, 1100.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Roca, A., Cabeo, M., Enguidanos, C., Martínez-Checa, F., Sampedro, I. & Llamas, I. (2024) Potential of the quorum-quenching and plant-growth promoting halotolerant *Bacillus toyonensis* AA1EC1 as biocontrol agent. *Microbial Biotechnology*, 17, e14420. Available from: https://doi.org/10.1111/1751-7915.14420