Antagonistic activity of endophytic actinobacteria from native potatoes (Solanum tuberosum subsp. tuberosum L.) against Pectobacterium carotovorum subsp. carotovorum and Pectobacterium atrosepticum

Natalia Padilla-Gálvez1, Paola Luengo-Uribe1, Sandra Mancilla2, Amandine Maurin1,3, Claudia Torres1, Pamela Ruiz1,4, Andrés France5, Ivette Acuña2 and Homero Urrutia1,6*

Abstract

Background: The native potatoes (Solanum tuberosum subsp. tuberosum L.) grown in Chile (Chiloé) represent a new, unexplored source of endophytes to find potential biological control agents for the prevention of bacterial diseases, like blackleg and soft rot, in potato crops.

Result: The objective of this study was the selection of endophytic actinobacteria from native potatoes for antagonistic activity against Pectobacterium carotovorum subsp. carotovorum and Pectobacterium atrosepticum, and their potential to suppress tissue maceration symptoms in potato tubers. This potential was determined through the quorum quenching activity using a Chromobacterium violaceum ATCC 12472 Wild type (WT) bioassay and its colonization behavior of the potato plant root system (S. tuberosum) by means of the Double labeling of oligonucleotide probes for fluorescence in situ hybridization (DOPE-FISH) targeting technique. The results showed that although Streptomyces sp. TP199 and Streptomyces sp. A2R31 were able to inhibit the growth of the pathogens, only the Streptomyces sp. TP199 isolate inhibited Pectobacterium sp. growth and diminished tissue maceration in tubers (p ≤ 0.05). Streptomyces sp. TP199 had metal-dependent acyl homoserine lactones (AHL) quorum quenching activity in vitro and was able to colonize the root endosphere 10 days after inoculation.

Conclusions: We concluded that native potatoes from southern Chile possess endophyte actinobacteria that are potential agents for the disease management of soft rot and blackleg.

Keywords: Endophytic actinobacteria, Streptomyces sp., Quorum quenching, Confocal laser microscopy, Blackleg, Soft rot, Potato

Background

The world potato (S. tuberosum) production in 2018 was 368.2 million tons, with a yield of 209.4 hg ha−1[1]. One of the greatest threats to productivity is loss caused by infectious bacterial diseases, which, once introduced into the crop, may persist and be disseminated in agricultural environments unnoticed or through latent
(asymptomatic) infection of seed tubers [2, 3]. Research has estimated that 60% of these losses are due to rot, produced during the cultivation, transport, and storage of the tubers [4].

Pectobacterium carotovorum subsp. carotovorum and Pectobacterium atrosepticum are the etiological agents that cause blackleg [5] and soft rot (tubers) diseases on potato crops [6, 7]. They may enter the host through natural apertures (lenticels) or wounds and colonize the plant tissue without causing apparent signs or symptoms of the disease until the environmental conditions (free water, anaerobiosis, and temperature) are propitious for the disease development [4, 8]. The virulence of Pectobacterium sp. relies on plant cell wall-degrading enzymes (PCWDE), which disrupt the host cell integrity; however, they activate the synthesis of these exoenzymes when reaching a high population density coordinated by quorum sensing [9] through a complex set of transcription factors and posttranscriptional regulators [10].

There is no efficient chemical control against these diseases, since the ability of bactericidal compounds to disinfect seed tubers is limited [3, 4]. The method to prevent latent infection in the mother tuber is to use seed tubers derived from material not contaminated by Pectobacterium sp., which can be obtained by cutting, rapid multiplication in vitro, or by botanical seeds [4, 7, 11, 12]. Biological control is proposed as an alternative and sustainable tool for preventing and controlling infectious diseases in plants [13, 14]. This involves studying the interactions of the controlling agent with the host and the pathogen to reduce the pathogen inoculum or to control the severity of disease symptoms by avoiding the expression of their virulence factors [14, 15].

In potato crops, the rhizospheric bacterial community appears to be dominated by alphaproteobacteria and actinobacteria [16], as does the endophytic bacterial community. However, this may vary according to management systems [17, 18]. On the other hand, different studies have found different isolates of actinobacteria with demonstrated antagonist activity against phytopathogens in vitro and in vivo [9, 19–23]. Facultative endophytes could be crucial to obtain plant probiotic agents because their adaptive behavior to colonize the root surface could be a decisive step for the expression of beneficial effects for the host plant [24–26].

The study of endophytes has emerged as an alternative method to control vascular wilting diseases [27]. Endophytes have been shown to stimulate plant growth [28–30] in addition to inducing biotic stress resistance [31], suppressing disease [32], and effectively competing for the space available for pathogens [13]. The potential of bacterial endophytes to inhibit different fungal and bacterial plant pathogens has been reported [33–35].

There is a unique germplasm of native potatoes in Chile on the island of Chiloé, which is considered a sub-center for the origin of potatoes grown worldwide. The island’s isolation has allowed the proliferation of a variety of native potatoes preserved in small fields, characterized by their shapes, sizes, colors, and phenological characteristics [36]. Currently there is no information on the endophytic microbiota associated with these native varieties; however, according to previous results in different varieties, endophytic actinobacteria is a promising bacterial group for the engineering approaches of the endospheric microbiome [37]. Therefore, we investigated the in vitro antagonist capacity of actinobacteria endophytes present in Chilean native potatoes against P. carotovorum subsp. carotovorum and P. atrosepticum to assess their potential as agents for the preventive disease management of blackleg and soft rot in potato crops.

**Results**

**Characterization of endophytic actinobacteria**

Ten isolates of putative endophyte actinobacteria were obtained from native Chiloé potatoes differentiated by their phenotypic characterization and 16S ribosomal gene analysis (Table 1, Fig. 3). Of these, nine isolates were related to the genus Streptomyces sp., and one isolate was related to the genus Nocardiia sp. (Table 1, Fig. 3).

For the following ultramicroscopic visualization using scanning electron microscopy, three bacterial isolated were selected. Nocardiia sp. CR34 was the only isolation obtained from this genus and was observed according the typical fragment mycelia in liquid (ISP1- tryptone-yeast extract broth) (Fig. 1A) and solid (ISP2- yeast extract and malt extract agar) culture (Fig. 1B).

Then, Streptomyces sp. TP199 and Streptomyces sp. A2R31 were selected as they showed antimicrobial activity in vitro (Fig. 2), and their ultramicroscopic visualization showed the long chains of spore growth only in ISP2-agar typical of Streptomyces sp. (Fig. 1D and F).

For the Nocardiia sp. CR34 isolate (Fig. 3), the morphology corresponded to characteristic pseudomycelial growth, both in ISP2-agar culture (Fig. 1B) and in liquid ISP1 culture (Fig. 1A). The other nine isolates were related to Streptomyces sp. (Fig. 3), showing mycelial morphology in liquid culture for isolates TP199 (Fig. 1C) and A2R31 (Fig. 1E). The spores could be distinguished on ISP2-agar culture, with a smooth texture in the A2R31 isolate (Fig. 1F) and a warty texture for the TP199 isolate (Fig. 1D).

**Antagonism**

Of the ten isolates obtained, only Streptomyces sp. TP199 and Streptomyces sp. A2R31 inhibited the growth of P. carotovorum subsp. carotovorum and P. atrosepticum.
Table 1  Macro- and microscopic phenotype descriptions of putative endophyte actinobacteria of native Chilean *Solanum tuberosum* subsp. *tuberosum* with their taxonomic identity according to 16S ribosomal gene analysis

| Isolate code | Origin of plant tissue | Culture media a | Aerial mycelia b | Substrate mycelia b | Soluble pigment c | Filament diameter | Spore diameter | Spore disposition d | Identity by 16S ribosomal DNA partial gene | Closest taxon strain in NCBI | Nucleotide sequence | Similarity in Blast analysis | Accession in NCBI database |
|--------------|------------------------|-----------------|-----------------|-------------------|------------------|------------------|---------------|--------------------|--------------------------------|-----------------|----------------|----------------------------|---------------------------|
| H2TP199      | Leaf                   | B               | MB              | MB                | gray             | orange           | 0.77 um      | 0.62 um            | Rectiflexibiles              | Streptomyces galilaeus strain P-YIM40 (GenBank: MT533915.1) | 1051 bp         | 100%                     | KY242591                  |
| G1TP199      | Root                   | MB              | MB              | B                 | gray             | orange           | < 0.5 um     | < 0.5 um           | ND d                         | Streptomyces sp. | 1036 bp         | 99.2%                     | KY242592                  |
| KR31         | Stem                   | B               | B               | B                 | white            | brown            | 0.72 um      | < 0.5 um           | Retinaculaperti              | Streptomyces sp. | 803 bp          | 98.9%                     | KY242593                  |
| DP143        | Tubercle               | MB              | MB              | MB                | white/gray       | brown            | 0.67 um      | < 0.5 um           | Retinaculaperti              | Streptomyces sp. YRA147 (GenBank: LC479916.1) | 1104 bp         | 99.8%                     | KY242596                  |
| A2R31        | Stem                   | MB              | EX              | B                 | gray             | brown            | 0.77 um      | 0.89 um           | Rectiflexibiles              | Streptomyces achromogenes strain NBRC 12735 | 969 bp          | 100%                     | KY242590                  |
| CR34         | Tubercle               | MB              | B               | B                 | ND e              | cream            | < 0.5 um     | < 0.5 um           | ND e                         | Nocardia sp. | 750 bp          | 99.9%                     | KY296348                  |
| HP171        | Root                   | MB              | MB              | EX                | white            | orange           | 0.67 um      | 0.66 um           | Rectiflexibiles              | Streptomyces galilaeus strain PSWC-10 (GenBank: MT275786.1) | 1027 bp         | 99.9%                     | KY242594                  |
| MP136        | Leaf                   | MB              | MB              | MB                | black            | yellow           | 0.56 um      | 0.66 um           | Spira                        | Streptomyces sp. CH9-7 (GenBank: LC489240.1) | 1078 bp         | 99.8%                     | KY242595                  |
| Isolate code | Origin of plant tissue | Culture media a | Aerial mycelia b | Substrate mycelia b | Soluble pigment c | Filament diameter | Spore diameter | Spore disposition d | Identity by 16S ribosomal DNA partial gene | Closest taxa strain in NCBI | Nucleotide sequence | Similarity in Blast analysis | Accession in NCBI database |
|--------------|------------------------|-----------------|-----------------|-------------------|-----------------|------------------|---------------|-------------------|----------------------------------------|-----------------------------|------------------------|--------------------------|------------------------|
| TP199        | Tubercle               | EX, B, MB       | gray            | yellow            | –                | 0.56 µm          | <0.5 µm       | Straight to flexuous chains | Streptomyces sp.             | ISPK13-6 (GenBank: LC487830.1) | 1147 bp                | 99.6%                    | KY228978                 |
| NP199        | Tubercle               | MB, MB, MB      | gray            | yellow            | –                | 0.77 µm          | 0.58 um       | ND e                        | Streptomyces sp.             | ISPK13-6 (GenBank: HST09: KX130883.1) | 1114 bp                | 99.3%                    | KY242597                 |

a Culture media according to [38], ISP2 (yeast extract and– malt extract agar), ISP3 (oatmeal agar) and ISP4 (inorganic salts and– starch agar). Furthermore, excellent (EX, 76 to 100%), very good (MB, 51 to 75%), good (B, 26 to 50%), poor (P, 1 to 25%), and negative (N, 0%).

b Determined on ISP2 medium according to [38]

c Negative: -, Positive: +. Determined in ISP1 medium according to [38]

d Description according to [39] e ND Not determined
in vitro. *Streptomyces* sp. TP199 generated inhibition halos of $13 \pm 1.29$ mm and $14 \pm 1.71$ mm against *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum*, respectively, according to the cross-streak method, while *Streptomyces* sp. A2R31 generated inhibition halos of $7 \pm 2.37$ mm and $10 \pm 1.71$ mm against the same phytopathogens (Fig. 2A). Using the agar disc diffusion method, *Streptomyces* sp. TP199 generated inhibition halos of $14 \pm 0.5$ mm and $19 \pm 0.96$ mm against *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum*, respectively, while the corresponding values for *Streptomyces* sp. A2R31 were $13 \pm 0.96$ mm and $20 \pm 1.71$ mm (Fig. 2B). Ampicillin, which was used as the positive control, generated inhibition halos of $37 \pm 1.29$ mm and $37 \pm 0.96$ mm against *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum*, respectively (Fig. 2B).

The effects of *Streptomyces* sp. TP199 and *Streptomyces* sp. A2R31 to inhibit plant tissue maceration were screened by a potato tuber slice assay, which showed, for the control treatments: maceration halos...
of 4.29 ± 1.03 mm for the well with the sterile nutrient broth, 14.97 ± 0.49 mm for the well inoculated with *P. atrosepticum*, and 15.95 ± 2.83 mm for the well inoculated with the *P. carotovorum* subsp. *carotovorum* alone. On the other hand, when mixing pathogens and antagonists, the inocula resulted in macerated tissue halos of 11.87 ± 7.25 mm for the well inoculated with a mix of *P. atrosepticum* and *Streptomyces* sp. A2R31 in a 1:1 ratio, 5.16 ± 1.97 mm for the well inoculated with a mix of *P. atrosepticum* and *Streptomyces* sp. TP199 in a 1:1 ratio (Fig. 4), 11.03 ± 4.79 mm for the well inoculated with a mix of *P. carotovorum* subsp. *carotovorum* and *Streptomyces* sp. A2R31 in a 1:1 ratio, and 6.19 ± 3.25 mm for the well inoculated with a mix of *P. carotovorum* subsp. *carotovorum* and *Streptomyces* sp. TP199 in a 1:1 ratio (Figs. 4 and 5).

Endosphere colonization of *Streptomyces* sp. TP199 in roots potato plants

Two-photon laser scanning confocal microscopy was used to obtain insights into the *Streptomyces* sp. TP199 root colonization pattern, which was targeted by 23S rRNA with a Cy5 dye double-labeled HGC69a probe (Fig. 7D, H, J, P, T). The identification of the colonization zones in the plant was possible by the autofluorescence of the plant tissue, which is visualized in blue (Fig. 7B, F, L, N, R) and green (Fig. 7C, G, K, O, S). Therefore, in the merged image, the signal from *Streptomyces* sp. TP199 (visualized in red) is contrasted with the autofluorescence of plant tissue (Fig. 7A, E, I, M, Q). The first step of colonization was visualized on a rhizodermal (Fig. 7I) and in the root piliferous...
zone cells 3 days after inoculation (Fig. 7M). In the case of the histological sections, which correspond to 10 days after inoculation, the greater intensity in the fluorescence corresponding to the HGC69a probe was observed in the area of root hair growth and the metaxylem (Fig. 7Q). The zoom of the vascular bundle's plant
cells allowed us to visualize that the points of greater intensity of the HGC69a probe’s fluorescence were presented in the cell walls and intercellular spaces of eukaryotic cells.

**Discussion**

Actinobacteria is a bacterial phylum differentiated by the high content of G+C in its DNA, recognized as one of the main producers of natural bioactive compounds...
of microbial origin [41] and its biotechnological application prospects [42].

In this study, the presence of facultative endophytic actinobacteria was determined for the first time in native Chilean potatoes by the selective humic acid medium (HV) culture, most of them from the \textit{Streptomyces} genus, which is consistent with the results from cultivation and cultivation-independent population analysis of bacterial endophytes in potato (\textit{S. tuberosum}) [17, 34, 43–47]. According to our results, the morphological and molecular characterization of the isolates obtained was consistent with the genera \textit{Streptomyces}, and not related to pathogenic species or \textit{Nocardia} [39, 48].

Ten isolates of actinobacteria were selected by considering that the disinfection controls presented no growth when cultured. However, this validation is subject to the restrictions governing microorganism culturability [49], as they might be epiphytes resistant to the disinfection process [50, 51]. Thus, the isolates reported here should be considered facultative endophytes, with the exception of \textit{Streptomyces} sp. TP199, which was validated as an endophyte by microscopy [30].

The in vitro results for antagonism (cross streak and diffusion with agar discs) resulted in the selection of \textit{Streptomyces} sp. A2R31 and \textit{Streptomyces} sp. TP199 as antagonists of \textit{Pectobacterium carotovorum} subsp. \textit{carotovorum} and \textit{Pectobacterium atrosepticum}. We observed that only the \textit{Streptomyces} sp. TP199 isolate produced a significant reduction in the tuber tissue maceration assay relative to inoculation with the two phytopathogens alone. This phenomenon may,

![Inhibition of the violacein production of Chromobacterium violaceum ATCC 12472 (WT) by culture of Streptomyces sp. TP199. This according to the metal used. The bars represent the diameter sizes generated (inhibition halo in mm) of the colorless areas. Plot mean with SD. Different letters on the top of error bars indicate statistical significative differences (Tukey-test, \(p \leq 0.05\)), \(n = 3\)](See figure on next page.)

![Streptomyces sp. TPP199 root colonization patterns targeted by 23S rRNA with Cy5 dye double-labeled HGC69a. Probe visualized by two-photon laser scanning microscopy where autofluorescence of the plant tissue is shown in blue (B, F, L, N, R), and green (C, G, K, O, S) separately, while the probe is in red separately (D, H, J, P, T) and in a merged image (A, E, I, M, Q). A-D Apical root from a not-inoculated plant, scale bar of 50 μm (day 3 of the assay). E-H Longitudinal histological section root from a not-inoculated plant, scale bar of 90 μm (day 10 of the assay). I-L Rhizodermis from a 3 day post-inoculated plant, scale bar of 20 μm. M-P Root hair growth area from a 3 day post-inoculated plant, scale bar of 60 μm. Q-T Longitudinal histological section root from a 10 day post-inoculated plant, scale bar of 60 μm. A, E, I, M, and Q merged image. B, F, J, N, and R blue (excitation-emission: 405/410–480 nm). C, G, K, O, and S green (excitation-emission: 488/490–560 nm) separately, while the 23S rRNA with Cy5 dye double-labeled HGC69a probe is in D, H, L, P, and T red separately (excitation-emission: 633/638–747 nm). Rz: rhizodermis, Pr: root hair. Mx: metaxylem. Yellow arrows indicate \textit{Streptomyces} sp. TP199 targeted with Cy5 dye double-labeled HGC69a probe fluorescence, and white arrows indicate the plant morphology.](See figure on next page.)
Fig. 7 (See legend on previous page.)
that some AHL-lactonases need a metal in order to degrade the AHL in an optimum way. Different metals were tested, in this experiment, in addition to AHL including: manganese, calcium, magnesium, and zinc. The choice of these metals was made according to their frequency in different strains, in the AHL degradation [61]. Through the results in C. violaceum plates, violacein inhibition appeared only for 0.2 mM for each metal, which is a promising result for the inhibition quorum sensing mechanism possibly related to an AHL degrading enzyme metal-dependent and not currently defined for Streptomyces sp. species [59, 60].

Finally, this taxa has been described as part of the plant microbiota, colonizing different compartments, such as the endosphere, phyllosphere, and rhizosphere [28, 62]. To determine the colonization capacity of Streptomyces sp. TP199 in a commercial potato crop, like an endophyte, it was inoculated in the root system of potato cv. Pukará-INIA plants, which were obtained by micropropagation to obtain plant material free of pathogens. Bearing in mind that the combination of the use of DOPE-FISH with the use of confocal laser microscopy (CLSM) facilitated the exploration of micro-habitats and allowed the observation of microorganisms associated with their host in situ with excellent results, these techniques were used and resulted in the visualization of Streptomyces targeted with a double-labeled Cyt5 dye HGC69a probe in the piliferous zone and into the xylem vessels 10 days post-inoculation, similar to the results of [63]; however, it is necessary to make a distinction regarding the passive or active mechanism and to determine if this could confer protection to the plants against Pectobacterium sp. infection [62, 64].

To understand this behavior, future studies must determine the chemical nature, concentrations, and diffusion properties of the assemblage of active molecules secreted by the antagonists, interpret more precisely the potential of Streptomyces sp. TP199 against P. carotovorum subsp. carotovorum and P. atrosepticum, and validate the mechanism related to blocking the virulence expression of Pectobacterium sp. inside the plant and in the tubers [14, 59].

Conclusions
In conclusion, two isolates of endophytic actinobacteria from native Chilean potatoes (Solanum tuberosum L.), Streptomyces sp. TP911 and Streptomyces sp. A2R31, were found to possess antagonistic activity in vitro against Pectobacterium carotovorum subsp. carotovorum and Pectobacterium atrosepticum; however, only Streptomyces sp. TP199 decreased the tuber tissue maceration (approximately 38 and 34%, respectively), and responded to the presence of antimicrobial compounds and AHL signal interference with metal-dependent enzymatic action. Streptomyces sp. TP199 also acted as a vegetable...
 probiotic by being able to colonize the root endosphere of potato plants of cv. Pukará-INIA. This suggests that the native Chilean potato possesses endophytic actinobacteria that could be potential agents for the management of the diseases of blackleg and soft rot in potato crops.

**Methods**

**Isolation of endophyte actinobacteria**

Tubers of native potato (*S. tuberosum* subsp. *tuberosum*) obtained from Chiloé (Southern Chile) were made available by the National Agricultural Research Institute (INIA) Remuehe, Osorno (accession number Ch. P11). This institution made the formal identification of the plant material used. A voucher specimen of this material has been deposited in a publicly available herbarium of INIA Remuehe (Deposition number not available). They were sown in pots containing sterile truamao soil (Soil derived from frequent volcanic ash in southern Chile) and kept in a greenhouse for 5 months at INIA Quilamapu, Chillán (VIII Region of Chile) to collect plant tissue. Leaves, stems, roots, and tubers were collected from plants that presented no visible disease symptoms in the greenhouse. After collection, the tissues were washed in a soapy solution, and then 2 to 3 g of each tissue was resuspended in phosphate-buffered saline (PBS) solution with Tween-20 (0.01%) and sonicated at 60 Hz for 3 min [65, 66]. The tissues were then immersed in ethanol 70% for 1 min, sodium hypochlorite (NaOCl) at 3% for 4 min, washed three times in sterile water, and finally dried on sterile absorbent paper under a laminar airflow hood. To obtain only endophytes from the phylum Actinobacteria, slices of the samples were then observed under an optical microscope. In each case, the filaments and spore morphology was observed, and the diameter was measured [39, 48]. Isolates representing different taxonomic genera were selected for the examination of cell morphology by scanning electron microscopy [39], both as solid cultures on ISP2 after 14 days of incubation at 28°C and as liquid cultures in ISP1 (tryptone-yeast extract broth) after 120 h of incubation with shaking at 120 rpm and 28°C. The products were preserved in 4% glutaraldehyde at 5°C and processed with scanning electron microscopy at the Microscopy Centre of the University of Concepción, Concepción, Chile.

The taxonomy was determined via 16S ribosomal genes by culturing the actinobacterial isolates in ISP1 for 120 h at 28°C and inoculating them to a final concentration of 2.5 × 10⁸ spores/mL. The samples were centrifuged at 12,000 rpm and 4°C, and the DNA was extracted from the pellets using the ZR Soil Microbe DNA MiniPrepTM kit following the manufacturer’s instructions. For isolates that did not form spores, DNA was extracted from an even covering on ISP2 medium. The 16S ribosomal gene was amplified with the universal primers for bacteria: 9-27F (5′-GAGTTTTGAT CCTGGCTCAG-3′) and 1541R (5′-AAGGAGGTG ATCCACC-3′) [70]. The PCR product was recovered with electrohoresis in 1.2% (w/v) agarose gel with ethidium bromide (10 mg/mL) in a transilluminator with UV light [71]. Sequencing services were provided by Macrogen Korea (http://www.macrogen.com/). The rDNA 16S sequences were analyzed using the BLASTn tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences produced by Blast were selected with >98% identity.

MEGA7 software was used to construct the phylogenetic tree [72] using the maximum likelihood method based on the model of [73]. The tree was validated statistically by bootstrapping with 1000 iterations [74], and the initial tree for the heuristic search was obtained by applying the Neighbor Joining method to an estimated distance matrix using the compound maximum probability approach.
Antibacterial activity

The strains of *Pectobacterium carotovorum* subsp. *carotovorum* and *Pectobacterium atrosepticum* were supplied by the Phytopathology Laboratory of INIA CRI Remehue, Osorno, Chile. The antagonistic activity of the actinobacterial isolates was evaluated using the cross streak agar diffusion technique and agar discs [53, 54]. The cross streak agar procedure is a simple way to test antagonistic properties of actinobacteria through an easy and a rapid semi-quantitative screening, where the inhibition halos could be measure, then let establish a spectrum of inhibiting properties of actinobacteria [75]. The *Pectobacterium* strains were grown in the exponential phase (10^6 cells/mL) cultured in nutrient broth under shaking at 110 rpm and 28 °C for 18 h. For cross streak evaluation, the actinobacterial isolates were sown in a strip across the diameter of the dish on nutrient agar and incubated at 28 °C until sporulation. Then, the *Pectobacterium* was sown in a strip perpendicular to the actinobacteria and incubated for 48 h at 28 °C to observe the formation of the inhibition halos. For evaluation on agar discs, *Pectobacterium* was soon evenly on nutrient agar and then placed on an agar disc (6 mm) with sporulated actinobacteria on ISP2, as well as an agar disc with sterile ISP2 as a blank and a disc with ampicillin (10 μg) as a positive control. These were incubated at 28 °C for 48 h to observe the formation of inhibition halos. Both assays were performed in triplicate for each isolate, and the measured halos were analyzed by means of Tukey HSD test [76].

Maceration assay in tuber slices

Tubers of Pukará-INIA potatoes were washed with a soapy solution and resuspended in PBS with Tween-20 (0.01%), then sonicated at 60 Hz for 10 min. The tubers were disinfected in sequence in 3% sodium hypochlorite (NaOCl) for 4 min and 70% ethanol for 1 min and flamed. Then, the slices were placed in a laminar flow chamber (for drying). Slices 7 to 8 mm thick were cut and placed in Petri dishes with sterile absorbent paper, and three wells (6 mm) were made in each slice [77–79]. A pathogenic suspension of each strain of *Pectobacterium* (10^8 cells/mL) was mixed with a suspension of actinobacteria; spores (10^8 spores/mL) at a 1:1 ratio. The control suspensions were prepared in the same way but with only the pathogen or only the antagonist, mixing the strains with sterile PBS at a 1:1 ratio. A volume of 30 μL of each inoculum was added to each well, and the absorbent paper under the tuber slices was moistened with sterile water. The sterile nutrient broth was used as a negative control. The dishes were then incubated at 28 °C for 72 h, and the macerated tissue diameter around the wells was measured. Each assay was performed in triplicate [77–79], and the mean diameter per treatment was compared using Tukey HSD test \((p \leq 0.05 [76])\).

Metals tests and AHL- inhibition assays

Bioassay plates were made with LB-Agar 1,5% medium, covered by a solution of *Chromobacterium violaceum* ATCC 12472 (WT) at 10^7 cells/mL from the inoculum, in Soft Luria Bertani (LB)-agar 0,7% [80]. In the first experiment, filter discs were filled with 20 μL of each sampling time supernatant (every 12 h of *Streptomyces* sp. TP199 for 7 days) previously passed through 0,22 μm filters to eliminate the possible presence of bacteria. Two serial dilutions at ½ and ¼ were made from these supernatants [57, 58]. In the second experiments, *Streptomyces* sp. TP199 were grown on ISP2 agar culture for 3 days at 28 °C and then subcultured in ISP1 medium for 1 day. An inoculum of 10^8 cells/mL of this strain was made. One hundred fifty microliter of both cultures were further incubated in two 96 well microplates at 28 °C with 120 rpm shaking for 3 days.

The metals solutions were adjusted at pH = 7 (MnCl2, ZnSO4, and MgSO4) and added at final concentrations of 0,2, 0,5, 1, and 2 mM, according to the experiment. One triplicate of wells did not receive a metal solution. Negative controls were made with filling two triplicates of wells, respectively, with metal solutions only and ISP1 medium. Forty microliters of each well were mixed with an equal volume of a 40 μM-N-Hexanoyl-DL homoserine lactone (C6-AHL) [40]. The mixture was incubated at 28 °C with a 120 rpm shaking during 2 h and the reaction was stopped by heating at 95 °C during 10 min. Negative controls were made with the LB liquid medium and positive controls with vanillin (0,01 g/mL), previously proven to have AHL-inhibition in the laboratory. All plates were incubated 24 h at 30 °C to allow the purple development. An inhibition halo of the purple pigment was observed when AHLs production by *C. violaceum* ATCC 12472 (WT) was inhibited. We measured and recorded the results. The mean diameter per treatment was compared using Tukey HSD test \((p \leq 0.05 [76])\).

Plant inoculation

Twenty plants were obtained by micropropagation and acclimated in a sterile sand substrate under controlled environmental conditions. The photoperiod was maintained with 16 h light and 8 h dark, 70% relative humidity (R.H.), and 20 ± 2 °C for approximately 3 weeks, until vigorous root growth was observed [81]. Then, 10 plants were selected to control treatment, and 10 plants were inoculated with 10^8 spores/mL of *Streptomyces* sp. TP199. The root system was inoculated by the dipping method for 10 min, keeping the spores' movements softly. Then, they were transplanted to independent pots in the
sterile substrate, and the conditions of controlled growth were maintained.

Root tissue samples were collected from the control and inoculated plants on days 3 and 10 post-inoculation and were fixed in 4% paraformaldehyde for 24 h and subsequently stored in PBS/EtOH 95.5° (1: 1) at −20°C until processing. The HM525 cryostat was used to obtain 30 μm thick cross-sections, which were placed on microscope slides that were previously treated with polylysine (1 mg/mL).

Double labeling of oligonucleotide probes for fluorescence in situ hybridization (DOPE-FISH)
The probe HGC69a (5’-TAGGTATACCAACCCGCT-3’) [82] was used for the 16S rRNA gene whose specificity was verified in the Probe Check database (http://microbial-ecology.net/) and was labeled with fluorophore Cy5 at the 5’ and 3’ ends of the nucleotide sequence (emission at the 5’ and 3’ ends of the nucleotide sequence (emission outside the autofluorescence range of the plant tissue) [83]. Whole root samples and histological root sections on microscope slides (the edge was drawn with hydrophobic pencil) were processed and were pre-treated with a-chromoproteinase (1 mg/mL) at 37°C for 15 min and then serially dehydrated with ethanol (50–99.9%, 30 min each stage). A first hybridization step was performed (0.02 mM Tris-HCl, 0.01% SDS, 0.9 M NaCl, and 25% formamide), the probe was added to a final concentration of 15 ng/μL in the dark, and then the samples were incubated at 48 ± 1°C for 3h. Subsequently, the samples were drained in a washing solution (0.02 mM Tris-HCl, 0.01% SDS, and 5 M NaCl), preheated to 51°C, and incubated in the same solution at 51°C for 20 min. Finally, the samples were dried in the dark in the laminar flow chamber, the immersion solution was added to maintain fluorescence (Prolong, Sigma), and the coverslip was placed.

Visualization by two-photon confocal laser microscopy
A two-photon confocal laser microscope LSM780 NLO Zeiss (Advanced Microscopy Center, CMA BIO-BIO, CONICYT PIA ECM-12 Project) linked to the ZEN blue software was used for the visualization and image capture from the treated root samples. Different lasers were used for different ranges of excitation and emission, corresponding to the excitation/emission spectra: 488/490–560 nm, 405/410–480 nm (UV light), and 633/638–747 nm for the probe (far-red light). The Image J program [69] was used to process the images.

Abbreviations
ATCC: American Type Culture Collection; WT: Wild type; DOPE-FISH: Double labeling of oligonucleotide probes for fluorescence in situ hybridization; AHL: Acyl homoserine lactones; PCWDE: Pectobacteria relies on plant cell wall-degrading enzymes; ISP1: Tryptone-yeast extract broth; ISP2: Yeast extract and– malt extract agar; ISP3: Oatmeal agar; ISP4: Inorganic salts and– starch agar; EX: Excellent; MB: Very good; B: Poor; N: Negative; NCBI: National Center for Biotechnology Information; ND: Not determined; Pcc: P. carotovorum subsp. carotovorum; Pba: P. atrosepticum; 23S rRNA: 23S ribosomal RNA; HV: Humic acid medium; INIA: National Agricultural Research Institute; PBS: Phosphate-buffered saline; HSD: Honestly significant difference; RH: Relative humidity; SDS: Sodium dodecyl sulfate; UV: Ultraviolet.

Acknowledgements
Not Applicable.

Authors’ contributions
Conceptualization N.P., S.M., A.F., and H.U.; methodology, N.P., P.L., A.M., and C.T.; software, N.P. and A.M.; validation, N.P., P.L., A.M., and H.U.; formal analysis, N.P., C.T., P.R., and H.U.; investigation, N.P., P.L., and A.M.; resources, H.U., S.M., A.F., and I.A.; data curation, N.P. and H.U.; writing—original draft preparation, N.P. and H.U.; writing—review and editing, N.P., P.R., and H.U.; visualization, N.P. and P.R.; supervision, H.U. and S.M.; funding acquisition, H.U., S.M., A.F., and I.A. All authors have read and agreed to the published version of the manuscript.

Funding
This research was funded by Fundacion para la Innovacion Agraria (FIA), grant number PYT20150093 and by the Agencia Nacional de Investigación y Desarrollo (ANID) Of Chile through the Fondef ID N° 1910382.

Availability of data and materials
The sequencing data generated in this study are submitted to the GeneBank Database (https://www.ncbi.nlm.nih.gov/nuccore, accessions numbers: KY242591, KY242592, KY242593, KY242596, KY242590, KY296348, KY242594, KY242595, KY228978 and KY242597).

**Salmon**
Salmon tuberosum Pukará-INIA is available according to the number 1-20-09-2016-G0-128 of the Registry of Varieties Suitable for Certification of Chile, (https://www.sag.gob.cl/).

**Availability**
Salmon tuberosum (native from the Chiloe region, Chile) was obtained from the Chilean germplasm network belonging to the National Institute of Agricultural Research (https://web.inia.cl/blog/tag/red-de-bancos-de-germplasma/). Accession number Ch. P11_9.

Declarations
Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Author details
1Laboratorio de Biopelículas y Microbiología Ambiental, Centro de Biotecnología, Universidad de Concepción, Victor Lamas 1290, P.O. Box: 160 C, Concepción, Chile. 2Instituto de Investigaciones Agropecuarias, INIA Remehue, Ruta 5 Norte Km 8-, Osorno, Región de Los Lagos, Chile. 3University of Montpellier, Montpellier, France. 4Departamento de Ciencias Biológicas, Facultad de Ciencias de la Vida, Universidad Andres Bello, Autopista Concepción Talcahuano # 7100, 4300866 Talcahuano, Chile. 5Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Región de Ribeuf, Chillán, Chile. 6Departamento de Microbiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Received: 4 May 2021   Accepted: 15 November 2021
Published online: 07 December 2021
79. Zamani M, Behboudi K, Ahmadvazdeh M. Quorum quenching by Bacillus cereus U92: a double-edged sword in biological control of plant diseases. Biocontrol Sci Tech. 2013;23(5):555–73. https://doi.org/10.1080/09583157.2013.787046.

80. Morohoshi T, Kato M, Fukamachi K, Kato N, Ikeda T. N-acylhomoserine lactone regulates violacein production in Chromobacterium violaceum type strain ATCC 12472. FEMS Microbiol Lett. 2008;279(1):124–30. https://doi.org/10.1111/j.1574-6968.2007.01016.x.

81. Volkova L, Urmantseva V, Burgutin A. Stress-protective effect of phenylpropanoid complex on potato plants in vitro. Russ J Plant Physiol. 2014;61(2):255–61. https://doi.org/10.1134/s1021443714010166.

82. Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, et al. Defining the core Arabidopsis thaliana root microbiome. Nature. 2012;488(7409):86–90. https://doi.org/10.1038/nature11237.

83. Compant S, Muzammil S, Lebrini A, Mathieu F. Visualization of grapevine root colonization by the Saharan soil isolate Saccharothrix algeriensis NRRL B-24137 using DOPE-FISH microscopy. Plant Soil. 2013;370(1-2):583–91 https://doi.org/10.1007/s11104-013-1648-6.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.