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REGULAR ARTICLE

Siderophore and chitinase producing isolates from the rhizosphere of *Nicotiana glauca* Graham enhance growth and induce systemic resistance in *Solanum lycopersicum* L.

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Abstract A screening for Plant Growth Promoting Rhizobacteria (PGPR) was carried out in the rhizosphere of wild populations of *Nicotiana glauca* Graham in south-eastern Spain. Nine hundred and sixty strains were isolated and grouped in four parataxonomic groups: Gram positive endospore forming bacilli, Gram positive non-endospore forming bacilli, Gram negative bacilli and others. Two groups were selected to continue the study: Gram negative bacilli since it was the most abundant, and Gram positive sporulated bacilli, seeking their sporulating capacity as an advantage for inoculants formulation. The ability of these to release siderophores and chitinases in vitro was evaluated. Ninety six isolates were siderophore producers, and 56 of them were also able to produce chitinases. Fifty percent of these were tested for growth promotion in tomato. The best results were obtained with 5 Gram negative bacilli and one Gram positive sporulated bacilli; 5 strains increased all growth parameters while one of them, N21.4, severely compromised plant growth. The ability of these 6 strains to induce systemic resistance against the leaf pathogen *Xanthomonas campestris* in tomato was evaluated. Five of them effectively reduced disease symptoms (up to 50%). The six strains were identified by 16s rDNA sequencing resulting in 3 *Pseudomonas*, 1 *Bacillus* and 2 *Stenotrophomonas*; it's striking that 2 *Pseudomonas* protected up to 50% while the other increased disease incidence. This indicates that systemic induction is strain specific and not necessarily related to production of siderophores and chitinases.

Keywords Induced systemic resistance · Plant protection · PGPR · Priming · Solanaceae · Tomato

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Introduction

Today it is a widely accepted fact that rhizobacteria play a key role in plant health and nutrition. The term Plant Growth Promoting Rhizobacteria (PGPR) coined by Kloepper et al. (1980) includes those strains that benefit plant fitness by many different mechanisms (Ramos Solano et al. 2008a). This term includes those bacteria that enhance plant growth, either by indirect mechanisms such as improving plant nutrition, or by direct mechanisms, that is, involving plant's metabolism, such as producing plant growth regulators (Gutierrez Mañero et al. 2001), or preventing successful invasion of pathogenic microorganisms triggering plant's defensive metabolism (Bowen and Rovira 1999; Ramamoorthy et al. 2001; Zhang et al. 2004;

van Loon et al. 1998; Ramos Solano et al. 2008b). Knowledge of the rhizosphere and its implications on plant physiology have dramatically changed traditional crop management practices regarding plant nutrition and defensive mechanisms (Ramamoorthy et al. 2001; Lucas García et al. 2004; Domenech et al. 2007; Raaijmakers et al. 2009; Bari and Jones 2009; Lucas et al. 2009). Moreover, biotechnological applications of the rhizosphere are yet to be unravelled as there is increasing evidence of bacterial communication involved in the beneficial effects of PGPR on plant growth (Barriuso et al. 2008).

Nowadays, intensive production of *Solanum lycopersicum* L. (tomato) is fully optimised as regards to nutrient requirements, although sudden pathogen attack accounts for huge economic losses in greenhouses in south-eastern Spain (Lucas García et al. 2004). In order to palliate these pathogen attacks, large amounts of chemicals are used. However, sustainable alternatives need to be developed due to the incoming prohibition of some chemicals for agriculture (CE 2037/2000). Therefore, the use of PGPR in agricultural crops appears as a challenging alternative, contributing to environmentally safe agricultural practices.

It has been shown that some PGPR strains inoculated on plant roots trigger the plant's defensive metabolism, and this effect is evidenced only upon pathogen challenge. This physiological status of the plant has been termed **priming** (Conrath et al. 2002) and involves redirection of carbon sources to defensive metabolism, which may compromise plant growth (van Hulten et al. 2006). However, the cost of priming is by far overcome under natural conditions where disease exists. In view of this, it seems interesting to find effective strains.

The rhizosphere of wild plant species is a good source for this purpose (Lucas García et al. 2001; Gutierrez Mañero et al. 2002; Donate-Correa et al. 2004; Barriuso et al. 2005; Ramos Solano et al. 2007) since plants select those bacteria that are more beneficial for their health by releasing organic compounds through exudates (Lynch 1990), creating a very selective environment where diversity is low (Marilley and Aragno 1999; Barriuso et al. 2005). Since our goal was to improve performance of *Solanum lycopersicum* L. the ideal screening would be carried out in wild tomato plants; however, this is not a wild species in Spain, and the screening was performed in the rhizosphere of *Nicotiana glauca* Graham, a plant species from the same family (Solanaceae). Native from South America and naturalized in the Mediterranean area, the *Nicotiana* genera, includes over 40 different species, some of which are relevant in agriculture. Able to colonize poor soils, *N. glauca* contains anabasin, an alkaloid that confers some toxicity, and indicates the existence of a complex secondary metabolism, inducible and probably related to defense (Sinclair et al. 2004; DeBoer et al. 2009). Consequently, our rationale was that the rhizosphere of wild populations of *N. glauca* would be a good source for putative PGPR effective in other Solanaceae with an agronomic interest.

A screening of 960 strains in the rhizosphere of *N. glauca* was carried out to isolate PGPRs associated to this genus. A subset of 442 isolates constituted by the most abundant parataxonomic groups were characterised based on metabolic activities regarded as putative PGPR traits (siderophores and chitinase production). Fifty percent of the isolates that tested positive for both traits (56 isolates) were tested for growth promotion in *Solanum lycopersicum* L. seedlings and outstanding strains were tested for induction of systemic resistance against *Xanthomonas campestris*. The six strains that performed best were identified by partial sequencing of 16s rDNA.

Materials and methods

Origin of bacteria

The bacterial screening was carried out in the rhizosphere of wild populations of *Nicotiana glauca* Graham in three different soils (calcareous, volcanic and cuaternary) in south-eastern Spain (Coordinates UTM N37º00′00″ W1º58′00″; San José, Almeria; N36º45′00″ W3º10′00″, respectively) along 2 years, 1999 and 2000, in the hot (spring-summer, average temperature 22°C) and cold season (autumn-winter, average temperature 13.2°C) to achieve the maximum edaphic and environmental variability.

Sixteen plants were sampled in each soil (3 soils: calcareous, volcanic and cuaternary) and sampling moment (2 sampling moments: hot and cold). The soil intimately adhered to roots and the thinner roots (diameter 1–2 mm) of four plants were pooled at random and constituted a replicate (4 replicates); each

replicate was named with the N from *Nicotiana* and the number of the replicate (N1 to N24). All were brought to the lab in plastic bags at 4°C.

Two grams of rhizosphere soil and thinner roots were suspended in 2 mL sterile distilled water and homogenized for 1 min in an omnimixer. One hundred μL of the soil suspension was used to prepare serial 10-fold dilutions in a final volume of 1 mL; 500 μL were plated on Standard Medium Agar (Pronadisa SPAIN) and incubated for 4 days at 28°C. Individual colonies were selected after 36 h and after 4 days of incubation to select fast and slow growing strains. To avoid duplication, isolated colonies were marked on the plate after selection.

Forty colony forming units (cfu) were selected from each serial-dilution series, that is, from each replicate (4), in each soil (calcareous, cuaternary and volcanic), in the two sampling moments (hot and cold season), constituting 960 cfu. All were isolated and grouped according to Gram staining, morphological characteristics and sporulating capacity into four parataxonomic groups: Gram positive endospore forming bacilli, Gram positive non-endospore forming bacilli, Gram negative bacilli and other morphologies were grouped under "Others". All isolates were kept at −20°C on glycerol: water (1:4).

In vitro tests

All isolates from the selected groups (Gram positive endospore forming bacilli and Gram negative bacilli) were tested for in vitro production of siderophores (Alexander and Zuberer 1991) and chitinases (Frändberg and Shnurer 1998; Rodríguez-Kábana et al. 1983). Fifty percent of the strains able to produce both siderophores and chitinases, were randomly selected to carried out the growth promoting assay.

Inoculum preparation

Bacterial suspensions for inoculation were prepared as follows: each bacterial strain was grown in 100 mL nutritive broth (DIFCO) in a 250-mL Erlenmeyer flask on a shaker (125 rpm) at 28°C for 24 h. The culture was centrifuged (350 g for 10 min), washed with sterile water and pellets were resuspended in sterile $MgSO_4$ 10 mM to achieve 10^8 cfu/mL. The enumeration and calculations were carried out following the "drop method" (Hoben and Somasegran 1982).

The leaf spot pathogen *Xantomonas campestris* CECT 95 was used for the induction of systemic resistance trials. The strain was grown in nutrient broth for 48 h at 28°C. The culture was centrifuged (350 g for 10 min), washed with sterile water and pellets resuspended in sterile $MgSO_4$ 10 mM to achieve 10^8 cfu/mL.

Growth promotion assay

The biological effect of 28 selected strains, based on their in vitro measured capacities, on the growth of *Solanum lycopersicum* var. Marmande seedlings was determined. For this purpose, seeds were sown on peat according to the following experimental design: 1392 plants were organized following a random block design with 3 replicates and 8 repetitions: 16 seeds (two per pot) were placed in an eight-pot tray filled with peat (Flora Gard) and covered with a layer of vermiculite; other two trays were prepared in the same way. Each tray was considered a replicate and each plant, a repetition in this replicate ($n=8$); one seedling was removed to adjust to 8 repetitions in each replicate. A total of three trays (24 plants) were prepared for each bacteria (28). The controls were designed in the same way but in this case, these trays were mock-inoculated with $MgSO_4$ 10 mM.

Inoculation of putative PGPR was done on the seedbeds by soil drench upon sowing (Domenech et al. 2006), with one mL of a bacterial suspension containing 10^8 cfu/mL. Five weeks after sowing, seedlings were harvested and shoot fresh weight, shoot length and calliper were measured. The conditions were the same as those used by plant producing companies during the seedbed phase before transplanting into the production greenhouse. From sowing until germination, trays were kept in darkness, at 30°C, and 70% of relative humidity. When seeds germinated, trays were placed in greenhouse under natural photoperiod; watering was done on alternative days. Once a week, plants were supplied with Hoagland solution.

Systemic induction of resistance assay

Six strains were selected for induction of systemic resistance based on their performance on growth trials. Seeds were sown and grown as described above.

Two hundred and ten plants were used. Seven groups were made, one for each of the bacteria (N21.4, N6.8, N5.18, N17.35, N19.27, N11.37) and one for the non-inoculated controls. Four weeks after sowing, each group was divided in two subsets, leaving one block for pathogen challenge and the other block as the non-pathogen controls, to ensure disease progress. Pathogen blocks were inoculated by deeping plants in the pathogen solution and kept at 100% HR 24 h before and after pathogen challenge to ensure estomatal opening. One week after, disease symptoms were recorded. Results are expressed as relative disease incidence, calculated as number of diseased leaves per plant (n=15) over total number of leaves, relative to pathogen control plants that represent 100% disease incidence.

PCR amplification of bacterial 16s rDNA, sequencing and phylogenetic analysis

Those strains that demonstrated a positive effect on plant growth and were able to induce systemic resistance were identified by 16S rDNA partial sequencing and phylogenetic analysis.

Each bacterial strain was amplified with 16s rDNA primers: P1F (AGA GTT TGA TCC TGG CTC AG) *E. coli* and P2R (AAG GAG GTG ATC CAG CCG CA) (Ulrike et al. 1989). Amplification reactions were made with 5 µL DNA (20 ng/µL), 3 ud Taq polymerase (Roche Expand HighFidelityTM PCR system), 5 µL 10× PCR buffer, primers 1 and 2 at 0.5 µM and ultrapure water at a 50 µL volume. The reaction mixtures were incubated in a thermocycler (PE Cetus DNA thermal cycler) at 95°C for 5 min and then subjected to 30 cycles consisting of 95°C for 60 s, the annealing temperature 64°C for 60 s, and 72°C for 2 min. Finally, the mixtures were incubated at 72°C for 6 min. Two microliters of each amplification mixture were verified by agarose (1.2% *wt*/*vol*) gel electrophoresis in Tris-acetate-EDTA (TAE) buffer containing 0.5 g of ethidium bromide per mL.

Subsequently, PCR products were purified with the QIAquick Gel Extraction Kit (Qiagen), resolved in agarosa gel electrophoresis and sequenced in a ABI PRISM-377 sequencer (Applied Biosystems). The 16s rDNA sequences were combined with Bioedit Sequence Alignment editor 5.0.3®, checked manually, corrected, and then analysed by BLAST. Phylogenetic tree performed with ClustalX 1.8 from the 16s rDNA gene partial sequences of the following bacteria: N11.37, N5.18, N6.8, N17.35, N19.27 and N21.4. The tree was carried out with the neighbour joining method using sequences of bacteria in GenBank with the greatest homology to ours and *Chloroflexus aurantiacus* (GenBank accession number AJ308501) as negative control (outgroup) Sequence data from this article have been deposited at the DDBJ/EMBL/GenBank database under the accession numbers in Table 1.

Statistical analysis

One way analysis of variance with replicates was used to evaluate the effect of treatments on plant growth parameters and plant protection (Harmann 1967). When significant differences appeared (p<0.05), a Fisher test was used.

Results

The nine hundred and sixty isolates sampled from the rhizosphere of *N. glauca* from the three different soils and in the hot and cold seasons were classed into the following parataxonomic groups: Gram positive

Table 1 16s rDNA sequences accession numbers of strains tested for induction of systemic resistance (END: sporulated non deforming)

Strain	Morphology	Gram	Spores	Most significant alignment	Gene-bank accession number
N6.8	Rod	G−	No	*Stenotrophomonas maltophilia* strain 6B2-1	AY748889
N17.35	Rod	G−	No	*Pseudomonas aeruginosa*	AY748891
N19.27	Rod	G−	No	*Pseudomonas corrugata*	AY748892
N11.37	Rod	G+	END	*Bacillus subtilis* strain HJ19	AY748890
N5.18	Rod	G−	No	*Stenotrophomonas maltophilia*	AY748888
N21.4	Rod	G−	No	*Pseudomonas fluorescens*	AY748893

endospore-forming bacilli, Gram positive non-espore forming bacilli, Gram negative bacilli and all other morphologies were grouped together under the name "others". Two groups were selected to continue the study: Gram negative bacilli, since it was the most abundant, and Gram positive sporulated bacilli, seeking their sporulating capacity as an advantage for inoculants formulation. The 96 siderophore producing isolates were tested for chitinase production, resulting that only 56 of them were able to perform both activities in vitro; most of them were Gram negative except for 5 g positive sporulated bacilli.

Fifty percent of these strains (28) were selected at random for growth promotion assays. Table 2 shows data from all assayed strains on shoot fresh weight, shoot height and calliper. Only eight strains of the 28 assayed significantly affected the three evaluated parameters, 7 enhanced growth (the Gram positive endospore forming bacilli N11.37, and 6 Gram negatives), while one of them, the gram negative N21.4, significantly decreased all parameters.

Among these eight isolates, those with outstanding chitinase production capacity (N5.18, N6.8, N11.37, N21.4, N17.35, N19.27) were selected and assayed for protection effect against *Xanthomonas campestris*. Interestingly, results in growth promotion were not necessarily associated to protection, as shown by N19.27-inoculated plants with even higher disease incidence than controls (Fig. 1) associated to a strong growth promotion (Table 2). All other strains protected

Table 2 Growth parameters of 5 week old *Solanum lycopersicum* L. seedlings inoculated with chitinolytic and siderophore producing isolates

Bacterial strain	Shoot fresh weight (g)	Shoot height (cm)	Calliper (mm)
Control	1.96±0.18	13.22±0.26	3.08±0.08
N 2.13	1.49±0.11	11.56±0.30[b]	2.70±0.09[b]
N 5.18[a]	2.92±0.08[b]	18.10±0.29[b]	3.31±0.05[b]
N 6.8[a]	2.67±0.04[b]	18.29±0.40[b]	3.36±0.04[b]
N 9.13	1.75±0.13	11.80±0.30[b]	2.98±0.08
N 9.14	2.28±0.27	13.14±0.44	3.31±0.11
N 10.19	4.25±0.35[b]	17.75±0.54[b]	3.63±0.10[b]
N 10.27	1.86±0.12	12.63±0.19	2.77±0.08
N 10.34	2.05±0.14	13.20±0.25	3.11±0.06
N 10.36	2.27±0.30	13.52±0.45	2.89±0.17
N 11.30	2.22±0.15	13.90±0.35	3.05±0.07
N 11.37[a]	2.66±0.06[b]	17.16±0.37[b]	3.34±0.06[b]
N 13.8	3.69±0.31[b]	16.47±0.34[b]	3.56±0.08[b]
N 13.31	2.67±0.24	15.35±0.29[b]	3.28±0.12
N 15.15	1.86±0.06	16.55±0.22[b]	2.73±0.06
N 15.30	1.89±0.17	12.65±0.29	2.65±0.08[b]
N 16.34	1.59±0.17	12.41±0.34	2.63±0.09[b]
N 17.22	2.17±0.2	13.25±0.30	2.81±0.10
N 17.35[a]	2.87±0.22[b]	15.03±0.45[b]	3.66±0.10[b]
N 19.22	1.46±0.12	11.74±0.26[b]	2.49±0.05[b]
N 19.23	1.91±0.13	12.18±0.31	2.62±0.09[b]
N 19.27[a]	3.18±0.31[b]	15.07±0.59[b]	3.50±0.14[b]
N 20.6	2.09±0.20	13.61±0.37	2.93±0.11
N 21.2	1.85±0.18	12.58±0.32	2.68±0.10
N 21.4[a]	1.02±0.07[b]	10.75±0.34[b]	2.35±0.06[b]
N 21.13	2.81±0.17[b]	14.04±0.44	2.98±0.09
N 21.26	1.80±0.19	13.92±0.27	3.00±0.10
N 22.18	1.94±0.21	12.41±0.39	3.30±0.14
N 22.29	1.55±0.12	11.63±0.21[b]	2.45±0.07[b]

[a] denotes strong chitinolytic activity

[b] indicates significant differences with non-inoculated controls according to Fisher test ($p<0.05$) ($n=24$)

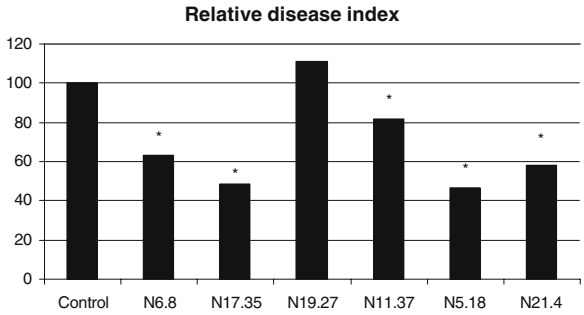

Fig. 1 Relative disease index in *Solanum lycopersicum* L. plants inoculated with the six PGPR and the non-inoculated control, 1 week after challenge with the leaf spot pathogen *Xanthomonas campestris*; the control is expressed as 100% of disease incidence (disease index for pathogen challenged control plants = 75%). *Asterisks* indicate the existence of significant differences according to Fisher's test ($p<0.05$) ($n=15$)

against the pathogen reducing disease index up to 50%, being the sporulated bacilli less effective with only a 20% protection (Fig. 1).

All six strains were identified by 16s rDNA sequencing (Table 1) and the phylogenetic tree is presented in Fig. 2. Three were identified as *Pseudomonas* (N21.4, N19.27, N17.35), two *Stenotrophomonas* (N5.18 and N6.8) and one *Bacillus* (N11.37).

Discussion

This study was carried out to isolate strains with potential to enhance plant fitness in intensive greenhouse production in south-eastern Spain, where *Solanum lycopersicum* is grown in a great part of the area, being the main goal to lower chemical inputs.

Nicotiana glauca was the plant species sampled since it belongs to the Solanaceae family, as tomato, and a certain degree of specificity between plant species and bacterial strains has been reported (Bent et al. 2001; Ramos et al. 2003). Among the species in this genera, *N. glauca* is the most resistant to different fungal pathogens, and due to this fact, its germoplasm is being used to identify pathogen-resistance related genes in this genera (DeBoer et al. 2009). Based on this good resistance traits and in the inducible nature of secondary metabolism, our rationale was that strains present in the rhizosphere of *N. glauca* would be good candidates to trigger secondary defensive metabolism, so our objective was to isolate them. It's striking that only 10% of the sampled isolates (96) were able to produce siderophores, and only 62 strains were able to produce siderophores and chitinases in vitro. This supports the notion of the plant selecting microorganisms to perform one function that is necessary for its survival and reinforces our hypothesis to find the best candidates in the rhizosphere (Barriuso et al. 2005).

Plants encounter a vast array of pathogenic microorganisms that usually deliver effector molecules to cause plant disease (Bari and Jones 2009). PGPR can improve plant fitness by different mechanisms, including nutrient mobilization, alteration of plant hormonal balance and systemic induction of plant defense (Bowen and Rovira 1999; van Loon et al. 2006; Ramos Solano et al. 2008a). Considering the agricultural practices in intensive greenhouse production, where plant nutrient requirements are precisely supplied through irrigation, nutrient mobilization was not considered as a criterion to select putative PGPR. However, due to the artificial environment created to maximize plant growth, plant roots are free of natural soil microorganisms that usually colonize roots (Bari and Jones 2009), preventing contact between pathogenic microorganisms and triggering basal protection mechanisms (van Loon et al. 2006). Therefore, the criteria set for putative PGPR addressed traits related to plant protection: siderophore and chitinase production (Cattelan et al. 1999; Adesina et al. 2007). Siderophore producing microorganisms protect plants at two levels, first, limiting growth of pathogenic microorganisms (Bevivino et al. 1998) and secondly, triggering plant's defensive metabolism (Gang et al. 1991). Chitin, an insoluble linear polymer, is a major structural component of most fungal cell walls, and therefore, many species of microorganisms and plants produce chitinolytic enzymes to protect themselves against fungi, constituting good biocontrol agents (Lorito et al. 1993; Sid et al. 2003; Adesina et al. 2007). With these criteria, benefits in greenhouse would be achieved at three levels: i) niche colonization (Lucas García et al. 2004), ii) preventing pathogen growth by iron chelation and/or hydrolization of fungal cell walls (Lorito et al. 1993; Chernin et al. 1995; Adesina et al. 2007), and iii) inducing systemic resistance, activating plant's defensive mechanisms (van Loon et al. 1998; Ramamoorthy et al. 2001; Ramos Solano et al. 2008b).

All isolates triggered plant growth, indicating that communication has been established between both

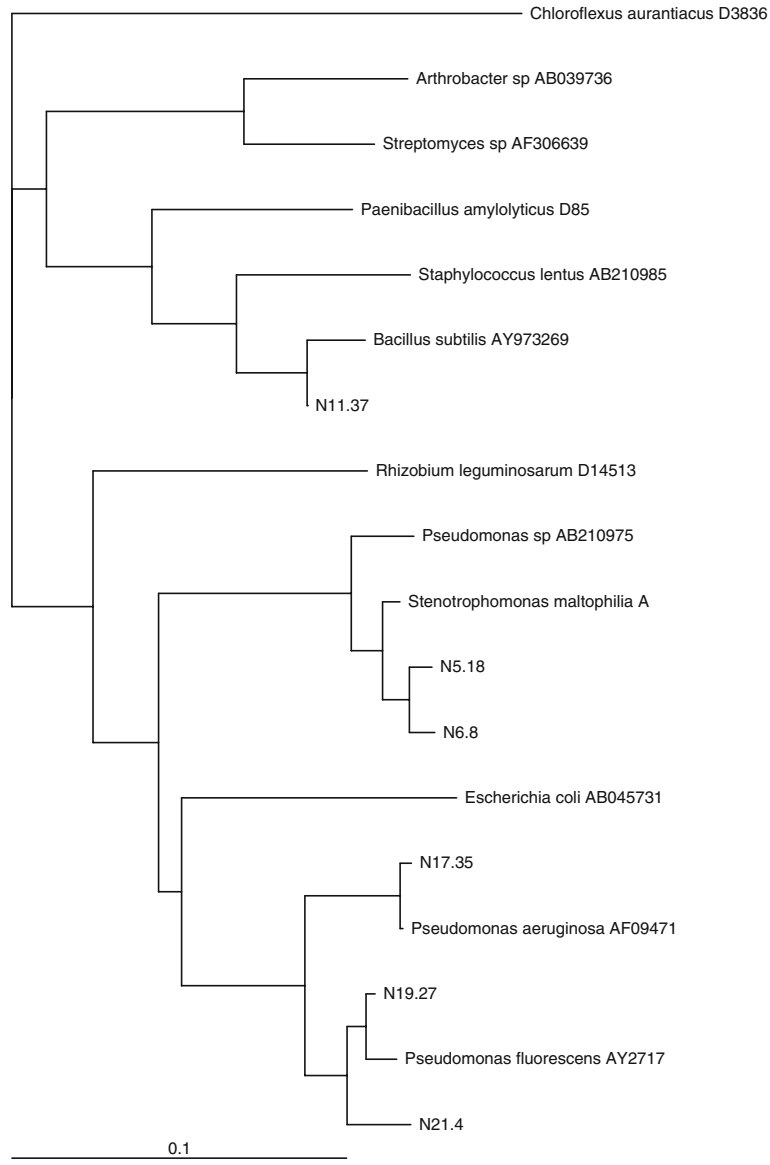

Fig. 2 Phylogenetic tree performed with ClustalX 1.8 from the 16s rDNA gene partial sequences of the following bacteria: N11.37, N5.18, N6.8, N17.35, N19.27 and N21.4. The tree was carried out with the neighbour joining method using sequences of bacteria in GenBank with the greatest homology to ours and *Chloroflexus aurantiacus* (GenBank accession number AJ308501) as negative control (outgroup)

partners, confirming our hypothesis of plant-bacteria specificity based on the phylogenetic relationship. However, only 8 isolates caused significant variations in all 3 biometric parameters. Among these 8 isolates, those with outstanding chitinase production capacity (N5.18, N6.8, N11.37, N21.4, N17.35, N19.27) were selected for evaluation of protection against *Xanthomonas campestris*. All belong to three bacterial genera according to 16s rDNA sequencing (Fig. 2) and representatives of all of these genera have been reported in the literature as PGPR, showing different mechanisms of action (Marten et al. 2001; Gutierrez Mañero et al. 2001; Sid et al. 2003).

Results in growth promotion were not necessarily associated to protection. All strains were able to induce systemic resistance protecting the plant up to 50% except N19.27 (Fig. 1), and all strains enhanced growth except N21.4 (Table 2). Interestingly, among the 3 isolates identified as *Pseudomonas* only two (N17.35 and N21.4) protected effectively against the pathogen, while the other strain (N19.27) even increased disease symptoms (Fig. 1). This reveals that induction of systemic resistance is strain-specific and that siderophores and chitinases are not necessarily the elicitors, suggesting other bacterial metabolites or structural cell wall

molecules from bacteria (Ramos Solano et al. 2008b) as putative elicitors. Effects of N21.4 on growth and plant protection is consistent with the priming effect described by van Hulten et al. (2006). These authors showed that show that upon certain stimuli, plants detour C metabolism to defensive metabolism and growth may be compromised. However, the advantage of this physiological state and the metabolic changes associated to this physiological state is not shown until pathogen challenge, when all defensive metabolism is activated and the plant is protected, revealing a systemic induction of resistance triggered by the elicitor. Interestingly, despite the negative effect on growth registered only for N21.4 treated plants, it protected against the pathogen, like the other 4 strains, speaking of different mechanisms of action depending on the combination plant species-bacterial strain (Bari and Jones 2009). This is supported by data from Domenech et al. (2007) who demonstrated that these 6 strains were able to trigger systemic resistance also in *Arabidopsis thaliana*, although to a different extent. These authors also showed with *A.thaliana* transgenic and mutant plants that N6.8 triggered systemic protection through a salicylate-dependent pathway while N11.37 seemed to trigger salicylate-dependent and SA-independent pathways (Domenech et al. 2007). It is also worth mentioning that although N11.37 inoculated plants only showed 20% protection in tomato, it is the same protection degree as in *A. thaliana,* indicating that this strain is quite unspecific. Considering this unespecificity, the effects on growth, its intense chitinolytic activity and siderophore production together with its mechanism of action (Domenech et al. 2007) and being a Gram positive sporulated bacilli, N11.37 appears as a good candidate for formulation of biofertilizers.

In summary, and based on the results presented here, the hypothesis to search PGPR in the rhizosphere of wild species that share a phylogenetic relationship and the specific traits set for the search have proved to be suitable for our objective, resulting in 5 effective strains to enhance tomato protection. Except N19.27, all other strains trigger defensive metabolism in tomato effectively. This confirms that the strong pressure in the rhizosphere selects strains able to improve plant fitness by triggering different metabolic pathways to improve performance upon biotic and abiotic challenges. Further studies need to be carried out to unravel underlying mechanism and optimize performance to develop biofertilizers in greenhouse production.

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