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Endophytic Bacteria Isolated from Common Bean (*Phaseolus vulgaris*) Exhibiting High Variability Showed Antimicrobial Activity and Quorum Sensing Inhibition

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Abstract Endophytic bacteria play a key role in the biocontrol of phytopathogenic microorganisms. In this study, genotypic diversity was analyzed via repetitive element PCR (rep-PCR) of endophytic isolates of the phylum *Actinobacteria* that were previously collected from leaves of cultivars of common bean (*Phaseolus vulgaris*). Considerable variability was observed, which has not been reported previously for this phylum of endophytic bacteria of the common bean. Furthermore, the ethanol extracts from cultures of various isolates inhibited the growth of pathogenic bacteria in vitro, especially Gram-positive pathogens. Extracts from cultures of *Microbacterium testaceum* BAC1065 and BAC1093, which were both isolated from the 'Talismã' cultivar, strongly inhibited most of the pathogenic bacteria tested. Bean endophytic bacteria were also demonstrated to have the potential to inhibit the quorum sensing of Gram-negative bacteria. This mechanism may regulate the production of virulence factors in pathogens. The ability to inhibit quorum sensing has also not been reported previously for endophytic microorganisms of *P. vulgaris*. Furthermore, *M. testaceum* with capacity to inhibit quorum sensing appears to be widespread in common bean. The genomic profiles of *M. testaceum* were also analyzed via pulsed-field gel electrophoresis, and greater differentiation was observed using this method than rep-PCR; in general, no groups were formed based on the cultivar of origin. This study showed for the first time that endophytic bacteria from common bean plants exhibit high variability and may be useful for the development of strategies for the biological control of diseases in this important legume plant.

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Introduction

Bacteria that colonize the interior of the host plant and cause no apparent damage to the plant are known as endophytic bacteria [5]. A variety of interactions between endophytic bacteria and plants as well as a large richness of endophytic species has been described [12]. Although the endophytic bacterial community of seeds and roots has been reported in common bean (*Phaseolus vulgaris*) [6], little is known about the endophytic bacteria of the shoot tissues of *P. vulgaris*. Costa et al. [2] described the culturable endophytic bacteria from leaves of the 'Vermelhinho,' 'Talismã,' and 'Ouro Negro' cultivars of *P. vulgaris* and reported isolates of Proteobacteria (36.7 %), Firmicutes (32.9 %), Actinobacteria (29.7 %), and Bacteroidetes (0.6 %). Those results also revealed differences in the structure of the endophytic bacterial community across different bean cultivars.

Microbial diseases of common bean are a leading cause of reduced yields. *Xanthomonas axonopodis* pv. *phaseoli*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, and *Pseudomonas syringae* pv. *tabaci* are responsible for common bacterial blight (and its variant, fuscous blight), bacterial wilt, and wildfire disease, respectively, which are among the most important bacterial diseases of bean plants. Several phytopathogenic bacteria regulate the production of virulence factors through the population density-

dependent gene expression control mechanism known as quorum sensing, which is mediated by self-produced molecules, typically *N*-acyl-homoserine lactone (AHL) for Gram-negative bacteria, such as *Pseudomonas* [4]. However, endophytic bacteria have the capacity to reduce the symptoms of diseases caused by phytopathogens through several mechanisms, including quorum sensing inhibition of phytopathogenic bacteria [10].

This study aimed to (1) analyze the genotypic diversity of leaf endophytic actinobacteria from three cultivars (i.e., 'Talismã', 'Ouro Negro,' and 'Vermelhinho') of *P. vulgaris* using repetitive element PCR (rep-PCR); (2) assess the antimicrobial activity of the obtained isolates against pathogenic bacteria; (3) evaluate the potential of the obtained isolates to inhibit the quorum sensing of Gram-negative bacteria, including *P. syringae*; and (4) analyze the genomic profile of endophytic *Microbacterium testaceum* using pulsed-field gel electrophoresis (PFGE).

Materials and Methods

Isolated and Bacterial Strains

Endophytic bacterial isolates, which were previously collected from surface sterilized leaves of the 'Talismã,' 'Ouro Negro,' and 'Vermelhinho' cultivars of *P. vulgaris* [2], and *Brevibacillus* sp., which was isolated from bean pods and used as positive control in the in vitro antimicrobial activity assays, are included in the bacterial collection of the Laboratory of Microbial Molecular Genetics of the Federal University of Viçosa (Universidade Federal de Viçosa (UFV)). *Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 14028, and *Escherichia coli* MG1655 were obtained from the American Type Culture Collection. *X. axonopodis* pv. *phaseoli*, *C. flaccumfaciens* pv. *flaccumfaciens,* and *P. syringae* pv. *tabaci* were provided by the Laboratory of Plant Bacteriology at UFV. *Chromobacterium violaceum* CV026 [8] and *E. coli* pSB403 [16] were used as reporter strains and are capable of detecting AHL via violacein production and bioluminescence, respectively. *Hafnia alvei* 071 was provided by Viana et al. [15]. *Enterobacter cloacae* 067T [7] was the reference strain in the quorum quenching assay.

rep-PCR Fingerprinting

Total DNA was extracted from endophytic actinobacteria using a previously described protocol [2]. Genomic fingerprints were generated using the primers BOXA1R (5′-CTACGGCAAGGCGACGCTGACG-3′) and ERIC1R (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC2 (5′-AAGTAAGTGACTGGGGTGAGCG-3′). PCR was performed using 50 ng of total DNA in a final volume of 25 µl, as described previously [14]. The reactions were incubated at 95 °C for 7 min for an initial denaturation, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C (BOX-PCR) or 52 °C (ERIC-PCR) for 1 min, extension at 72 °C for 8 min, and a final extension at 72 °C for 8 min. The PCR products were separated in a 1.5 % agarose gel containing ethidium bromide (0.2 µl/ml). The band pattern was analyzed using Bionumerics software version 6.0 (Applied Maths, Inc., Austin, TX, USA) and the Jaccard similarity coefficient, and clustering was performed using the UPGMA clustering algorithm for a combined gel.

In Vitro Antimicrobial Activity

The isolates were cultured at 28 °C for 72 h in 10 % trypticase soy agar (TSA) and nutrient broth media. The supernatant was extracted with ethanol, and the organic solvent was evaporated under reduced pressure to yield the ethanol extract. The ethanol extract suspension was filtered through a 0.22-µm membrane and used for antimicrobial activity screening against pathogenic bacteria using the paper-disk agar-plate method. The inhibition zone diameter was measured after 24 h. A 25-µl volume of ethanol was used as a negative control. The test was performed with three independent replicates. The mean inhibition zone diameter values were subjected to analysis of variance (ANOVA) and the Scott-Knott test with a significance level of $P < 0.05$ using SAEG software version 9.1 (UFV).

Quorum Quenching Test

The AHL produced by *H. alvei* 071 and *P. syringae* pv. *tabaci* was extracted as described previously [11]. The growth cultures of endophytic isolates in 10 % TSA were diluted 1:5 in fresh medium, and 3-ml aliquots were added to 100 µl of AHL and incubated for 18 h. Then, the decrease of AHL in the supernatant was evaluated using the biosensors *C. violaceum* CV026 and *E. coli* pSB403 inoculated in plates. The diameter of the inhibition zones of the biosensors was determined after a 24-h incubation. The test was performed with three independent replicates.

PFGE

The overnight cultures were rinsed three times in a 0.85 % NaCl solution, and the cell suspension was standardized to an optical density of 1.2 (OD 620 nm) by mixing 500 µl of the cell suspension with an equal volume of 2 % low melting agarose (Bio-Rad Laboratories, Inc., Hercules, CA,

USA). The cell suspension was then allowed to set in molds (Bio-Rad Laboratories). The cells were lysed in situ by incubating the plugs in a lysozyme solution (2 mg lysozyme/ml, 0.05 % *N*-lauryl sarcosine, and 50 mM EDTA, pH 8.0) for 4–5 h at 37 °C. The plugs were then rinsed three times in 50 mM EDTA, pH 8.0, and incubated overnight at 50 °C in NDS buffer (10 mM Tris–HCl, 1 % sodium dodecyl sulfate (SDS), and 50 mM EDTA, pH 8.0) with 2 mg proteinase K/ml. Following the incubation, the plugs were rinsed again from five to seven times in 50 mM EDTA, pH 8.0, for at least 30 min at room temperature. The plugs were equilibrated for 30 min in Tris–EDTA (TE) buffer prior to electrophoresis, and DNA digestion was performed overnight using 30 U of *Xba*I (Promega). PFGE was performed using the CHEF-DR III system (Bio-Rad Laboratories) and 1 % agarose gel electrophoresis in ×0.5 Tris–Borate-EDTA (TBE) buffer at 12 °C. A voltage of 6.0 V/cm was applied at a 120° angle with 1- to 15-s pulse times for 24 h. Following the PFGE, the gel was stained in an ethidium bromide solution for 30 min and photographed using a molecular imaging system (Loccus Biotecnologic L-Pix Chemi, São Paulo, SP, Brazil). The obtained restriction profiles were compared using the Bionumerics 6.0 software, and an analysis of Dice similarity coefficients was performed based on the UPGMA algorithm to generate a dendrogram. Profiles with Dice similarity coefficients of at least 80 % were clustered together.

Results and Discussion

The BOX- and ERIC-PCR patterns were used to distinguish the different isolates of *Actinobacteria* (Fig. 1), a phylum of Gram-positive bacteria which includes the most economically important prokaryotes, due to production of many bioactive compounds described. The fingerprints obtained using BOX-PCR showed DNA bands ranging from 100 to 4000 bp, while the products amplified by ERIC-PCR had an estimated size of approximately 100–3000 bp. The clustering analysis generated 18 groups (A to R) with at least 65 % similarity. The main isolates were *M. testaceum* and they formed groups B to M, with groups B, C, D, F, G, H, K, and M consisting of only one or two isolates that were collected exclusively from the 'Talismã' or 'Ouro Negro' cultivars. Groups E, I, and J included *M. testaceum* isolates from both cultivars, while group L was formed by isolates from the 'Talismã,' 'Ouro Negro,' and 'Vermelhinho' cultivars. The other groups consisted of other actinobacteria species that were represented by fewer isolates. Our results revealed significant genotypic diversity among the analyzed isolates. Furthermore, most isolates were not clustered according to the cultivar of origin. These findings revealed a genotypic diversity higher than that found by Yuan et al. [17] for other species of endophytic actinobacteria from different host plants using BOX-PCR and other methods.

A variation in the microbial antagonism was observed when the isolates were grown in 10 % TSA and nutrient broth media (Table 1). The antimicrobial activity of most ethanol extract against *X. axonopodis* pv. *phaseoli* exhibited no difference when the cells were grown on 10 % TSA or nutrient broth. However, the extracts from 11 cultures that were grown in 10 % TSA showed greater antagonism than those from other cultures in the same medium against *C. flaccumfaciens* pv. *flaccumfaciens*. Furthermore, 22, 18, and 17 extracts from cultures that were grown in 10 % TSA showed greater *S. aureus* ATCC 25923, *E. coli* MG1655, and *S.* Typhimurium ATCC 14028 growth inhibition than the extracts from other cultures in the same medium, respectively. However, most extracts from cultures that were grown in nutrient broth exhibited no difference in antagonism against *S. aureus* ATCC 25923, and only 9 and 12 extracts exhibited greater antimicrobial activity than the extracts from remaining cultures in this medium against *E. coli* MG1655 and *S.* Typhimurium ATCC 14028, respectively. Those results demonstrated that the isolates were more efficient against Gram-positive pathogens, particularly when grown in 10 % TSA. As the 10 % TSA medium has lower concentration nutrients than the nutrient broth medium, this suggested that the reduced availability of nutrients may stimulate the production of compounds which inhibit the growth of other microorganisms. *M. testaceum* BAC1065 and *M. testaceum* BAC1093, which were both isolated from the 'Talismã' cultivar, were considered promising antagonists because their culture extract strongly inhibited most of the pathogenic bacteria tested. The antimicrobial action of endophytic bacteria can occur by mechanisms such as synthesis of antibiotics [1].

Furthermore, the isolates exhibited difference in quorum quenching potential against Gram-negative bacteria, as revealed by difference in the ability to inhibit the response of AHL reporter strains to *P. syringae* pv. *tabaci* or *Hafnia alvei* 071 (Table 1). The isolates with greater quorum quenching activity were *M. testaceum* BAC1065, BAC1100, and BAC2153, *Bacillus thuringiensis* BAC3151, and *Rhodococcus erythropolis* BAC2162. The ability of *M. testaceum* [9] and *R. erythropolis* [13] to cleave AHL has been reported. Further, Dong et al. [3] demonstrated that lactonase-producing *B. thuringiensis* strains suppress the quorum sensing-dependent virulence of the bacterial pathogen *Pectobacterium carotovorum*. Those results revealed the potential of antagonists that inhibit bacterial cellular communication mechanisms for disease control. Moreover,

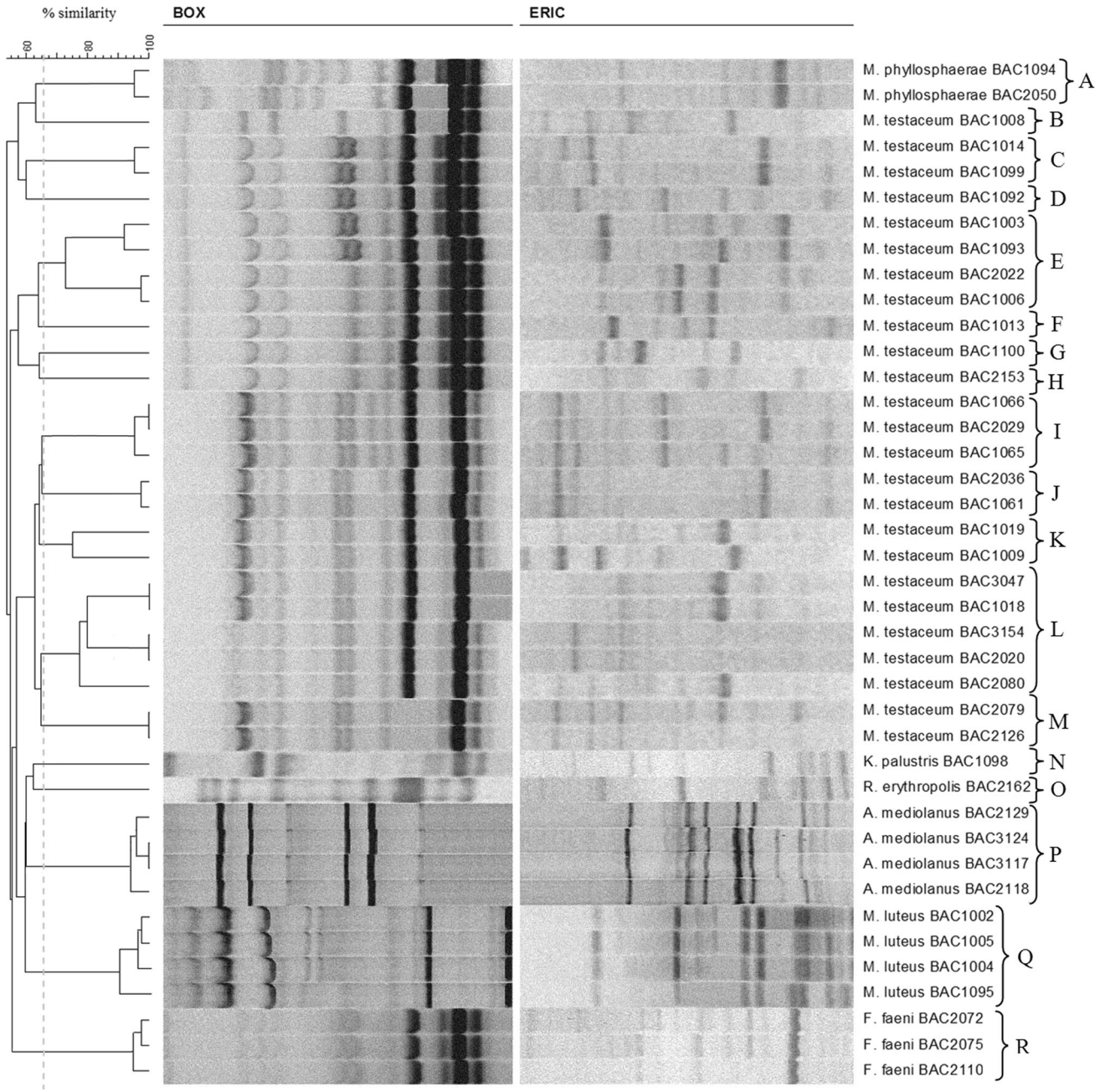

Fig. 1 Dendrogram generated from the band profiles of the BOX and ERIC-PCR fingerprints of endophytic actinobacteria. The dendrogram was constructed using the Bionumerics 6.0 software. The UPGMA algorithm and the Jaccard similarity coefficient were applied to the resulting matrix, and the clusters were set at a similarity level ≥65 %

because most *M. testaceum* isolates exhibited moderate or strong quorum quenching of the tested bacteria, it is possible that *M. testaceum* with quorum quenching potential may also be widespread in common bean.

Because most isolates used in this study were *M. testaceum* and because some of those isolates had greater antimicrobial activity and exhibited greater quorum quenching potential, we analyzed the genomic profiles of isolates of that species using PFGE, which is a more accurate molecular typing method. The profiles generated using this approach revealed 13–21 bands per isolate, ranging from 48.5 to 436.5 kb. The resulting dendrogram showed the presence of 11 clusters (I to XI) (Fig. 2). Our results demonstrated that the *M. testaceum* isolates exhibit greater differentiation via PFGE than via rep-PCR and fail to cluster according to the cultivar of origin, confirming the

Table 1 Antagonism against pathogenic bacteria and quorum quenching by endophytic bacteria from leaves of *Phaseolus vulgaris*

Bacteria	Cultivar[a]	Halo (mm) ± SD[b,c]					
		Sa		Ec		St	
		10 % TSA	NB	10 % TSA	NB	10 % TSA	NB
Microbacterium testaceum BAC1003	TAL	14.0 ± 4.0a	8.5 ± 3.0a	12.5 ± 3.0b	10.5 ± 3.0b	13.0 ± 2.0b	11.0 ± 2.0b
Microbacterium testaceum BAC1006	TAL	14.0 ± 2.0a	13.0 ± 2.0a	13.5 ± 3.0b	12.0 ± 2.0a	13.5 ± 3.0a	14.0 ± 4.0a
Microbacterium testaceum BAC1008	TAL	12.5 ± 3.0b	11.0 ± 2.0a	12.0 ± 2.0b	10.5 ± 3.0b	13.0 ± 2.0b	11.0 ± 2.0b
Microbacterium testaceum BAC1009	TAL	13.0 ± 2.0b	13.0 ± 4.0a	14.0 ± 4.0a	8.0 ± 2.0b	17.0 ± 4.0a	13.5 ± 3.0a
Microbacterium testaceum BAC1013	TAL	12.5 ± 3.0b	12.0 ± 2.0a	14.5 ± 5.0a	11.5 ± 3.0a	12.0 ± 2.0b	13.5 ± 3.0a
Microbacterium testaceum BAC1014	TAL	13.0 ± 2.0b	11.5 ± 3.0a	11.0 ± 2.0b	10.0 ± 2.0b	11.0 ± 2.0b	12.0 ± 2.0b
Microbacterium testaceum BAC1018	TAL	13.0 ± 3.0b	10.5 ± 3.0a	12.5 ± 3.0b	13.5 ± 3.0a	15.0 ± 4.0a	12.0 ± 2.0b
Microbacterium testaceum BAC1019	TAL	8.5 ± 3.0c	11.0 ± 2.0a	9.0 ± 2.0b	12.5 ± 3.0a	9.0 ± 2.0b	11.0 ± 2.0b
Microbacterium testaceum BAC1061	TAL	7.5 ± 3.0c	10.0 ± 2.0a	8.0 ± 2.0b	11.0 ± 2.0b	13.0 ± 2.0b	10.0 ± 1.0b
Microbacterium testaceum BAC1065	TAL	17.5 ± 3.0a	14.5 ± 3.0a	16.0 ± 4.0a	14.0 ± 2.0a	15.5 ± 3.0a	16.0 ± 4.0a
Microbacterium testaceum BAC1066	TAL	16.5 ± 5.0a	14.0 ± 4.0a	15.0 ± 4.0a	11.0 ± 2.0b	13.0 ± 2.0b	14.0 ± 2.0a
Microbacterium testaceum BAC1092	TAL	14.5 ± 3.0a	11.0 ± 4.0a	14.0 ± 2.0a	11.5 ± 3.0b	14.5 ± 3.0a	13.0 ± 1.0b
Microbacterium testaceum BAC1093	TAL	14.0 ± 2.0a	13.0 ± 2.0a	14.5 ± 3.0a	12.5 ± 3.0a	15.0 ± 4.0a	14.0 ± 2.0a
Microbacterium testaceum BAC1099	TAL	15.0 ± 4.0a	10.5 ± 3.0a	13.5 ± 3.0b	9.5 ± 3.0b	14.5 ± 3.0a	12.5 ± 5.0b
Microbacterium testaceum BAC1100	TAL	16.0 ± 5.0a	10.0 ± 2.0a	14.0 ± 4.0a	10.0 ± 2.0b	15.5 ± 5.0a	15.0 ± 4.0a
Microbacterium testaceum BAC2020	ONG	15.5 ± 3.0a	12.0 ± 2.0a	15.5 ± 5.0a	13.5 ± 3.0a	10.0 ± 2.0b	12.0 ± 2.0b
Microbacterium testaceum BAC2022	ONG	12.0 ± 2.0b	9.0 ± 2.0a	14.0 ± 2.0a	12.0 ± 2.0a	15.0 ± 4.0a	12.0 ± 2.0b
Microbacterium testaceum BAC2029	ONG	14.0 ± 4.0a	11.5 ± 3.0a	14.5 ± 5.0a	11.0 ± 2.0b	14.5 ± 3.0a	9.5 ± 3.0b
Microbacterium testaceum BAC2036	ONG	5.0 ± 2.0d	4.0 ± 2.0b	4.5 ± 3.0c	4.5 ± 3.0c	9.0 ± 2.0b	10.0 ± 4.0b
Microbacterium testaceum BAC2079	ONG	15.5 ± 5.0a	10.5 ± 3.0a	12.0 ± 2.0b	10.5 ± 3.0b	8.0 ± 2.0b	12.0 ± 2.0b
Microbacterium testaceum BAC2080	ONG	13.0 ± 2.0b	8.5 ± 3.0a	15.5 ± 3.0a	13.0 ± 4.0a	14.5 ± 3.0a	13.5 ± 3.0a
Microbacterium testaceum BAC2126	ONG	14.5 ± 3.0a	11.5 ± 3.0a	13.0 ± 2.0b	8.0 ± 2.0b	13.0 ± 2.0b	12.5 ± 3.0b
Microbacterium testaceum BAC2153	ONG	15.0 ± 4.0a	11.0 ± 2.0a	14.5 ± 3.0a	11.5 ± 3.0b	14.0 ± 4.0a	10.0 ± 2.0b
Microbacterium testaceum BAC3047	VER	4.0 ± 1.0d	5.0 ± 2.0b	8.0 ± 2.0b	4.5 ± 3.0c	8.0 ± 2.0b	12.0 ± 2.0b
Microbacterium testaceum BAC3154	VER	15.0 ± 3.0a	12.5 ± 3.0a	12.0 ± 2.0b	11.5 ± 3.0b	12.5 ± 3.0b	11.5 ± 3.0b
Bacillus thuringiensis BAC3151	VER	16.0 ± 2.0a	10.0 ± 2.0a	15.0 ± 4.0a	10.0 ± 2.0b	15.5 ± 5.0a	14.0 ± 4.0a
Microbacterium phyllosphaerae BAC2050	ONG	8.5 ± 3.0c	4.5 ± 3.0b	4.0 ± 2.0c	4.5 ± 3.0c	9.0 ± 2.0b	11.5 ± 3.0b
Rhodococcus erythropolis BAC2162	ONG	13.0 ± 4.0b	12.0 ± 4.0a	13.0 ± 2.0b	11.5 ± 3.0b	13.0 ± 2.0b	12.0 ± 2.0b
Micrococcus luteus BAC1002	TAL	12.0 ± 3.0b	11.5 ± 3.0a	13.0 ± 3.0b	9.0 ± 2.0b	13.0 ± 2.0b	10.5 ± 3.0b
Micrococcus luteus BAC1004	TAL	12.0 ± 2.0b	11.0 ± 4.0a	13.0 ± 2.0b	12.0 ± 2.0a	14.5 ± 3.0a	14.0 ± 2.0a
Micrococcus luteus BAC1005	TAL	10.0 ± 2.0c	11.0 ± 2.0a	12.5 ± 3.0b	9.0 ± 2.0b	11.0 ± 1.0b	12.5 ± 3.0b
Micrococcus luteus BAC1095	TAL	15.5 ± 5.0a	12.5 ± 3.0a	14.5 ± 3.0a	11.0 ± 2.0b	15.0 ± 3.0a	13.0 ± 1.0a
Agromyces mediolanus BAC2118	ONG	14.5 ± 5.0a	11.5 ± 3.0a	14.0 ± 2.0a	8.5 ± 3.0b	11.5 ± 3.0b	12.5 ± 3.0b
Agromyces mediolanus BAC2129	ONG	15.0 ± 4.0a	11.5 ± 2.0a	14.5 ± 5.0a	10.0 ± 2.0b	11.0 ± 2.0b	12.5 ± 3.0b
Agromyces mediolanus BAC3117	VER	15.5 ± 3.0a	11.5 ± 3.0a	12.5 ± 3.0b	8.5 ± 3.0b	12.5 ± 3.0b	12.0 ± 2.0b
Agromyces mediolanus BAC3124	VER	14.5 ± 3.0a	11.0 ± 4.0a	12.5 ± 3.0b	9.5 ± 3.0b	10.5 ± 3.0b	14.0 ± 2.0a
Frigoribacterium faeni BAC2072	ONG	12.0 ± 2.0b	8.0 ± 2.0a	12.0 ± 1.0b	11.0 ± 2.0b	14.0 ± 2.0a	10.5 ± 3.0b
Frigoribacterium faeni BAC2075	ONG	12.0 ± 2.0b	11.0 ± 2.0a	15.5 ± 3.0a	7.5 ± 3.0b	13.5 ± 3.0a	10.0 ± 2.0b
Frigoribacterium faeni BAC2110	ONG	15.5 ± 3.0a	11.0 ± 3.0a	12.0 ± 2.0b	8.0 ± 2.0b	11.0 ± 2.0b	11.0 ± 2.0b
Kocuria palustris BAC1098	TAL	15.5 ± 5.0a	11.5 ± 3.0a	14.5 ± 3.0a	10.0 ± 2.0b	12.5 ± 3.0b	12.5 ± 3.0b
Brevibacillus sp.		14.5 ± 3.0a	11.0 ± 2.0a	14.0 ± 2.0a	13.5 ± 3.0a	14.5 ± 4.0a	12.0 ± 4.0b
Escherichia coli MG1655							
Enterobacter cloacae 067*T*							

Bacteria	Cultivar[a]	Halo (mm) ± SD[b,c]				Quorum quenching[d]			
		Cf		Xa		Ps		Ha	
		10 % TSA	NB	10 % TSA	NB	*Cv*	pSB403	*Cv*	pSB403
Microbacterium testaceum BAC1003	TAL	13.5 ± 3.0b	10.0 ± 2.0a	12.5 ± 3.0a	10.0 ± 2.0a	+++	++	++	+
Microbacterium testaceum BAC1006	TAL	8.0 ± 2.0b	11.0 ± 2.0a	16.0 ± 4.0a	14.0 ± 2.0a	+	+	+	–
Microbacterium testaceum BAC1008	TAL	12.0 ± 2.0b	10.5 ± 3.0a	15.5 ± 3.0a	13.5 ± 3.0a	++	++	++	+

Table 1 continued

Bacteria	Cultivar[a]	Halo (mm) ± SD[b,c]				Quorum quenching[d]			
		Cf		Xa		Ps		Ha	
		10 % TSA	NB	10 % TSA	NB	*Cv*	pSB403	*Cv*	pSB403
Microbacterium testaceum BAC1009	TAL	10.5 ± 3.0b	13.5 ± 3.0a	10.0 ± 2.0a	12.0 ± 4.0a	++	++	++	+
Microbacterium testaceum BAC1013	TAL	8.0 ± 2.0b	8.0 ± 2.0a	15.5 ± 3.0a	13.0 ± 2.0a	+++	++	++	+
Microbacterium testaceum BAC1014	TAL	14.5 ± 3.0b	8.5 ± 3.0a	12.0 ± 2.0a	9.5 ± 3.0a	++	+	+	+
Microbacterium testaceum BAC1018	TAL	14.0 ± 2.0b	10.0 ± 4.0a	13.0 ± 2.0a	10.0 ± 4.0a	+	–	+	–
Microbacterium testaceum BAC1019	TAL	10.5 ± 3.0b	11.0 ± 4.0a	11.0 ± 2.0a	10.5 ± 3.0a	+++	++	+	+
Microbacterium testaceum BAC1061	TAL	11.5 ± 3.0b	10.0 ± 2.0a	10.5 ± 3.0a	5.0 ± 2.0b	+++	+++	++	++
Microbacterium testaceum BAC1065	TAL	15.5 ± 5.0a	12.5 ± 3.0a	12.0 ± 2.0a	9.5 ± 3.0a	+++	+++	+++	+++
Microbacterium testaceum BAC1066	TAL	14.0 ± 4.0b	12.0 ± 2.0a	12.5 ± 3.0a	12.0 ± 4.0a	++	+	+	+
Microbacterium testaceum BAC1092	TAL	12.0 ± 2.0b	11.0 ± 2.0a	14.0 ± 2.0a	10.5 ± 3.0a	++	++	–	–
Microbacterium testaceum BAC1093	TAL	16.5 ± 3.0a	13.5 ± 3.0a	12.5 ± 3.0a	11.5 ± 3.0a	+	–	+	–
Microbacterium testaceum BAC1099	TAL	14.5 ± 3.0b	13.0 ± 4.0a	11.0 ± 2.0a	12.0 ± 4.0a	+	+	–	–
Microbacterium testaceum BAC1100	TAL	16.0 ± 4.0a	13.5 ± 5.0a	12.0 ± 4.0a	13.5 ± 5.0a	+++	+++	+++	+++
Microbacterium testaceum BAC2020	ONG	15.0 ± 2.0a	13.0 ± 2.0a	15.0 ± 4.0a	11.0 ± 2.0a	+	–	+	–
Microbacterium testaceum BAC2022	ONG	13.5 ± 3.0b	12.5 ± 3.0a	13.5 ± 5.0a	11.5 ± 3.0a	+	+	–	–
Microbacterium testaceum BAC2029	ONG	18.0 ± 4.0a	9.0 ± 2.0a	13.0 ± 2.0a	11.0 ± 2.0a	++	++	+	+
Microbacterium testaceum BAC2036	ONG	5.0 ± 2.0c	4.0 ± 2.0b	5.5 ± 3.0b	4.0 ± 2.0b	++	++	++	+
Microbacterium testaceum BAC2079	ONG	13.5 ± 3.0b	11.0 ± 2.0a	13.5 ± 3.0a	9.5 ± 3.0a	++	+	++	+
Microbacterium testaceum BAC2080	ONG	14.0 ± 2.0b	12.5 ± 3.0a	13.0 ± 2.0a	10.5 ± 5.0a	+++	++	+	+
Microbacterium testaceum BAC2126	ONG	14.0 ± 4.0b	12.0 ± 2.0a	14.0 ± 2.0a	9.0 ± 2.0a	+++	++	+	+
Microbacterium testaceum BAC2153	ONG	16.5 ± 5.0a	12.5 ± 3.0a	13.0 ± 4.0a	11.5 ± 3.0a	+++	+++	+++	+++
Microbacterium testaceum BAC3047	VER	5.5 ± 0.0c	4.5 ± 3.0b	5.0 ± 2.0b	4.0 ± 2.0b	++	++	++	++
Microbacterium testaceum BAC3154	VER	13.5 ± 3.0b	12.0 ± 2.0a	12.0 ± 2.0a	10.0 ± 2.0a	++	+	+	+
Bacillus thuringiensis BAC3151	VER	18.5 ± 5.0a	14.0 ± 4.0a	13.5 ± 3.0a	9.5 ± 3.0a	+++	+++	+++	+++
Microbacterium phyllosphaerae BAC2050	ONG	5.0 ± 2.0c	5.0 ± 2.0b	4.0 ± 2.0b	4.5 ± 3.0b	++	++	++	+
Rhodococcus erythropolis BAC2162	ONG	13.0 ± 2.0b	11.5 ± 3.0a	13.0 ± 2.0a	10.0 ± 4.0a	+++	+++	+++	+++
Micrococcus luteus BAC1002	TAL	12.2 ± 3.0b	8.5 ± 3.0a	12.0 ± 2.0a	10.5 ± 3.0a	–	–	–	–
Micrococcus luteus BAC1004	TAL	15.0 ± 2.0a	8.0 ± 3.0a	14.5 ± 5.0a	13.0 ± 2.0a	–	–	–	–
Micrococcus luteus BAC1005	TAL	11.0 ± 2.0b	11.5 ± 3.0a	12.0 ± 4.0a	9.0 ± 2.0a	–	–	–	–
Micrococcus luteus BAC1095	TAL	14.5 ± 3.0b	12.0 ± 2.0a	13.5 ± 3.0a	9.5 ± 3.0a	–	–	–	–
Agromyces mediolanus BAC2118	ONG	14.0 ± 2.0b	11.5 ± 3.0a	14.0 ± 4.0a	10.5 ± 3.0a	–	–	–	–
Agromyces mediolanus BAC2129	ONG	18.5 ± 5.0a	10.5 ± 3.0a	14.5 ± 3.0a	12.0 ± 4.0a	–	–	–	–
Agromyces mediolanus BAC3117	VER	15.5 ± 3.0a	10.5 ± 5.0a	15.5 ± 5.0a	10.0 ± 2.0a	–	–	–	–
Agromyces mediolanus BAC3124	VER	13.0 ± 2.0b	11.5 ± 3.0a	13.0 ± 2.0a	12.5 ± 5.0a	–	–	–	–
Frigoribacterium faeni BAC2072	ONG	12.0 ± 2.0b	12.0 ± 2.0a	12.5 ± 3.0a	11.0 ± 2.0a	–	–	–	–
Frigoribacterium faeni BAC2075	ONG	13.0 ± 4.0b	9.5 ± 3.0a	10.0 ± 2.0a	5.0 ± 2.0b	–	–	–	–
Frigoribacterium faeni BAC2110	ONG	16.5 ± 5.0a	11.5 ± 3.0a	11.5 ± 3.0a	9.5 ± 3.0a	–	–	–	–
Kocuria palustris BAC1098	TAL	14.0 ± 2.0b	12.0 ± 2.0a	12.5 ± 5.0a	10.0 ± 2.0a	–	–	–	–
Brevibacillus sp.		14.5 ± 3.0b	12.0 ± 2.0a	13.0 ± 4.0a	11.0 ± 2.0a				
Escherichia coli MG1655						–	–	–	–
Enterobacter cloacae 067*T*						+++	+++	+++	+++

[a] 'Talismã' (TAL), 'Ouro Negro' (ONG), and 'Vermelhinho'(VER) cultivars of *P. vulgaris*

[b] Mean ± standard deviation (SD) followed by the same letter in the same column does not differ by the Scott-Knott test ($P < 0.05$)

[c] 10 % trypticase soy agar (TSA) and nutrient broth (NB) culture media used. *In vitro* antimicrobial activity against *Staphylococcus aureus* ATCC 25923 (*Sa*), *Escherichia coli* MG1655 (*Ec*), *Salmonella* Typhimurium ATCC 14028 (*St*), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (*Cf*), and *Xanthomonas axonopodis* pv. *phaseoli* (*Xa*)

[d] AHL from *Pseudomonas syringae* pv. *tabaci* (*Ps*) and *Hafnia alvei* 071 (*Ha*). The biosensors *Chromobacterium violaceum* CV026 (*Cv*) and *E. coli* pSB403 (pSB403) were used. Lack of quorum quenching (-) (mean induction zone of the biosensors >6.0 cm), weak (+) (4.0–6.0 cm), moderate (++) (2.0–4.0 cm), and strong (+++) (<2.0 cm)

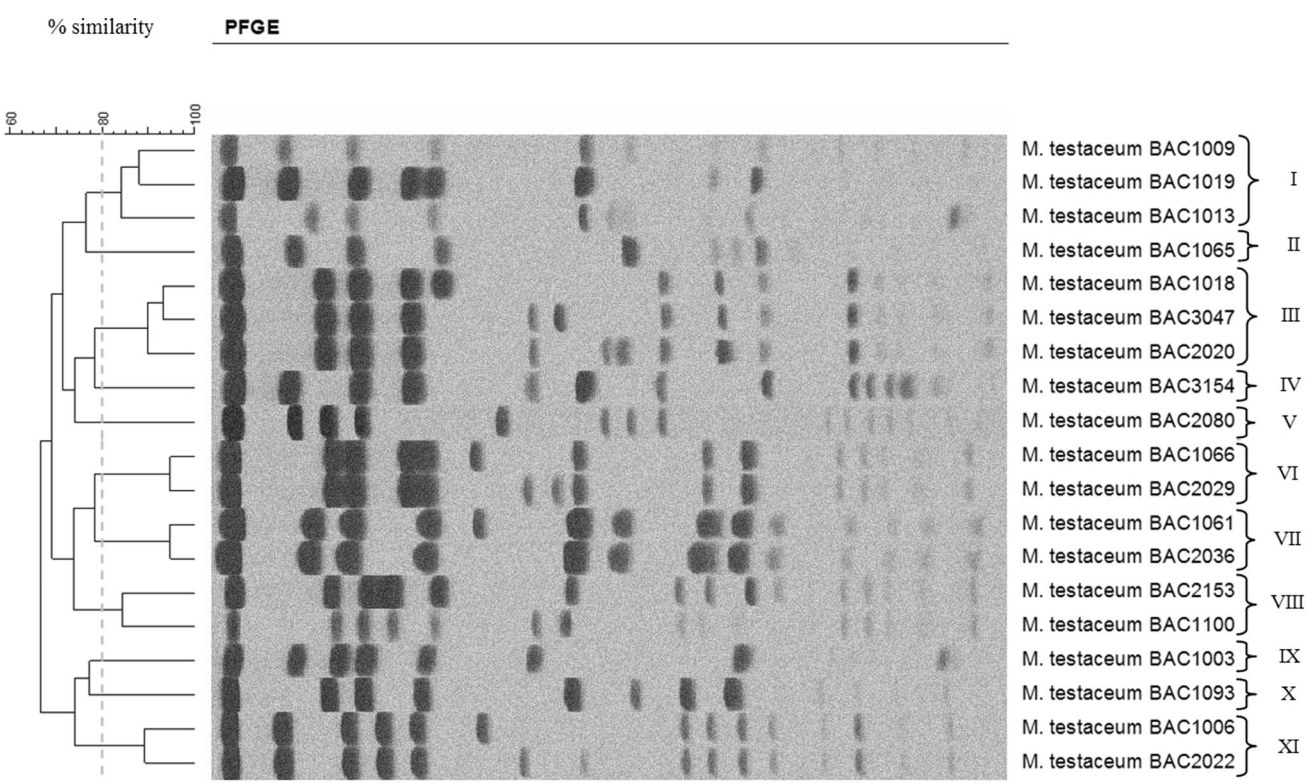

Fig. 2 Dendrogram showing the relationships between strains of *M. testaceum* obtained via PFGE. The dendrogram was generated using the UPGMA algorithm based on the Dice similarity coefficient. The clusters were set at a genomic profile similarity level of ≥80 % after macro-restriction with *Xba*I

high variability of this species in the endophytic *P. vulgaris* community. To our knowledge, the genomic profiles of *M. testaceum* have never been evaluated by PFGE and no reports of the macro-restriction profiles of these bacteria are available.

This paper is the first report of the variability of endophytic actinobacteria from common bean plants and the potential of these endophytes for the biocontrol of phytopathogenic bacteria of common bean and other types of pathogens. The results of this study will contribute to the development of new strategies for the biological control of diseases of *P. vulgaris* and to the reduction of the use of agrochemicals, which are harmful to the environment.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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