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Occurrence of endophytic bacteria in Vietnamese Robusta coffee roots and their effects on plant parasitic nematodes

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Abstract

Several plant parasitic nematode genera were identified in the Robusta coffee (*Coffea canephora* Pierre ex A. Froehn) tree roots and surrounding soil samples from three different coffee groups: coffee planted at 5 years (CYG), 18 years (CBG) and 40 years (COG) in Central Highland, Vietnam. They included *Meloidogyne* spp., *Pratylenchus* spp., *Apratylenchus* spp., *Criconemella* spp., *Xiphinema* spp. and *Rotylenchulus* spp. *Meloidogyne* spp. was the most abundant genus, at 77% across all three groups. Endophytic bacteria were isolated from healthy tissues of roots of the same Robusta coffee trees. In total, 77 bacterial strains were isolated and determined to be *Bacillus* spp., *Serratia* spp., *Paenibacillus* spp., *Enterobacter* spp. and *Streptomyces* spp. based on colony morphological and 16S rRNA gene sequence analysis. Overall, *Streptomyces* was the dominant genus and accounted for 49.35% of total isolated strains. Using statistical methods, we found a tendency in the abundance of endophytic bacterial isolates with the elevation or decrease of several nematode populations, indicating a relation between endophytic bacteria occurrence and nematode distribution. In in vitro anti nematode screening test, a *Streptomyces* sp. strain named CBG9 showed significant nematicidal activities against *Meloidogyne incognita*, inhibiting egg hatching (85.8%) and causing mortality of secondary stage juveniles (85%). This study explored the anti-nematode potency of endophytic bacteria isolated from coffee trees, which could provide a future application in suppression and management of pathogenic nematodes without the use of chemical pesticides.

Keywords Anti-nematode · Coffee root · Endophytes · Meloidogyne incognita · Streptomyces

1 Introduction

Coffee is one of the most exchanged commodities in international agricultural trade and represents a large source of income for several countries (Waller et al., 2007). Vietnam is the second largest producer of coffee in the world, accounting for 17.6% of the total global production in 2017 (Statistics, May 2018 – International Coffee Organization). Among the two main coffee production species, Arabica and Robusta, Vietnam is the largest Robusta producer in the world (Wirryadiaputra & Tran, 2008). According to the statistics from the Western Highlands Agriculture & Forestry Science Institute, 198,000 ha of coffee plantations in Vietnam contained trees that were more than 20 years old, which accounted for 30.4% of coffee production area. These old coffee trees produce less than 2 ton of coffee per hectare per year which is a decrease in yield of 80% compared to the yield produced by younger trees. To revitalize and increase production, these older coffee trees were replanted in a World Bank programme associated with the Vietnam Ministry of Agriculture and Rural Development (MARD) called the Vietnam Sustainable Agriculture Transformation (VnSAT), which aims to replant a total of 120,000 ha of coffee farms in the Central Highlands between 2014 and 2020 (Truong 2018).

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However, plant-parasitic nematodes pose a major problem for coffee replanting in various parts of the world (Chapman 2014). In Vietnam, replanted coffee plantations face serious challenges by root-knot nematode *Meloidogyne* spp. and the lesion nematode *Pratylenchus* spp. (Chapman 2014; Le et al., 2019; Trinh et al., 2009; 2013; 2019; Villain et al. 2018; Wirryadiaputra & Tran, 2008), which are one of the most aggressive and harmful plant-parasitic nematodes attacking coffee plantations (Bell et al., 2018; Bertrand et al. 2000). The leaves of trees infected with nematodes become yellow, and show symptoms of rot root or galling. Infected trees are stunted in growth and can eventually die (Vu et al., 2014). It was reported to the Food and Agriculture Organization (FAO) in Vietnam that the amount of coffee trees that will suffer and eventually will die from nematode-induced disease could reach 80% or higher during the replantation process (Chapman 2014).

Meanwhile, the control of parasitic nematodes following the 2016 replanting protocol by the MARD has been largely based on methods such as soil sanitation before planting, rotation of crops, soil resting and the use of nematicides (MARD, 2016). These methods are labor intensive, expensive (Truong 2018), hazardous and not fully effective based on experiences in Indonesia (Chapman 2014). On the other hand, bacterial endophytes are promising candidates for the long-term biocontrol of plant-parasitic nematodes in agriculture (Reys et al., 2017; Ryan et al., 2008; Vetrivelkalai et al., 2010). Endophytic bacteria that secrete anti-nematode metabolites have been found in agricultural crops and have been shown to exhibit strong and stable anti-nematode activity (Ruanpanun et al., 2011; Ward & Allenby, 2018). Bacterial endophytes colonize an ecological niche similar to that of phytopathogens, which makes them suitable as biocontrol agents, and numerous studies have shown that endophytic microorganisms can have the capacity to suppress damage from plant pests such as fungi, insects and nematodes (Hallmann et al., 2009; Lacava et al., 2014; Maheshwari et al., 2017; Shiomi et al., 2006; Su et al., 2017; Taechowisan et al., 2003). In some cases, they can also accelerate seedling emergence, promote plant establishment under adverse conditions and enhance plant growth (Maheshwari et al., 2017). Bacterial endophytes have been shown to prevent disease development through endophyte-mediated de novo synthesis of novel compounds and antifungal metabolites. Investigation of the biodiversity of endophytic strains for novel metabolites may identify new drugs for effective treatment of diseases in humans, plants and animals (Campbell 2016; Magarvey et al., 2006; Mohammed et al., 2017).

Endophytic bacteria have been isolated from coffee (Oliveira 2013; Vega et al., 2005) and reported to have potential in controlling diseases such as leaf rust (Shiomi et al. 2006; Silva et al. 2012) and *Meloidogyne* nematodes (Hallmann et al. 2009). However, studies focused on the endophytic microbiota in coffee plants are scarce with limited follow-up results. In particular, there have been no reports on endophytic bacteria in coffee plants in Vietnam to date; and as a result, many potential endophytic species and their effects on plant health remain unknown. Therefore, the present study was conducted to extract, isolate and characterize endophytic bacteria in Robusta coffee roots, and subsequently investigate their potential as biocontrol agents against pathogenic nematodes. We selected three coffee tree groups that were successfully replanted and classified by their agricultural age from a study field in Dak Lak province in Vietnam, and analysed endophytic bacteria and plant nematode community diversity as well as the correlation between plant nematode occurrence and culturable endophytic bacteria using statistical methods. The most significant endophytic bacterial strains identified were then screened against the most predominant nematode species present to estimate their biocontrol value. Our results indicate novel options for the development of biocontrol agents to replace the current nematicides.

2 Material and methods

1.1. Sample collections

Successful replanted coffee trees at 5 years old (pre-production, CYG group), 18 years old (production, CBG group) and 40 years old (old trees, COG group) were identified in a study field in Dak Lak province in May, 2016. These plants showed normal growth and no sign of disease. Soil characteristics, such as pH, physical and chemical properties, were measured and analyzed to confirm homogeneity across the field (see supplementary data Table S5). In each age group, three plants were chosen randomly for sample collection. Soil and roots from these plants were collected at three separated spots surrounding the tree trunk and were combined to make a composite sample. A soil auger was used to collect soil from 0 to 20 cm depth at each sampling site, from which pathogenic nematodes were immediately extracted. At the same time, root samples were stored in sterile plastic bags, kept at 8 °C and brought to the laboratory for further analysis within 24 h of collection.

1.2. Nematode extraction and community study

Nematodes were extracted from 100 g of collected soil and 5 g of root from each site using decanting and modified Baermann tray method (Whitehead & Hemming 1965). The extracted nematodes were relaxed, killed by hot water (70 °C), and fixated in 4% formaldehyde. Total nematode numbers were counted under a stereomicroscope (×50 magnification). The fixed individuals were gradually transferred to anhydrous glycerol following the de Grisse's (1969) technique and mounted on permanent glass slides. The nematodes were identified to the genus level by morphological analysis using the keys from Nguyen & Nguyen (2000), Vovlas & Castillo (2007) and Adams et al. (2009).

Furthermore, *Meloidogyne* species were identified by molecular techniques. DNA was extracted from individual second-stage juveniles (J2) using the Lysis buffer protocol (Holterman et al., 2009). The reaction components were as follows: 5 μl of Taq PCR MasterMix 2X, 0.5 μl of primers (10 mM: Far/Rar (5'-TCGGCGATAGAGGTAAATGAC-3′; 5'-TCGGCGATAGACACTACAAACT-3′); Mi2F4/Mi1R1 (5'-ATGAAGCTAAGACTTTGGGCT-3′; 5'-TCCCGCTACACCCTCAACTTC-3′); Fjav/Rjav (5'-GGTGCGCGATTGAACTGAGC-3′; 5'-CAGGCCCTTCAGTGGAACTATA-3′), 1 μl of total DNA and 1 μl of molecular grade water. PCR conditions were as follows: 95 °C for 2 min, 40 cycles of 96 °C for 35 s, 59 °C for 30 s and 72 °C for 45 s, and continued at 72 °C for 3 min. The products were run on 1.5% agarose gel, 100 V, for 60 min, dyed with ethidium bromide and photographed under UV lamp. DNA bands specific for species were determined based on standard 100 bp DNA ladder and compared with the results of Kiewnick et al. (2013).

1.3. Endophytic bacteria study

1.3.1. Endophytic bacteria isolation

The root samples were cleaned using running tap water, then air dried on sterile filter paper for 48 h. Surface disinfection was conducted by washing consecutively with 5% NaOCl for 4–10 min, 2.5% $Na_2S_2O_3$ for 10 min, 75% ethanol for 5 min, rinsed in sterile water and subsequently in 10% $NaHCO_3$ for 10 min. The surface-treated tissues were then dried at 100 °C for 15 min, under sterile conditions (Qin et al., 2009). In order to confirm the success of the disinfection process, 100 μL aliquots of the rinsed water from the final washing step were plated on Luria-Bertani (LB) agar and Yeast Malt Agar (ISP2) (Taechowisan et al., 2003) at optimized temperature (28 °C and 37 °C respectively) for seven days. The success of the surface sterilization protocol was confirmed by culturing of the sterile rinsed water culture after incubation which remained negative, indicating that the epiphytic microbes were completely removed with no observation of bacterial colonies.

The treated-plant roots were fragmented and grown on selective media: (M1) Nutrient Agar medium; (M2) Tap Water Yeast Extract Agar (Coombs & Franco 2003); (M3) ISP2 (Taechowisan et al., 2003). The plates were incubated at 28 °C for 2 to 30 days. The colonies recovered from each root were purified and grouped on the basis of phenotypic bacterial characteristics, cell shape and colony morphology observed from scanning electron microscope (SEM) images, motility, growth rate, and Gram reaction. Colonies with different morphological characteristics from the plates of each sample were chosen as different strains according to Bergey's Manual of Determinative Bacteriology (1994). Similar colonies were enumerated, and one was selected (see supplemental information Table S3).

1.3.2. 16S rRNA sequencing and phylogeny construction

Genomic DNA was extracted from single bacterial colonies using a QIAamp DNA mini kit (#51306, Qiagen, Germany) according to the manufacturer's instructions. The extracted genomic DNA was used to amplify the 16S rRNA gene fragment, using universal bacterial primers; 27F (5′-GAGTTTGATCACTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′) (Byers et al., 1998). The PCR reaction was performed in a volume of 25 μL containing 10 μL of GoTaq® Green Master Mix (# 9PIM712, Promega, USA), 0.5 μL of each primer, 100 ng of bacterial genomic DNA and 11 μL of nuclease-free water. The thermal conditions was as follows: an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s and a final extension at 72 °C for 5 min. The PCR products were analyzed on a 1% agarose gel to verify the target size. The PCR products were then purified using the GeneJET PCR Purification Kit (#K0701, Thermo Scientific, USA) following the manufacturer's manual. The Sanger sequencing reaction was performed with 15 ng of the purified template, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (#4337455, Thermo Scientific, USA) on a 3500xL Genetic Analyzer (Applied Biosystems, USA) at the National Key Laboratory of Gene Technology (IBT – VAST, Hanoi, Vietnam). The 16S rRNA gene sequencing results were analyzed with BLASTN (Altschul et al., 1990) and any 16S rRNA gene sequence with an identity score from 97% and above was imported into Mega 7.0 software and aligned using ClustalW (Kumar et al., 2016). The alignments were manually adjusted, and a phylogenetic analysis was performed using the maximum likelihood method. The phylogenetic distance was computed using the *p*-distance method and the robustness of the resulting trees and the statistical significance levels of the interior nodes were obtained by bootstrap analysis with 1000 replicates. Bootstrap values greater than 70% were kept, since nodes supported by values below this number are considered to have weak resolution (Pavlopoulos et al., 2010).

1.3.3. Screening of isolates against pathogens

The isolated *Streptomyces* strains were screened for inhibitory activity against eight pathogens: the bacteria *Staphylococcus aureus* 533R4 (reference: ACT12600) (Firmicutes), *Escherichia coli* WA321 (Dam mutant of *E.coli* K12 AB1157) (Gammaproteobacteria) and *Micrococcus luteus* ATCC49732 (Actinobacteria); the fungi *Rhizoctonia solani* (Basidiomycota), *Candida albicans* BSMY 212 (DSMZ 10697), *Fusarium oxysporum* and *Fusarium solani* (ARSEF 2597) (Ascomycota); and the oomycetes *Pytophthora capsici*. The pathogenic species were kindly provided by prof. Jos Raaijmakers by the Netherlands Institute of Ecology (NIOO). The pathogens and test conditions used here were previously described by Agamennone et al. (2018).

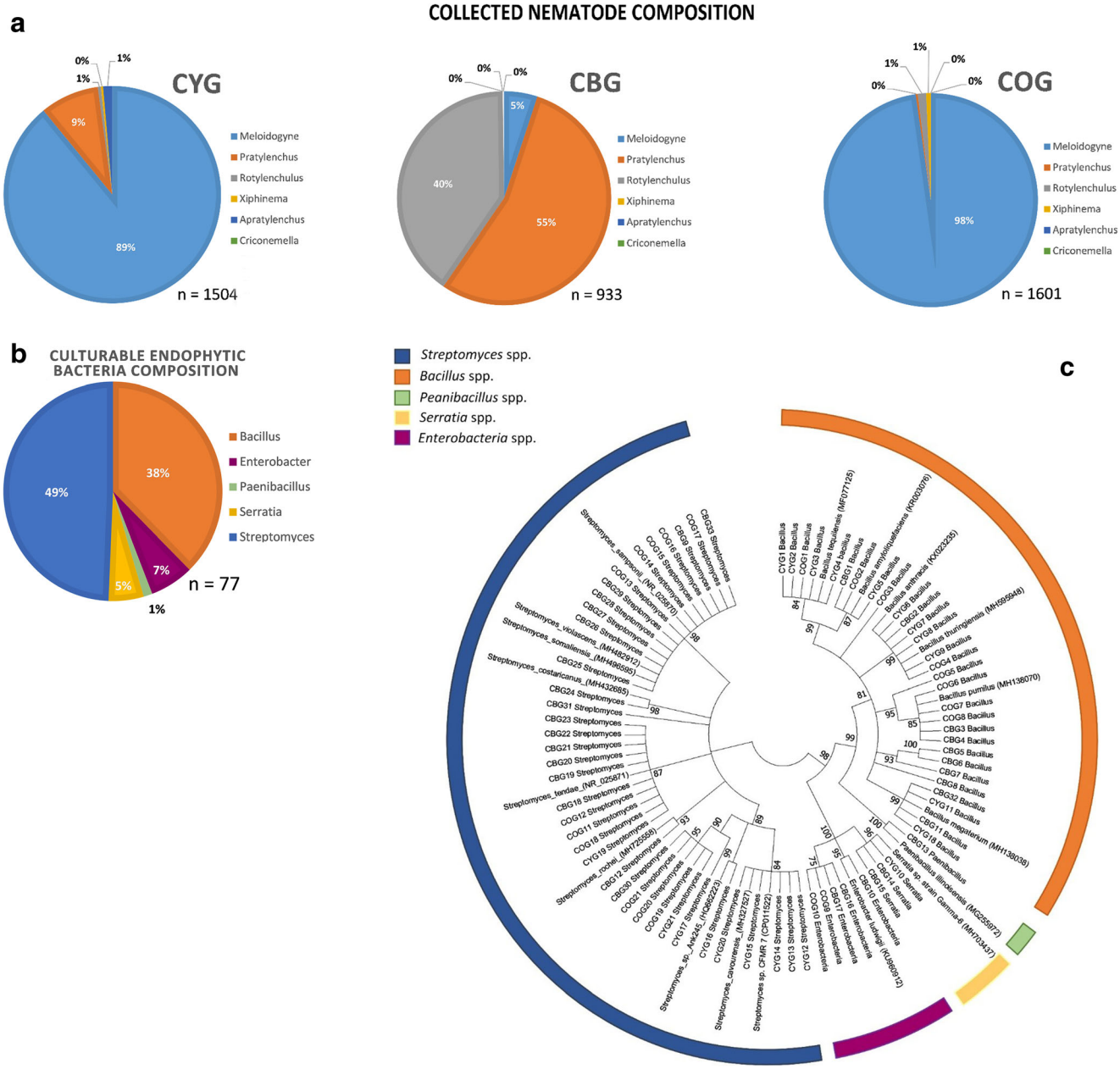

Fig. 1 Distribution of nematodes (a), endophytic bacteria (b) and phylogeny tree of endophytic bacteria (c) isolated from young coffee roots stage – CYG, business stage – CBG and old coffee stage – COG

1.4. In vitro screening of bacterial culture filtrates against the nematode *Meloidogyne incognita*

1.4.1. Nematode culturing for in vitro screening

Nematodes from original materials of sampled coffee tree roots and the surrounding soil were cultured on tomato plants (*Lycopersicon esculentum* L. cultivar Rutgers) in greenhouse (Southey 1986). Single egg mass isolates of each population were separated and plated in distilled water in a glass staining block in a refrigerated incubator (Shanghai Yuejin, China) until hatching, which were inoculated again in fresh tomato seedling 250 cm^3 pot.

Pure *Meloidogyne incognita* isolates were confirmed by PCR identification assay (Kiewnick et al., 2013). Eggs and J2 of *M. incognita* were harvested in accordance with methods described by Carneiro et al. (2004).

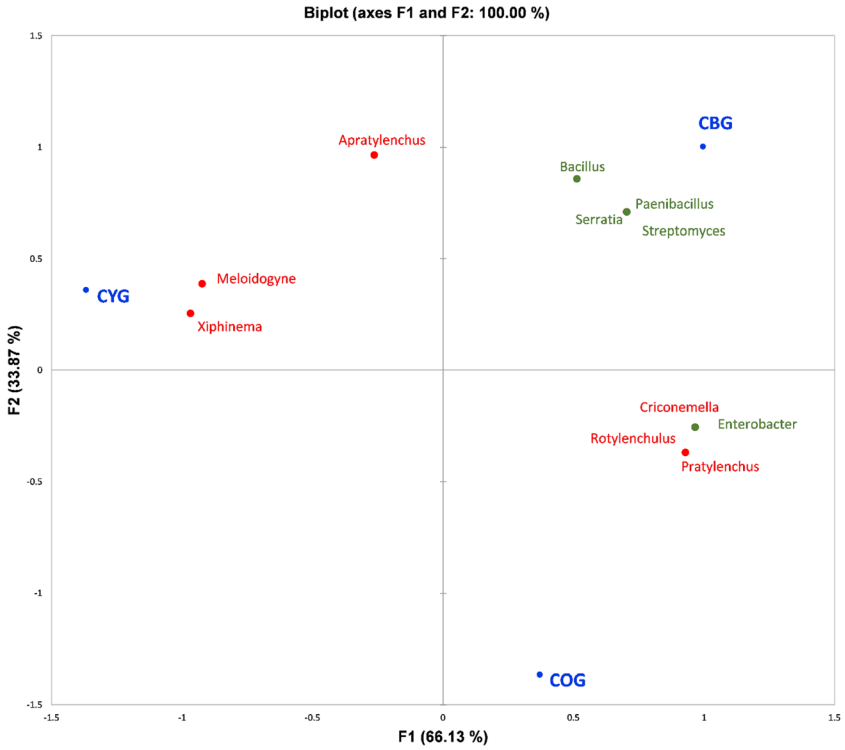

Fig. 2 Principle component analysis of the correlation of plant nematodes (red) and endophytic bacteria (green) in three coffee groups (blue)

1.4.2. Preparation of bacterial filtrates and in vitro screening with *Meloidogyne incognita*

Ten selected *Streptomyces* strains were grown on ISP2 medium (Taechowisan et al., 2003) at 28 °C for 72 h. The culture was centrifuged at 500 x g for 15 min and then filtered through a Whatman no.2 filter paper. Antagonizing effects of selected bacterial strains were tested against the eggs of *M. incognita* by using bacterial culture filtrates (CF) in three different volume ratios (10%, 20% and 30%) of total liquid medium. A dish with an equal volume of sterile water was used in place of CF prepared as a negative control for each level of bacterial concentrates. The CF were then added separately to Petri dishes (35 cm diameter) containing approximately 300 eggs. Hatching success was observed under a stereoscopic microscope 168 h after exposure. Similarly, the anti-nematode activity of bacterial strains against *M. incognita* J2 was performed, with 3 different rate ratios of bacterial liquid volume (10%, 20% and 30%). Dead J2 were observed and mortality was calculated as ((number of dead J2/total number of J2) × 100%) at 168 h after exposure (Yoon et al., 2012). Nematodes were considered dead if they did not move when probed with a fine needle. The experiment was performed with three replications and in triplicate.

1.5. Data analysis

In order to evaluate the effectiveness of bacterial isolation in collected samples and the potential effect of culturable endophytic bacteria on nematodes at different stages of growth, one-way ANOVA ($\alpha = 0.05$) for the number of isolates was performed.

Principal component analysis (PCA) was used to analyze the correlation between the endophytic bacteria and plant parasitic nematode communities at the genus level, using the XLSTAT 6.502 trial version (Addinsoft Inc., USA).

Data from all three anti-nematode activity test runs were pooled. The differences in J2 mortality and egg hatching between treatment and control solutions were analyzed by one-way ANOVA (SPSS for Windows version 14.0).

3 Results

1.6. Nematode community diversity

The nematode component that belonged to different genera was *Meloidogyne, Pratylenchus, Criconemella, Xiphinema* and *Rotylenchulus*, of which *Meloidogyne* was the most abundant genus ($p = 0.039$, $p < 0.05$) (see supplemental information Table S1). Group CYG (5-year-old) had the largest number of nematodes

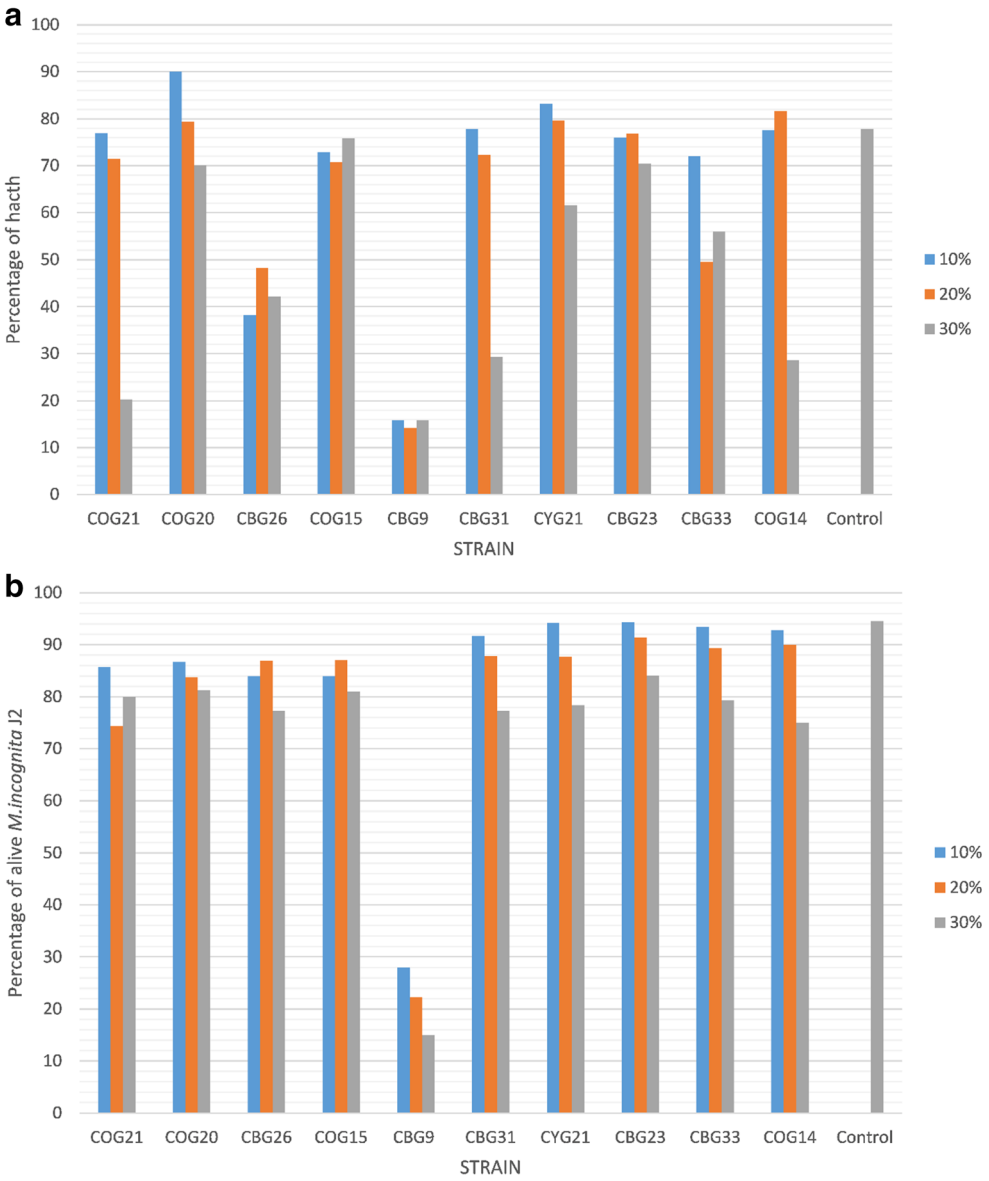

Fig. 3 The screening of antagonistic effects by 10 endophytic bacterial strains on *Meloidogyne incognita*'s egg hatching (a) and second-stage juvenile J2 mortality (b)

collected, followed by group COG (40-year-old), with *Meloidogyne* as the predominant genus (89% and 98% respectively, see Fig. 1a). On the other hand, in group CBG (18-year-old), *Meloidogyne* nematodes account for just 5.3% of the total nematode population, while *Pratylenchus* and *Rotylenchulus* nematodes are the most abundant in densities (Fig. 1a).

Meloidogyne populations collected from the field showed a 300 bp band from the multiplex PCR indicating *M. incognita* (Kiewnick et al., 2013).

3.1 Diversity of endophytic bacteria

Based on the visible morphological differences, a total of 77 bacterial strains were isolated from coffee root samples (Fig. 1b). 16S rRNA sequencing was used to confirm that these strains mainly belong to *Bacillus* spp., *Serratia* spp., *Paenibacillus* spp., *Enterobacter* spp. and *Streptomyces* spp. (Fig. 1c).

1.7. Correlation of plant nematodes with status of endophytic bacterial distribution

Principal component analysis (PCA) explained 100% of the data variability within the first two axes, with axis 1 accounting for 66.13% of variables (including *Meloidogyne*, *Pratylenchus, Rotylenchulus, Criconemella, Xiphinema* and *Enterobacter* spp.) and axis 2 accounting for 33.87% of variables (including *Apratylenchus* spp., *Paenibacillus* spp., *Serratia* spp., *Streptomyces* spp. and *Bacillus* spp.) (Fig. 2). The 18 year-old CBG production group showed a relationship between the occurrence of endophytic bacterial groups (*Bacillus, Paenibacillus, Serratia* and *Streptomyces*) and the absence of plant nematodes. On the other hand, the 40-year-old COG group was associated

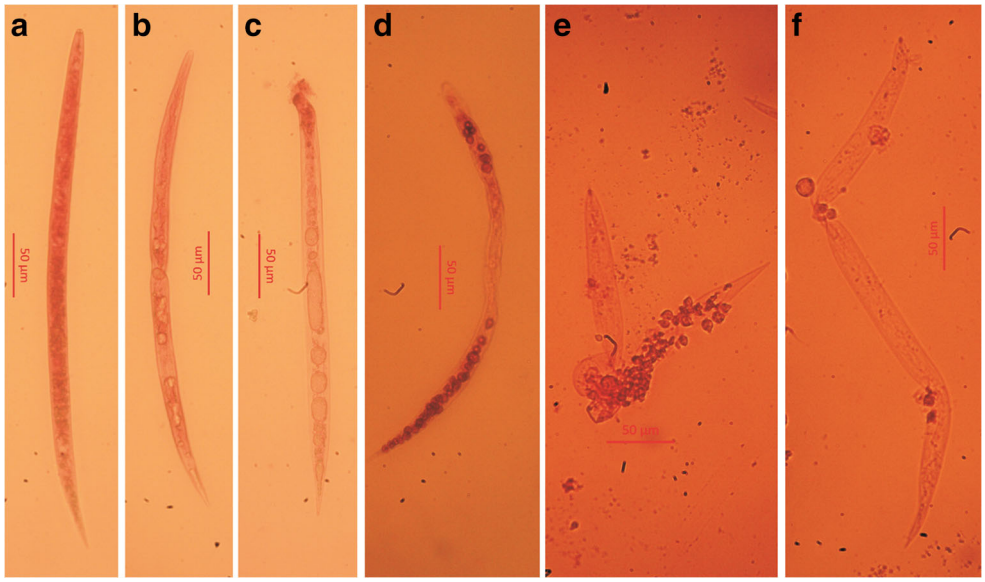

Fig. 4 The morphological pattern of dead *Meloidogyne incognita* J2, picture of dead nematode after 1 day (a), 3 days (b), the observation of shrunken body at 5 days (c, d) and with the body that were lysed at 7 days (e, f) after incubation

with the parasitic nematode genera *Pratylenchus, Criconemella* and *Rotylenchulus* while *Enterobacter* was the major bacterial occurrence. The young replant CYG group showed a tendency towards grouping with the occurrence of plant nematodes (*Meloidogyne, Xiphinema* and *Apratylenchus*) and showed an absence of endophytic bacteria.

1.8. In vitro screening of endophyte antagonistic activity against Meloidogyne incognita

Thirty-five isolated *Streptomyces* strains were screened for their ability to inhibit the growth of a variety of pathogenic microorganisms. The results of the inhibition assays are summarized in Table S4. Of the culturable strains, 91.4% inhibited at least one pathogen and the positive control *Pseudomonas protegens* Pf5 inhibited the growth of all pathogens on all media. Ten strains that showed the most significant antimicrobial activities were selected for antagonistic tests against the plant-parasitic nematode *M. incognita*. (see Fig. 3a and b) After 7 days, five strains showed no significant nematicidal activity with a normal hatching success, no sign of parasitic eggs and small numbers of dead J2 after incubation. The *Streptomyces* strain COG21, however, showed a high hatch success rate at low and medium addition concentrations (CF 10% (76.9%) and 20% (71.4%) respectively), but a substantial reduction in hatching rate at CF30% (20.3% hatching success). Similar results were observed for the *Streptomyces* strains CBG31 and COG14. All three CF levels of the *Streptomyces* CBG26 strain inhibited approximately 50% hatching. Treatment with *Streptomyces* CBG9 medium showed the highest effect on nematode hatching success at all CF levels with the lowest point at 14.2% hatching of the eggs at CF 20% (Fig. 3a). Additionally, while other strains had no significant effect on J2 survival compared to the control, *Streptomyces* CBG9 showed dramatic nematicidal activity. We observed that while the number of J2 in the control was unchanged, the survival rate of J2 decreased sequentially to approximately 15% ($p < 0.05$) with increasing bacterial medium concentration (10, 20 and 30% CF) (Fig. 3b). The effect on nematode physiology upon treatment with *Streptomyces* CBG9 medium with the suggested nematicidal mechanisms is shown in Fig. 4. When *M. incognita* eggs were exposed to bacterial culture filtrate of *Streptomyces* CBG9 we observed lysis of 85.8% of the eggs and a subsequent negative effect on the hatched juveniles. In the antagonistic experiments with *M. incognita* J2, 85% of the J2 were dead and showed a shrunk body after five days and were lysed after seven days of incubation time. This effect suggests a toxic mechanism of bacteria on nematode eggs and juveniles.

4 Discussion

Nematodes have been studied extensively, with *Meloidogyne* spp. and *Pratylenchus* spp. being established as both the dominant species and as important pests in coffee in Vietnam (Chapman 2014; Trinh et al., 2009; Trinh et al., 2019; Vallain et al. 2018) as well as in other countries (Barros et al., 2014; Bell et al., 2018; Vallian et al., 2018). The number of nematodes that we extracted from the field was relatively high (200+ individuals per 100 g of soil and 5 g of root of fresh sample) in all

studied groups. This highlights the problem that nematodes pose in coffee fields in Vietnam, as their population remains high at the time point of sample collecting (May), which is not the annual peak of population number (December) (Nguyen 2016).

Based on our observation, the abundance of nematode densities differed depending on the age of the coffee trees, with the highest number found in the COG group, followed by the older age CYG and CBG groups (Supplementary Table S1). This coincides with the varied number of endophytic bacterial isolates collected from the roots of these groups (Supplementary Table S2). Using selective growth media, we were able to culture several different endophytic bacterial species. *Streptomyces* spp. was identified as the predominant genera, with the combined proportion of 85% among the isolated strains in all the replanted coffee groups. Previous studies of culturable endophytic bacteria in coffee reported that different *Bacillus* spp. are the abundant genus in coffee trees in Brazil (Nunes et al., 2006), Mexico, Columbia, Hawaii (Vega et al., 2005) and Ethiopia (Hallmann et al., 2009). This difference in dominant species, however, can be explained by the media that we used. In our case, the selective medium (M3 medium) selects for the growth of *Actinomycetes* rather than other species leading to the increased number of *Streptomyces* genus in the isolated strains. More importantly, it can still be observed that in the CBG group, the number of extracted nematodes was lower as the number of endophytes was higher than in the other groups. This relationship was demonstrated by PCA (Fig. 2), showing the tendency of nematode occurrence and density associated with the presence of endophytic bacteria. Thus, we suspected that the residing endophytic bacterial community could affect the distribution of nematodes in these coffee trees with similar soil characteristics. In particular, *Meloidogyne* genus distribution varied significantly between CBG group and the other two groups, suggesting the multiple presences of endophytic bacterial genera in CBG negatively influenced the number of *Meloidogyne* nematodes, giving the genus *Pratylenchus* and *Rotylenchulus* opportunities to have higher population densities.

Previous report have shown *M. incognita* as the most widely distributed root knot nematode species in Vietnam (Trinh et al., 2013), agreeing with our finding that *Meloidogyne* was the majority genus by total number in all three groups. In addition, statistical analysis strongly suggested that endophytic bacteria have a specific effect on *Meloidogyne.* Hence, we chose *M. incognita* as the model species for our in-vitro antagonistic screening. The two antagonistic experiments showed that among the ten strains, four strains of *Streptomyces* (COG21, CBG26, CBG31 and COG14) inhibited the hatching of *M. incognita* eggs, while only *Streptomyces* CBG9 exhibited antagonistic ability on both eggs and juveniles of the plant-parasitic nematode *M. incognita*. Under in vitro conditions, *Streptomyces* CBG9 strain inhibited 85.8% of nematode hatching, which was higher than the inhibitory effect of *Streptomyces rubrogriseus* HDZ-9-47 reported by Jin et al. (2017). Also through in vitro screening, *Streptomyces* CBG9 killed 85% of *M. incognita* J2, compared to the 50% killed by the *Streptomyces* sp. in Su et al. (2017) and 80% mortality of J2 by *Streptomyces cacaoi* GY525 in Yoon et al. (2012). *Streptomyces* is a well-known actinomycete genus that acts against plant-parasites (El-Nagdi et al., 2004) by producing a wide variety of important antibiotics. Previously, secondary metabolite products that were isolated from *Streptomyces* species were avermectin (Burg et al., 1979), nanchangmycin, bafilolides, valinomycin, salinosporamide A, milbemycin, kalafungin, thiamycins and axenomycins. These compounds are reported to have high antagonistic activity against many harmful plant-parasitic nematodes. They colonize the same ecological niches as plant pathogens and therefore may be better suited than rhizosphere bacteria to outcompete or directly antagonize pathogens (Aravind et al., 2010; Ryan et al., 2008).

In conclusion, our results indicated endophytic *Streptomyces* strain as potential biocontrol agent of pathogenic nematodes (in particular, *M. incognita*) in coffee trees and open new opportunities for further research into the opportunity to develop biocontrol agents to combat and control pathogenic nematodes in e.g., coffee plants.

4.1 Nucleotide accession numbers

All 16S rRNA gene fragment sequences of isolated bacteria in this study were submitted to NCBI GenBank under the accession numbers shown in Table S6.

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References

Adams BJ, Dillman AR and Finlinson C. Molecular taxonomy and phylogeny. In: Moens M, Perry RN (2009) Root-knot nematodes. CABI, California, pp 119–135

Agamennone V, Roelofs D, van Straalen NM, Janssens TKS (2018) Antimicrobial activity in culturable gut microbial communities of springtails. J Appl Microbiol 125:740–752. https://doi.org/10.1111/jam.13899

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Aravind R, Eapen SJ, Kumar A et al (2010) Screening of endophytic bacteria and evaluation of selected isolates for suppression of burrowing nematode (*Radopholus similis Thorne*) using three varieties of black pepper (Piper nigrum L.). Crop Prot 29:318–324. https://doi.org/10.1016/J.Cropro.2009.12.005

Barros AF, Oliveira RDL, Lima IM et al (2014) Root-knot nematodes, a growing problem for Conilon coffee in Espírito Santo state, Brazil. Crop Prot 55:74–79. https://doi.org/10.1016/J.CROPRO.2013.10.004

Bell CA, Atkinson HJ, Andrade AC, Nguyen HX, Swibawa IG, Lilley CJ, McCarthy J, Urwin PE (2018) A high-throughput molecular pipeline reveals the diversity in prevalence and abundance of *Pratylenchus* and *Meloidogyne* species in coffee plantations. Phytopathology 108:641–650. https://doi.org/10.1094/phyto-10-17-0343-R

Burg RW, Miller BM, Baker EE, Birnbaum J, Currie SA, Hartman R, Kong YL, Monaghan RL, Olson G, Putter I, Tunac JB, Wallick H, Stapley EO, Oiwa R, Omura S (1979) Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob Agents Chemother 15:361–367

Byers HK, Stackebrandt E, Hayward C, Blackall LL (1998) Molecular investigation of a microbial mat associated with the great Artesian Basin. FEMS Microbiol Ecol 25:391–403. https://doi.org/10.1016/S0168-6496(98)00013-0

Campbell WC (2016) Lessons from the history of Ivermectin and other Antiparasitic agents. https://doi.org/10.1146/annurev-animal-021815-111209

Carneiro R, Randig O, Sarah J-L et al (2004) Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. Nematology 6: 287–298. https://doi.org/10.1163/1568541041217942

Chapman KR (2014). Final report: collaborative study of coffee rejuvenation strategies in Vietnam – technical aspects. FAO

Coombs JT, Franco CMM (2003) Isolation and identification of Actinobacteria from surface-sterilized wheat roots. Appl Environ Microbiol 69:5603–5608. https://doi.org/10.1128/AEM.69.9.5603-5608.2003

De Grisse AT (1969) Redescription ou modification de quelques techniques utilissée dans l'étude des nematodes phytoparasitaires. Meded Rijksfac der Landbouveten Gent 351–369

El-Nagdi WMA (2004) Youssef MMA (2004) soaking faba bean seed in some bio-agents as prophylactic treatment for controlling *Meloidogyne incognita* root-knot nematode infection. J Pest Sci 77:75–78. https://doi.org/10.1007/s10340-003-0029-y

Hallmann J, Mekete T, Sikora R, Kiewnick S (2009) Endophytic bacteria from Ethiopian coffee plants and their potential to antagonise Meloidogyne incognita. Nematology 11:117–127. https://doi.org/10.1163/156854108X398462

Jin N, Xue H, Li W et al (2017) Field evaluation of *Streptomyces rubrogriseus* HDZ-9-47 for biocontrol of Meloidogyne incognita on tomato. J Integr Agric 16:1347–1357. https://doi.org/10.1016/S2095-3119(16)61553-8

Kiewnick S, Wolf S, Willareth M, Frey JE (2013) Identification of the tropical root-knot nematode species *Meloidogyne incognita*. *M javanica* and *M arenaria* using a multiplex PCR assay Nematology 15:891–894. https://doi.org/10.1163/15685411-00002751

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for Bigger datasets. Mol Biol Evol 33: 1870–1874. https://doi.org/10.1093/molbev/msw054

Lacava PT, Azevedo JL (2014) Biological control of insect-Pest and diseases by Endophytes. In: Verma V, Gange A (eds) Advances in Endophytic research. Springer, New Delhi, pp 231–256

Le TML, Nguyen TD, Nguyen HT, Liebanas G, Nguyen AD, Trinh QP (2019) A new root-knot nematode, *Meloidogyne moensi* n. sp. (Nematoda: Meloidogynidae), parasitizing Robusta coffee from the Western highlands, Vietnam. Helminthologia. https://doi.org/10.2478/helm-2019-0014

Magarvey NA, Haltli B, He M, Greenstein M, Hucul JA (2006) Biosynthetic pathway for Mannopeptimycins, Lipoglycopeptide antibiotics active against drug-resistant gram-positive pathogens. Antimicrob Agents Chemother 50:2167–2177. https://doi.org/10.1128/AAC.01545-05

Maheshwari DK, Annapurna K (2017) Endophytes : crop productivity and protection. Volume 2. Springer International Publishing. DOI: https://doi.org/10.1007/978-3-319-66544-3

Mohammed SI, Patil MP, Patil RH, Maheshwari VL (2017) Endophytes: potential source of therapeutically important secondary metabolites of plant origin. In: Maheshwari D, Annapurna K (eds) Endophytes: crop productivity and protection, Sustainable development and biodiversity, vol 16. Springer, Cham, pp 213–237

Nguyen NC, Nguyen VT (2000): Plant parasitic nematodes in Vietnam. The Fauna of Vietnam volume 4. Hanoi, Vietnam, science and technology pub., 400 pp. (In Vietnamese)

Nguyen XH (2016) The effect of parasitic nematodes on the level of leaf yellowing and root knot in replanted Robusta coffee trees. Western highlands agriculture and forestry science institute (WASI).

Nunes FV, de Melo IS (2006) Isolation and characterization of endophytic bacteria of coffee plants and their potential in caffeine degradation. In: Environmental Toxicology. WIT Press, Southampton, UK, pp 293–297

Oliveira MN, Santos TM, Vale HM, Delvaux JC, Cordero AP, Ferreira AB, Miguel PS, Tótola MR, Costa MD, Moraes CA, Borges AC (2013) Endophytic microbial diversity in coffee cherries of *Coffea arabica* from southeastern Brazil. Can J Microbiol 59:221–230. https://doi.org/10.1139/cjm-2012-0674

Pavlopoulos GA, Soldatos TG, Barbosa-Silva A, Schneider R (2010) A reference guide for tree analysis and visualization. BioData Min 3:1. https://doi.org/10.1186/1756-0381-3-1

Qin S, Li J, Chen H-H, Zhao GZ, Zhu WY, Jiang CL, Xu LH, Li WJ (2009) Isolation, diversity, and antimicrobial activity of rare actinobacteria from medicinal plants of tropical rain forests in Xishuangbanna, China. Appl Environ Microbiol 75:6176–6186. https://doi.org/10.1128/AEM.01034-09

Reys T, Dumas B (2017) Plenty is no plague: *Streptomyces* Symbiosis with crops. Trends Plant Sci 22(1):30–37. https://doi.org/10.1016/j.tplants.2016.10.008

Ruanpanun P, Laatsch H, Tangchitsomkid N, Lumyong S (2011) Nematicidal activity of fervenulin isolated from a nematicidal actinomycete, *Streptomyces* sp. CMU-MH021, on *Meloidogyne incognita*. World J Microbiol Biotechnol 27:1373–1380. https://doi.org/10.1007/s11274-010-0588-z

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Shiomi HF, Silva HSA, de Melo IS et al (2006) Bioprospecting endophytic bacteria for biological control of coffee leaf rust. Sci Agric 63:32–39. https://doi.org/10.1590/S0103-90162006000100006

Silva HAS, Tozzi JPL, Terrasan CRF, Betiol W (2012) Endophytic microorganisms from coffee tissues as plant growth promoters and biocontrol agents of coffee leraf rust. Biol Control 63:62–67. https://doi.org/10.1016/j.micpath.2016.06.023

Southey JF (1986) Laboratory methods for work with plant and soil nematodes. Books, H.M.S.O

Su L, Shen Z, Ruan Y, Tao C, Chao Y, Li R, Shen Q (2017) Isolation of antagonistic Endophytes from Banana roots against *Meloidogyne javanica* and their effects on soil nematode community. Front Microbiol 8:2070. https://doi.org/10.3389/fmicb.2017.02070

Taechowisan T, Peberdy JF, Lumyong S (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity.

World J Microbiol Biotechnol 19:381–385. https://doi.org/10.1023/A:1023901107182

Trinh PQ, Pham KD, Nguyen NC (2013) Emerging *Meloidogyne* species (root-knot nematodes) threats to coffee in the western highlands in Vietnam. Proceedings of the second VAST-KAST workshop on bio-diversity and bio-active compounds. Pp 313-319

Trinh QP, Le M.T.L., Nguyen T.D., Nguyen HT (2019) *Meloidogyne daklakensis* sp. n. (Nematoda: Meloidogynidae), a new root-knot nematode associated with Robusta coffee (*Coffea canephora* Pierre ex a.Froehner) in the Western Highland, Vietnam, Journal of Helminthology 93 (2): 242–254. doi: https://doi.org/10.1017/S0022149X18000202

Trinh PQ, de la Pena E, Nguyen CN, Nguyen HX & Moens M (2009) Plant-parasitic nematodes associated with coffee in Vietnam. Russ J Nematol, 17:pp73–82

Truong H (2018) Areas of old coffee plants are increasing in Vietnam. The Western Highlands agriculture & forestry science institute (WASI) http://wasi.org.vn/thach-thuc-cua-san-xuat-ca-phe-o-viet-nam/.

Vega FE, Pava-Ripoll M, Posada F, Buyer JS (2005) Endophytic bacteria in *Coffea arabica* L. J Basic Microbiol 45:371–380. https://doi.org/10.1002/jobm.200410551

Vetrivelkalai P, Sivakumar M, Jonathan EI (2010) Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in Bhendi. Journal of Biopesticides 3(2):452

Vietnam Ministry of Agriculture and Rural Development (2016). Decision 2805/QĐ-BNN-TT on Rejuvenation protocol for Robusta coffee

Villain L, Lima Salgada SM, Trinh PQ. 2018. In : Sikora Richard A. (ed.), Coyne Danny (ed.), Hallmann Johannes (ed.), Timper Patricia (ed.). Plant parasitic nematodes in subtropical and tropical agriculture. CABI, Wallingford, pp. 536–583

Vovlas N, Castillo P (2007) Taxonomy and systematic. In: *Pratylenchus* (Nematoda: Pratylenchidae): diagnosis. Biology, Pathogenicity and Management. Brill, pp 36–49

Vu AT, Trinh QP, Nguyen VT (2014) Nematode in basalt soil replaces coffee and the relationship of nematode with yellow leaf coffee symptoms in Gia Lai. Journal of Agriculture and Agricultural Development 23:36–43 (in Vietnamese)

Waller JM, Bigger M, Hillocks RJ (2007) Nematodes. In: Waller JM, Bigger M, Hillocks RJ (eds) Coffee pests, diseases and their management. CABI, Wallingford, pp 258–276

Ward AC, Allenby NEE (2018) Genome mining for the search and discovery of bioactive compounds: the *Streptomyces* paradigm. FEMS microbiology letters, 365(24), fny240. https://doi.org/10.1093/femsle/fny240

Whitehead AG, Hemming JR (1965) A comparison of some quantitative methods of extracting small vermiform nematode from soil. Ann Appl Biol 55:25–38

Wirryadiaputra S, Tran LK (2008) Indonesia and Vietnam. In: Plant-parasitic nematodes of coffee. Springer Netherlands, Dordrecht, pp 277–292

Yoon GY, Lee YS, Lee SY et al (2012) Effects on *Meloidogyne incognita* of chitinase, glucanase and a secondary metabolite from Streptomyces cacaoi GY525. Nematology 14:175–184. https://doi.org/10.1163/138855411X584124