Endophytic Bacteria as an Alternative Agent for the Biological Control of Plant Parasitic Nematodes on Black Pepper

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Abstract

Plant parasitic nematodes cause damage and losses significantly to black pepper in Indonesia. Control of plant parasitic nematodes with pesticides is often restricted due to their high toxicity and negative impact on the environment. The need for environmentally safe control strategies has increased interest in developing biological control measures. Endophytic bacteria are ubiquitous in most plant species and reside within healthy plant tissue without producing symptoms of damage. The internal plant habitat provides several advantages for endophytic bacteria as biological control agents: 1) colonization of an ecological niche also used by plant pathogens, 2) less competition with other microorganisms, 3) sufficient supply wirth nutrients, 4) less exposure to environmental stress factors, and 5) better translocation of bacterial metabolites throughout the host plant. The objective of this work was to evaluate the effect of endophytic bacteria isolated from black pepper to control *Meloidogyne incognita* on black pepper. The results showed that some endophytic bacteria were able to reduce the root galls caused by *Meloidogyne incognita* and juveniles of nematodes in the soil up to 70% as well as to promote the growth of pepper seedlings.

Keywords: endophytic bacteria, Meloidogyne incognita*, black pepper, root gall*

Introduction

Black pepper (*piper nigrum* L) is one of the important export commodities in Indonesia. However, the production of black pepper is now threatened by pests and diseases. One of the main disease on black peper is yellow diseasae caused by plant parasitic nematodes i.e. *Meloidogyne* sp. and *Radopholus similis*. Several control methods have been developed to combat the nematodes, but they are still a serious problem of black pepper especially in Bangka Island, Indonesia. The use of chemical pesticides with persistent pesticides can result in negative impacts to the environment, pathogens become more resistant, disruptive presence of beneficial microbes in the soil, and human health. Use of pesticides also provides residual effects on pepper which is currently an one obstacle for consumers, mainly for export purposes that are very concerned about the health and environmental aspects. In connection with the need to develop a system of agricultural production, including plant disease control systems are environmentally sound one of them by optimizing the use of biological agents.

Endopyitic bacteria are bacteria living inside plant tissues without doing symptoms on these plants. As the internal plant habitat, endophytic bacteria provide several advantages as biological control agents: 1) colonization of an ecological niche also used by plant pathogens, 2) less competition with other microorganisms, 3) sufficient supply with nutrients, 4) less exposure to environmental stress factors, and 5) better translocation of bacterial metabolites throughout the host plant. Several studies have shown that endophytic bacteria isolated from various plant tissues are able to suppress plant parasitic nematodes *Meloidogyne incognita* on cotton and tomato plants (Hallmann 2001) as well as to control plant nematode *Pratylenchus* sp on patchouli (Harni 2010). In addition, some bacterial isolates endophytes have been reported to enhance plant growth because it can increase the availability of nutrients to induce plant resistance. The objective of this research was to determine the potential of biological agents bacterial endophytes isolated from pepper for controlling *Meloidogyne incognita* on pepper and their effects on the plant growth.

Materials and Methods

Isolation of Endophytic Bacteria

A total of 10 samples of healthy pepper plants were taken at random each of pepper plants and were cultivated in Bangka, Bogor and Sukabumi. The pepper plant roots were transported to the laboratory for immediate processing. The roots were washed with running tap water to remove adherent soil particles then blotted dry on tissue paper. The root material was weighed and surface sterilized with alcohol at 70% for 30 sec and in 2% of sodium hypochlorite (NaOCl) containing 0.01% of Tween 20 for 3 min, followed by four rinses with sterile 0.01 M of potassium phosphate buffer (PB) at pH of 7.0 (80 g NaCl, 2 g KCl, 11.5 g Na_2HPO_4, 2 g KH_2PO_4). To confirm complete surface sterilization (sterility check), the surface disinfected roots were imprinted on Tryptic Soy Agar (TSA). If bacterial growth occurred within 48 h, samples were discarded. The pepper roots were then macerated with a sterile mortar and pistil in three times PB (w/v). The macerate was decanted into sterile conical flasks and shaken for 30 sec. A dilution series was made and 100 µl of each dilution was plated onto 1/10 strength of TSA on petri disk. Petri plates were incubated at 24°C for 2-3 days and colony forming units (cfu) were determined. Three replicates were made for each dilution. On each petri plate containing approximately 10 bacterial strains was marked and all bacterial strains from this zone were transferred and purified on full strength of TSA. The bacterial strains were stored in Tryptic Soy Broth (TSB) plus 20% of glycerol at -20°C. Two isolates collection of endophytic bacteria were used in this experiment.

Inoculum of Parasitic Nematodes

Inoculum of nematode *Meloidogyne incognita* was isolated from the infected roots pepper of nematodes in Central Bangka, Province Bangka-Belitung Indonesia. Subsequently, the nematodes were cultured and propagated on susceptible tomato plants (cv. Ratna) for 2 months. After that plant was uprooted and the nematodes was extracted and used as a source of inoculum.

Effect of Endophytic Bacteria on Meloidogyne incognita

The effectiveness of biological agents against *M. incognita* on pepper seedlings was done in a greenhouse. Two months pepper cuttings with one segment were treated with isolates of endophytic bacteria. Eight isolates of selected bacterial endophytes isolated from root pepper and patchouli isolate MER7, AA2, HEN1, HEN3, MER9, ANIC, TT2 and EH11 were used in this study. The bacterial isolates were grown on TSA medium 100% for 24-48 h at room temperature. A single colony of the bacterial isolate was transferred into 100 ml of liquid TSB medium and shaken for 2 days with a speed of 150 rpm at a room temperature. Furthermore, the bacterial suspension was centrifuged at 11.000 rpm for 15 min at -4^{o}C to separate the supernatant/culture filtrate with a bacterial cell culture. Suspension of bacteria was made by diluted the bacterial cell with sterile water. Roots of 3 months old of pepper seedlings were soaken for 1 h in bacterial suspension with a population density of 10^9-10^{10} CFU. The treated pepper was planted subsequently in pots. One week after the bacterial treatment, the plants were inoculated with 1000 larvae of the nematode *M. incognita* per plant. Each treatment was repeated for 5 times and arranged in a completely randomized design, with positive control was the plant only nematode inoculation, while negative control was plants no inoculation with nematodes. Three months after inoculation the plants were harvested and the number of galls and the population of nematodes in the roots and the soil were observed as well as the plant height and weight, root weight, number of branches and number of leaves.

Results and Discussion

Effect of Endophytic Bacteria against *M. incognita* on Black Pepper

In this study, eight isolates of endophytic bacteria isolated from root pepper and patchouli were tested on seedling of black pepper against *M. incognita* in the greenhouse. All of isolates of bacterial endophyte were able to reduce gall and juveniles of *M. incognita*. Four out of eight isolates, i.e EH11, HEN1, HEN3 and TT2 significantly reduced gall nematodes compared to the control. Isolate EH11 showed the highest in reducing gall nematodes compared to other isolates (Table1). The isolates of bacteria were also able to suppress juveniles of nematode in the soil compared to control. Six isolates of EH11, AA2, HEN1, MER9, ANIC and TT2 significantly reduced number of juveniles compared to control. The highest influence in suppressing number of juveniles of *M.incognita* was showed by isolate HEN1 and followed by EH11. Five Isolates of HEN1, EH11, TT2, ANIC, and AA2 were able to reduce the population of *M. incognita* in the soil at more than 75%.

Table 1. Effect of isolates of biological agents bacterial endophyte against the number of galls and the population of nematodes larvae

Treatments	Number of galls	Population of the juveniles	Population of reduction (%)
Isolate MER 7	70,4 ab	1212 b	15,36
Isolate EH11	9,0 c	23 ef	96.97
Isolate ANIC	66,0 ab	312 cde	78,21
Isolate MER 9	57,4 ab	504 c	64,80
Isolate AA2	48,8 bc	352 cd	75,41
Isolate HEN1	42,2 bc	13 f	99,00
Isolate HEN3	38,4 bc	1176 b	17,87
Isolate TT2	32,6 bc	50 ef	96,50
Control + (with nematode)	101,0 a	1432 ab	-

Values followed by same small letters on the same column are not significantly different at 5% by DMRT.

Biological agents can suppress the development of plant diseases through a mechanism of competition, predation and the resulting antibiotics (Kloepper *et al*. 1991). Some research indicates that the use of biological agents endophytic bacteria through seed treatment reduce 30-50% of the amount of gall of *M. incognita* on cotton plants (Hallmann *et al.* 1997). Some bacterial endophytes isolated from the roots patchouli suppress populations of *Pratylenchus brachyurus* at 73.9% on patchouli plants in the greenhouse (Harni *et al*. 2007). Biological agents, including endophytic microbial may protect plants against plant-parasitic nematodes through various ways such as by producing toxic compounds that are nematicidals (Sikora *et al*. 2007; Yang *et al*. 2011). In addition, certain bacteria can also suppress the development of plant diseases due to the ability of bacterial endophytes in binding Fe (III) and to produce HCN (Keel *et al*. 1992). A treatment of culture filtrate of endophytic bacterial isolate isolated from patchouli was also able to reduce *Pratylenchus barchyurus* larvae up to 100%, 24 h after the treatment (Harni 2007).

Application of several bacterial endophytes can increase plant growth of black pepper. Isolate MER7 increased plant hight of pepper, number of branches and number of leaves (Table 2). Isolates EH11, MER9, TT2 increased plant height compared to control, while isolate HEN1 increased the number of brances compared to control. Vetrivelkalai *et al*. (2010) reported that bacterial endophytes suppressed the gall number of nematodes on bhendi, but not all of bacterial isolates increased the plant growth.

Table 2. The influence of endophytic bacteria on the growth of pepper plants inoculated with *M. incognita* 3 months after treatment

Treatments	Plant height addition (cm)	Numbers of branches increment	Numbers of leaves increment
Isolat MER7	24,20 a	4,6 a	12,6 ab
Isolat EH11	21,60 ab	2,0 bcd	9,4 abc
Isolat MER9	20,40 ab	2,0 bcd	10,0 abc
Isolat TT2	20,40 ab	1,6 bcd	7,8 bc
Isolat AA2	18,80 ab	2,0 bcd	7,8 bc
Isolat HEN1	17,60 ab	2,8 abc	9,2 abc
Isolat HEN3	16,40 ab	2,2 bcd	9,2 abc
Isolat ANIC	14,76 b	1,2 cd	5,2 c
Control + (with nematode)	16,00 b	0,4 d	6,4 c

Values followed by same small letters on the same column are not significantly different at 5% Duncan test.

Inoculation with nematodes caused damage from the stabbing stylet and secretion of enzymes released nematodes when the nematodes feed. Hallmann *et al*. (1997) reported that nematodes are taking root cells can reduce the ability of plants to absorb water and nutrients from the soil and cause symptoms such as lack of water and nutrients. Besides, the reduced concentration of plant growth regulators such as auxins, cytokinins and gibberellins can occur because the nematode secretes enzymes cellulose and pectinase that may degrade the cell up to the root tip injuries and broken, this leads to auxin is not active then growth of plant will be stunted.

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References

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. 1997. Bacterial endophytes in agricultural crops. *Canadian Journal of Microbiology* 43:895-914

Hallmann J, Quadt-Hallmann A, Miller WG, Sikora RA, Lindow SE. 2001. Endophytic colonization of plant by biocontrol agent *Rhizobium etli* G12 in relation to *Meloidogyne incognita* infection. *Pyhtopathology* 91:415-422

Harni R, Munif A, Supramana, Mustika I. 2007. Pemanfaatan bakteri endofit untuk mengendalikan nematode peluka akar (*Pratylenchus brachyurus*) pada tanaman nilam. *Jurnal Hayati* 14 (1): 7-12.

Keel C, Schneider U, Maurhofer M, Voisard C, Laville J, Burger U, Withner P, Hass D, Defago G. 1992. Suppression of root disease by *Pseudomonas fluorescens* CHAO: Importance of the bacterial secondary metabolite 2,4 diacetylphloroglucinol. *Mol. Plant-Microbe Interaction* 5:4-13.

Kloepper JW, Zablotowiz RM, Tipping EM, Lifshiftz R. 1991. Plant growth promoting mediated by bacterial rhizosphere colonizers. In: Keister DL and Cregan PB.(Eds.). The Rhizosphere and Plant Growth. Kluwer Academic Publisher. Dordrecht, The Netherland. P:315-326.

Sikora RA, Schafer K, and Dababat AA. 2007. Modes of action associated with microbially induced in planta suppression of plant parasitic nematodes. *Australasian Plant Pathol.* 36:124-134.

Vetrivelkalai P, Sivakumar M, Jonathan FI. 2010. Biocontrol potential of endophytic bacteria on *Meloidogyne incognita* and its effect on plant growth in bhendi. *Journal of Biopesticides* 3 (2): 452-457

Yang CJ, Zhang XG, Shi GY, Zhao HY, Chen L, Tao K, Hou TP. 2011. Isolation and Identification of endophytic bacterium W4 against tomato *Botrytis cinerea* and antagonistic activity stability. *African Journal of Microbiol*. *Res.* 5 (2): 131-136.

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