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Nematicidal and Plant Growth-Promoting Activity of *Enterobacter asburiae* HK169: Genome Analysis Provides Insight into Its Biological Activities ^S

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In the course of screening for microbes with nematicidal activity, we found that *Enterobacter asburiae* HK169 displayed promising nematicidal activity against the root-knot nematode *Meloidogyne incognita,* along with plant growth-promoting properties. Soil drenching of a culture of HK169 reduced gall formation by 66% while also increasing root and shoot weights by 251% and 160%, respectively, compared with an untreated control. The cell-free culture filtrate of the HK169 culture killed all juveniles of *M. incognita* within 48 h. In addition, the nematicidal activity of the culture filtrate was dramatically reduced by a protease inhibitor, suggesting that proteolytic enzymes contribute to the nematicidal activity of HK169. In order to obtain genomic information about the HK169 isolate related to its nematicidal and plant growth-promoting activities, we sequenced and analyzed the whole genome of the HK169 isolate, and the resulting information provided evidence that the HK169 isolate has nematicidal and plant growth-promoting activities. Taken together, these observations enable the future application of *E. asburiae* HK169 as a biocontrol agent for nematode control and promote our understanding of the beneficial interactions between *E. asburiae* HK169 and plants.

Keywords: *Enterobacter asburiae*, *Meloidogyne incognita,* biocontrol agent, plant growth promotion, genome analysis

Introduction

Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive plant parasites and have a very wide host range [1]. An infection of root-knot nematodes causes the formation of galls on the root system, which interrupt the root uptake of water and nutrients [2]. Resistant cultivars, crop rotation, and chemical nematicides have been widely used to control root-knot nematodes. However, plant resistance against root-knot nematodes is often unstable or ineffective against certain species of root-knot nematodes [3]. Furthermore, the overuse of chemical nematicides has resulted in environmental pollution and has led to the emergence of resistant strains [4, 5]. Therefore, instead of chemical control for root-knot nematodes, alternative strategies such as biological control agents are being explored [6].

A rhizosphere is a site where complex interactions among plant roots, soil, and soilborne microorganisms occur [6]. Plant parasitic nematodes and rhizobacteria occupy the same niche (rhizosphere), and some of these may have antagonistic or symbiotic relationships [6, 7]. Rhizobacteria can prevent the proliferation of nematodes by producing nematicidal substances such as antibiotics and cell-wall-degrading enzymes or by the induction of systemic resistance in the host plants [8, 9]. Rhizobacteria also enhance the growth of plants by producing growth-promoting substances, such as indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, and volatile compounds [10, 11].

The *Enterobacter* species are gram-negative bacteria belonging to the family Enterobacteriaceae, and the genus *Enterobacter* has been one of the most rapidly expanding genera in the last decade [12]. Members of this genus are most commonly isolated from the environment, in particular from soil, plants, and fruits, but also from human sources [13]. Several strains of *Enterobacter* have been investigated as plant growth-promoting rhizobacteria that colonize plant roots and improve the health or yields of plants [14, 15]. Although plant growth-promoting rhizobacteria have been investigated widely as biological control agents for plant parasitic nematodes, the nematicidal nature of *Enterobacter* has been rarely described [16].

The present study was designed to examine the agricultural benefits of *Enterobacter asburiae* HK169 isolated from the root system of tomato plants. The activities of the HK169 isolate in both stimulating plant growth and controlling root-knot nematodes have been explored and discussed. In addition, a whole-genome analysis of *E. asburiae* HK169 enables the comprehensive identification and profiling of potential factors contributing to its beneficial role in plant growth-promoting and nematicidal activities.

Materials and Methods

Antagonistic Bacterial Strain

The rhizobacterial strain HK169 isolated from tomato roots has been deposited into the Korean Agricultural Culture Collection (KACC) with accession number KACC 81020BP. The isolated bacterial strain was maintained on tryptic soy agar or Luria-Bertani (LB) agar medium for further study and was incubated at 30°C for 3 days to investigate its nematicidal activity.

Nematode Eggs and Juveniles

Root-knot nematode *Meloidogyne incognita*, identified previously by Hwang *et al.* [17], was proliferated on tomatoes (*Lycopersicum esculentum* Mill. cv. Seokwang; Seminis Vegetable Seeds, Korea) in a greenhouse at 28 ± 5°C. To obtain nematode eggs, tomato roots that had a severe case of galling due to an infection of *M. incognita* were submerged into a 1% sodium hypochlorite solution and then macerated with a blender. The ground plant materials were filtrated through a 45 and 25 μm sieve, sequentially, after which the eggs were collected and rinsed with distilled water. To obtain second-stage juveniles, the collected eggs were allowed to hatch using modified Baermann funnels [18] at room temperature.

In Vitro Mortality Bioassay

M. incognita juveniles and eggs were used for a bioassay using 96-well tissue culture plates (SPL Life Sciences, Korea). Aliquots of 25 μl suspensions containing approximately 50 juveniles and 150 eggs were placed in each well, after which the culture broth

(or culture filtrate) of *E. asburiae* HK169 was added to the wells to reach final concentrations of 5%, 10%, and 20% (v/v). As a cell treatment, pelleted cells after centrifugation of the culture broth were resuspended in distilled water at 2×10^8 cells/ml and then added to a juvenile solution to reach a final concentration of 10% (v/v). Abamectin (1 μg/ml; Sigma-Aldrich, USA) and LB medium were used as a positive and negative control, respectively. After incubation for 48 or 72 h, the juveniles were observed under a light microscope. Nematode juveniles were judged as dead if their bodies were straight and showed no movement. The rate of inhibition of nematode egg hatching was assessed under a light microscope at 7 days after treatment, and the egg hatch rate (EH) was calculated as follows: EH = [number of juveniles/(number of eggs + number of juveniles)] × 100. The experiment was conducted twice with three replicates. Mortality values were calculated according to Abbott's formula [19]: mortality (%) = [(mortality percentage in treatment – mortality percentage in control)/(100 – mortality percentage in control)] × 100. Additionally, hatch inhibition was calculated as follows: Hatch inhibition (%) = [(EH in control) – (EH in treatment)/(EH in control)] × 100.

To investigate if the protease of *E. asburiae* HK169 had an effect on the nematicidal activity, a protease-inhibitor cocktail (Sigma-Aldrich, USA) was added to the culture broth at a ratio of 1:9. The juvenile suspension (~50 juveniles/well) was placed in each well, followed by the addition of 2.5%, 5%, and 10% of the mixture containing the bacterial culture and the protease inhibitor. The nematicidal activity was evaluated 72 h after the treatment.

In Vivo Assessment of Plant Disease Control

To explore the degree of disease control efficacy, *M. incognita* eggs (~5,000) were inoculated into each pot containing a 3-week-old tomato seedling. After 1 h, a 2-day-old culture (20 ml) of *E. asburiae* HK169 was applied to each pot by drenching. A 2,000-fold dilution of sunchungtan (active ingredient: 30% fosthiazate; Farm Hannong, Korea) and a LB medium containing a 0.025% Tween 20 solution were used as positive and negative controls, respectively. After 6 weeks of incubation, the plants were carefully uprooted and washed free of sand particles. The galling index (GI) of the tomato roots was assessed in five phases depending on the formation level of galls (0, 0%; 1, 1−25%; 2, 26−50%; 3, 51−75%; 4, 76−100%) [20].

Whole-Genome Sequencing and Genome Analysis

The genomic DNA of *E. asburiae* HK169 was extracted using QIAamp genomic DNA kits (Qiagen, Germany), and the quantity and quality of the genomic DNA were determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). The 20-kb SMRTbell library was constructed and then sequenced with the PacBio RS II platform (Pacific Biosciences, USA). The resulting sequences were de novo assembled using the RS hierarchical genome assembly process 3.0 in the SMRT analysis program (ver. 2.3, Pacific Biosciences, USA). Genome annotation was predicted using Prokka ver. 1.11 and the prokaryotic genome annotation

pipeline (PAGP ver. 3.3) of NCBI. For a whole-genome comparison of closely related strains, the average nucleotide identities were calculated with currently available genome sequences of *Enterobacter* spp. using OAT ver. 0.90 (http://www.ezbiocloud.net/tools/orthoani). The gene clusters involved in the biosynthesis of secondary metabolites were analyzed with antiSMASH ver. 3.0 (http://antismash.secondarymetabolites.org).

Phylogenetic Analysis

To identify isolated bacterial strains, pairwise sequence similarities of the 16S rRNA gene were determined with the most closely related strains, using a global alignment algorithm on the EzTaxon server (http://www.ezbiocloud.net/identify). The consensus DNA sequence of the *gyrB* gene was subjected to sequence-similarity searches using BLASTn (http://www.blastn.ncbi.nlm.nih.gov). The sequences of *gyrB* were aligned using ClustalW implemented in MEGA ver. 7, and distances were estimated according to the model of Tamura and Nei [21]. The robustness of the inferred trees was evaluated by a bootstrap analysis consisting of 1,000 resamplings, and the phylogenetic tree was generated using the neighbor-joining method.

Statistical Analysis

Data ($n = 3$ independent experiments) were subjected to a one-way ANOVA, and the means of the treatments were separated by Duncan's multiple range test using the R-software package (ver. 3.3.1). All values are expressed as the mean ± standard deviation. Differences were considered statistically significant for p values of less than 0.05.

Results and Discussion

Isolation and Identification of *E. asburiae* HK169

In the course of screening for microbes with nematicidal activity, we found that rhizobacterial strain HK169 isolated from tomato roots showed strong nematicidal activities against the root-knot nematode *M. incognita*. This strain is gram-negative and rod-shaped with a cell size of 0.5–0.6 by 1.2–1.6 μm (Figs. S1A and S1B). The colonies grown on LB agar media are creamy white, mucoid, translucent, and 5–9 mm in diameter after 48 h of incubation at 30°C (Fig. S1).

Sequence alignment of the 16S rRNA gene revealed that the HK169 strain belongs to the genus *Enterobacter* and is most closely related to *Enterobacter cloacae* MNCRE16 (JYMH01000051), *Enterobacter tabaci* YIM Hb-3[T] (KP990658), *Enterobacter asburiae* JCM 6051[T], and *Enterobacter cancerogenus* LMG 2693[T] (Z96078), with 99.5%, 99.3%, 99.2%, and 99.0% similarity, respectively. A phylogenetic analysis based on *gyrB* gene sequences showed that the HK169 strain was the most close to *E. asburiae* strains (Fig. S1C). The HK169

strain shared very high average nucleotide identity values (>97%) with *E. asburiae* strains E20 (CP012999), CAV 1043 (CP011591), L1 (CP007546), and ATCC 35953 (CP011863). Thus, the HK169 strain was identified as *E. asburiae*.

In Vitro Nematicidal Activity of *E. asburiae* HK169

To investigate the nematicidal activity of *E. asburiae* HK169, an *E. asburiae* HK169 culture broth, cell-free filtrate, and cells were individually treated to nematode juveniles and eggs in vitro. The treatment with 10% culture broth (v/v) resulted in 98% juvenile mortality at 48 h after treatment and suppressed egg hatching by 54% at 7 days after treatment, rates that were comparable to those of the abamectin treatment (Table 1); the juvenile mortality and egg hatch suppression were increased depending on the concentration of the treatment. Furthermore, we observed that the nematode cuticle was thinner than those of the non-treatment control group at 72 h after the treatment (data not shown). When the culture filtrate of *E. asburiae* HK169 was exposed to juveniles, we observed that all juveniles were dead in 48 h (Fig. 1A). However, when *E. asburiae* HK169 cells were utilized, the mortality rate was less than 3% at 48 h after the treatment, although the mortality rate increased, reaching 49% at 72 h (Fig. 1A). Thus, our results showed that nematicidal substances may be released from the cells and may accumulate in the medium.

To identify the nematicidal compounds from the culture filtrate of *E. asburiae* HK169, the culture filtrate was partitioned with the organic solvents ethyl acetate and butanol. Unexpectedly, most of the nematicidal activity disappeared in all of the organic solvent fractions, through an aqueous layer remained after partitioning (data not

Table 1. In vitro nematicidal activity of the *Enterobacter asburiae* HK169 culture broth[a].

Treatment	Concentration	Mortality (%) of J2s	Hatching inhibition (%) of eggs
HK169	10%	98.2 ± 2.4 z[b]	53.7 ± 5.7 z
	5%	70.5 ± 10.5 y	40.6 ± 0 y
	2.5%	62.0 ± 9.4 xy	22.7 ± 2.4 x
Abamectin	1 μg/ml	97.6 ± 3.4 z	55.6 ± 4.7 z

[a]Mortality and egg hatching inhibition of *M. incognita* were investigated at 48 h and 7 days after treatment, respectively.

[b]Each value represents the mean ± standard deviation of three replications. Different small letters in each column indicate a significant difference at $p < 0.05$ (Duncan's test).
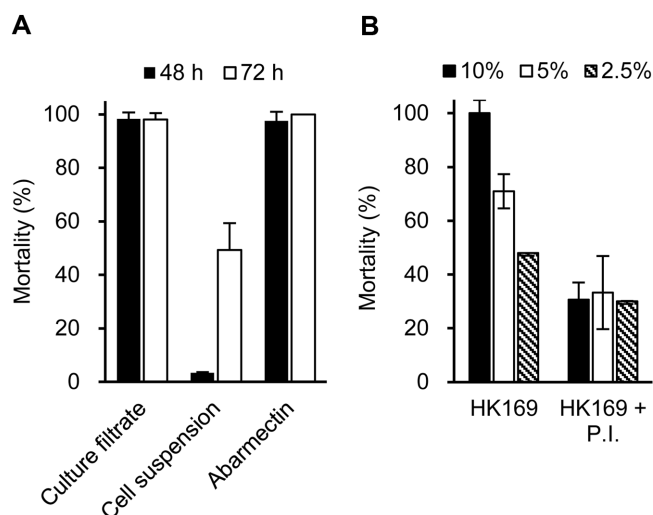
Fig. 1. In vitro nematicidal effects of *Enterobacter asburiae* HK169 on second-stage juveniles of *Meloidogyne incognita*.
(**A**) Nematicidal activity of cell-free culture filtrate and cell suspension of the HK169 strain. (**B**) Effects of a protease-inhibitor cocktail on the nematicidal activity of the culture filtrate of the HK169 strain.

Fig. 2. Nematicidal and plant growth-promoting activities of *Enterobacter asburiae* HK169.
(**A**) Root gall formation and shoot growth of tomato plants treated with the culture broth of HK169 or the nematicide fosthiazate. (**B**) In vivo nematicidal activity of the HK169 strain on tomato plants infected with *Meloidogyne incognita*, which was based on the gall formation. (**C**) Plant growth-promoting effect of the HK169 strain.

shown). Although the ethyl acetate fraction showed low nematicidal activity (20%), a possible active compound in the ethyl acetate fraction is likely to be responsible for a small portion of the nematicidal activity of the HK160 isolate, as the ethyl acetate fraction showed nematicidal activity at high concentrations exceeding 2,500 μg/ml (data not shown).

Extracellular enzymes, including serine proteases, chitinases, and collagenases, have been described as important factors that can degrade the structural constituents of nematode surfaces or eggshells [22]. Extracellular proteases have been intensively studied in relation to antagonistic fungi and bacteria against pathogenic nematodes [23]. In particular, a number of nematicidal proteases have been identified from *Bacillus* spp. [24, 25]. In this study, to determine whether proteases of the HK169 strain have nematicidal activity, bacterial cultures were exposed to juveniles with a protease-inhibitor cocktail. Treatments of 10%, 5%, and 2.5% of the bacterial culture without the protease-inhibitor cocktail showed 100%, 71%, and 48% mortality rates, respectively, with the mortality rate depending on the culture concentration. In contrast, bacterial cultures containing the protease-inhibitor cocktail showed dramatically reduced mortalities compared with the treatment without the protease-inhibitor cocktail, suggesting that the presence of extracellular proteases is associated with the nematicidal activity of *E. asburiae* HK169 (Fig. 1B). Unexpectedly, we
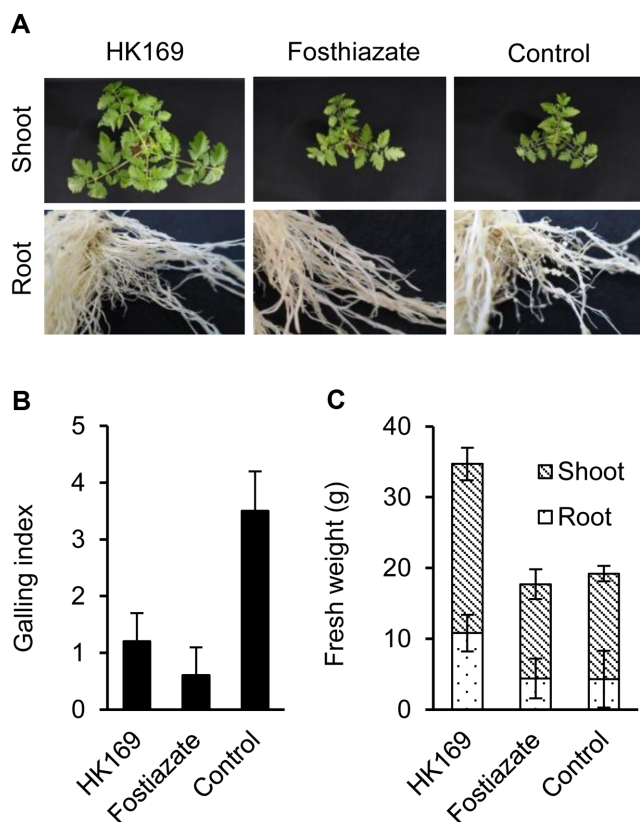
observed that the addition of the protease-inhibitor cocktail led to nearly 30% mortality regardless of the culture concentration (Fig. 1B). For this phenomenon, we cannot exclude the possibility that *E. asburiae* HK169 may possess other nematicidal mechanisms beyond proteases or that proteinase inhibitors may be toxic to nematodes and insects [26], although we did not observe nematicidal toxicity of the proteinase-inhibitor cocktail in this study.

In Vivo Nematicidal Activity and Plant Growth Promotion by *E. asburiae* HK169

The culture broth of *E. asburiae* HK169 exhibited a reduction in root-knot disease caused by the infection of *M. incognita* on tomato roots (Figs. 2A and 2B). Tomato plants treated with the HK169 culture broth showed 65% less root gall formation compared with the non-treatment

control (Fig. 2B). Furthermore, treatment with the HK169 culture broth stimulated the growth of tomato plants (Figs. 2A and 2C). The means of the root and shoot weights of the tomato seedlings treated with the HK169 culture broth were increased by 251% and 160% relative to the corresponding control values, respectively (Fig. 2C). As a control, when the commercial nematicide fosthiazate was applied to the tomato plants, the galling index was significantly reduced compared with that of the non-treatment control, whereas the plant growth outcome did not differ from that of the control plants (Fig. 2).

Duponnois *et al.* [27] described the potential of *E. cloacae* to stimulate plant growth and to reduce the egg formation and hatching of the root-knot nematode *M. incognita*. The possible mechanism was proposed to be *Enterobacter* spp. improving the levels of nitrogen fixation, IAA production, and ACC deamination of the plants [10, 13, 28]. Some plant growth-promoting rhizobacteria interfere with the relationship between plant-parasitic nematodes and their hosts, through which rhizobacteria produce metabolites that reduce egg hatching, nematode attraction, and degradation of specific root exudates controlling nematode behavior [29]. However, less is known about the possible mechanisms of action with regard to controlling plant-parasitic nematodes by *Enterobacter* species.

Genome Analysis of *E. asburiae* HK169

In order to obtain genomic information about *E. asburiae* HK169 related to its plant growth-promoting and nematicidal activities, the genome sequence of the HK169 isolate was analyzed. A total of 1,372,285,639 nucleotides based on 177,762 reads (N50 size 10,718 and mean subread length 7,719) with 221-fold coverage of the genome were generated, and the genome sequence was deposited into the GenBank database with accession number CP017087. The whole-genome sequence of HK169 features a single, circular chromosome of 4,551,186 bp in length, with 56.2% G + C content and with no plasmid detected. In total, 4,141 coding DNA regions were identified with 83 tRNA, 25 rRNA, 12 ncRNA, and 45 pseudo genes.

Based on RAST functional categories (http://rast.nmpdr.org), the carbohydrate metabolism subsystem (14.8%) ranks as the largest, followed by amino acid metabolism (12.4%) and protein metabolism (9.0%) (Fig. 3A). The subsystem of protein metabolism includes 43 protein-degradation-related open reading frames, including the putative TldE-TldD proteolytic complex, protein-degrading enzymes, aminopeptidases (E.C. 3.4.11), metallocarboxy-peptidases (E.C. 3.4.17), dipeptidases (E.C. 3.4.13), bacterial
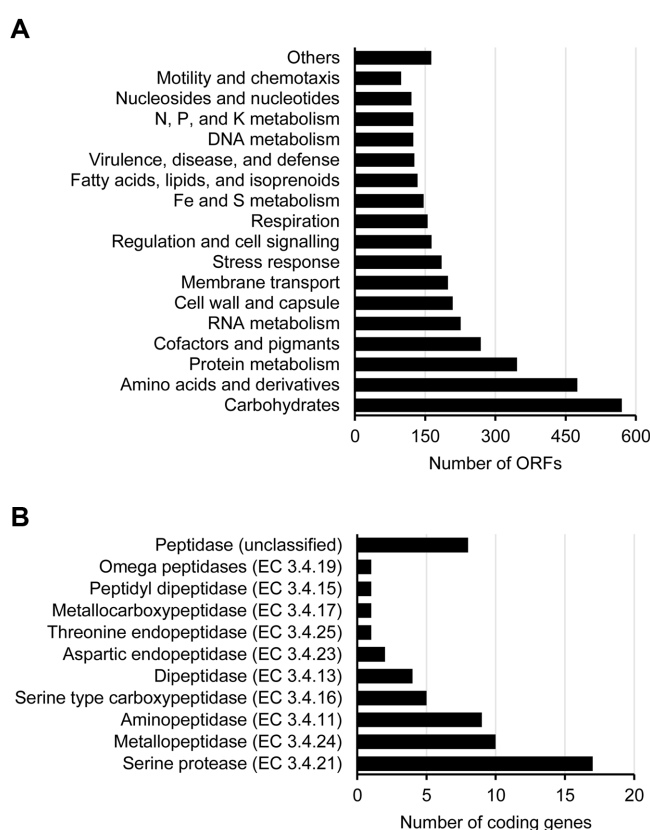
Fig. 3. Subsystem category distribution of major protein coding genes of *Enterobacter asburiae* HK169 as annotated by the RAST annotation server (**A**) and 59 putative genes encoding proteolytic enzymes in the HK169 genome (**B**).

proteasome, omega peptidases (E.C. 3.4.19), and bacterial-ATP-dependent proteolytic enzymes (Fig. 3B). Virulence factors such as toxins were not detected in the subcategories of virulence, disease, and defense.

Protease-Related Genes in *E. asburiae* HK169

The genome of the HK169 isolate contains 59 protease-related genes, including 17 serine proteases and 10 metallopeptidases (Fig. 3B). Several serine proteases have been proven to be involved in the antagonistic action of bacteria against plant parasitic nematodes, in which protein components hydrolyze the eggshell, cuticle, and intestine [24, 30]. The bacterial strain *Pseudomonas fluorescens* CHA0 produces extracellular serine protease AprA for its nematicidal activity [31]. The deficient mutant of the *aprA* gene or its regulatory *gacA* gene shows lost or impaired nematicidal activity compared with the wild type [31]. Paiva *et al.* [23] evaluated the nematicidal activities of 47 bacterial strains against the pinewood nematode and

identified two nematicidal proteins consisting of a subtilisin-type serine protease and a serralysin-like metalloprotease from *Serratia marcescens* A88copa13. Recently, the nematicidal metalloproteinases Bmp1 and ColB were identified from *Bacillus thuringiensis* [25, 32]. These examples support our hypothesis that proteolytic enzymes encoded in the genome of HK169 contribute to its nematicidal activity.

Secondary Metabolites of *E. asburiae* HK169

In the complete genome of the HK169 isolate, we found several gene clusters for secondary metabolites, such as siderophore and aryl polyene (Table 2). Biosynthetic gene loci for two siderophores, aerobactin (EnteroDNA1_01228–01236) and enterobactin (EnteroDNA1_03893–03912), were present in this genome. Bacteria have developed several distinct mechanisms by which to compete for iron, an element whose availability often limits microbial growth. Costa and Loper [8] reported that aerobactin and enterobactin are required for iron acquisition by *E. cloacae*. A mutant strain of *E. cloacae* deficient in the production of both aerobactin and enterobactin failed to grow on an iron-limited medium. Siderophore-mediated iron deficiency in the rhizosphere is often proposed as a mechanism of action in the reduction of soilborne pathogens [33]. The presence of an efficient iron uptake system can therefore contribute to protect the host plant against pathogenic nematode infections. In addition, the aryl polyene biosynthetic gene cluster (EnteroDNA1_02481–02499) was found in the HK169 genome. Both symbiotic and pathogenic bacteria of eukaryotic cells are likely to encounter oxidative stress frequently from their hosts during colonization or infection. Membrane-bound aryl polyene protects gram-negative bacterial cells from oxidative damage by reducing the concentrations of free radicals [34]. Many bacteria that interact with higher plants or animals harbor the aryl polyene biosynthetic gene cluster [35].

Table 2. Gene clusters found in the genome of *Enterobacter asburiae* HK169, which are related to the biosynthesis of secondary metabolites.

Gene cluster type[a]	Gene locus[b]	Most similar known gene cluster
Hserlactone	00630–00631	LuxI/R quorum sensing
Siderophore	01228–01236	Aerobactin
Aryl polyene	02481–02499	APE$_{Ec}$
Non-ribosomal peptide	03893–03912	Enterobactin

[a]Predicted by antiSMASH 3.0.

[b]Designated by locus tag numbers (the EnteroDNA1_prefix being deleted for clarity) that are based on HK169 genome annotations.

Genome Analysis of *E. asburiae* HK169 in Terms of Plant Growth Promotion

We also found that several key genes are involved in plant growth-promoting activities, such as in the production of plant growth-regulating substances, biofilm formation, and the production of volatile compounds. Endophytic and rhizosphere bacteria enhance plant growth through the synthesis of the auxin-class plant hormone IAA. Although the production of IAA by the HK169 isolate was not experimentally demonstrated in this study, the tryptophan degradation pathway would be capable of producing indole-3-pyruvate, through which the aromatic amino acid aminotransferase (EnteroDNA1_03084) catalyzes the transamination of L-tryptophan. The indole-3-pyruvate decarboxylase IpdC (EnteroDNA1_01540) converts indole-3-pyruvate into indole-3-acetaldehyde. Indole-3-acetaldehyde can also be synthesized from tryptamine, a decarboxylated product of L-tryptophan, by monoamine oxidase (EnteroDNA1_00463). The indole-3-acetaldehyde is further oxidized to IAA by indole-3-acetaldehyde dehydrogenase (EnteroDNA1_02926). Indole-3-acetylasparate hydrolase (EnteroDNA1_00376) is important in the regulation of indole-3-acetic acid metabolism in plants.

The *N*-acyl homoserine lactone is a signal molecule in bacterial quorum sensing that serves as a means of communication between bacteria [36]. The acyl homoserine lactone synthase (*luxI*, EnteroDNA1_00630) and its transcriptional activator (*luxR*, EnteroDNA1_00631) were found in the genome of HK169 (Table 2). The quorum-sensing system mediated by LuxI/LuxR-like proteins has been described as responsible for biofilm formation [37], and in this study, an early stage of biofilm formation of the HK169 isolate was observed by scanning electron microscopy (Fig. S1). In addition to biofilm formation, the quorum-sensing system mediated by LuxI/LuxR-like proteins is known to be involved in bacterial root colonization, which is an important factor in plant growth promotion by rhizobacteria and for the biocontrol activity of root-associated *Pseudomonas fluorescens* 2P24 [37].

Volatile compounds such as acetoin and 2,3-butanediol are emitted by some plant growth-promoting rhizobacteria to enhance plant growth [11]. The main pathway for the production of acetoin and 2,3-butanediol by HK169 is via the *budABC* operon. Acetolactate synthase (*budB*, EnteroDNA1_03878) converts pyruvate to acetolactate, which is subsequently converted by α-acetolactate decarboxylase (*budA*, EnteroDNA1_03877) into acetoin. Acetoin is released by bacteria or is subsequently converted into 2,3-butanediol by acetoin reductase (*budC*, EnteroDNA1_03879). Under

aerobic condition, acetolactate is spontaneously converted into diacetyl (2,3-butanedione), which in turn can be converted into acetoin by diacetyl reductase (*butA*, EnteroDNA1_00699).

In terms of nitrogen fixation for plant growth, unlike other nitrogen-fixing *Enterobacter* strains, HK169 lacks the *nif* genes required for nitrogen fixation. However, the HK169 genome contains the genes required for the nitrate reduction pathways. Nitrate transport and nitrate/nitrite reduction genes are present within two operons (*narIJHGKXL* and *nasAD-cmpCB-nrtA nasR*, EnteroDNA1_00946–00959) that can be separated by a putative invasin gene. Other regions involved in nitrite reduction (*nasD* and *nirD*, EnteroDNA1_02428–02429) and an ammonium uptake transporter (*amtB*, EnteroDNA1_03753) and its regulator (EnteroDNA1_03752), as well as a nitrate/nitrite sensor protein (*narX*, EnteroDNA1_01583), were also found on the HK169 genome.

In summary, we have isolated and identified *E. asburiae* HK169 from tomato roots, showing nematicidal activity against *M. incognita* and plant growth-promoting properties. In addition, our observations that the nematicidal activity of the culture filtrate was dramatically reduced by a protease inhibitor suggested that proteolytic enzymes contribute to the nematicidal activity of HK169. Based on a whole-genome analysis of the HK169 isolate, our study provides evidence that the HK169 isolate is capable of nematicidal and plant growth-promoting activities. Taken together, our results will contribute to the application of improved microbial agents in agriculture.

Acknowledgments

Conflict of Interest

The authors have no financial conflicts of interest to declare.

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