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Research Article

Isolation and identification of entomopathogenic nematodes and their symbiotic bacteria from Kurdistan province in Iran

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Abstract: Entomopathogenic nematodes (EPNs) are commonly used as biological agents for control of insect pests. This study was carried out to identify EPNs, determine dominant and frequent species in Kurdistan province, Iran and characterize their symbiotic bacteria. Identification of EPNs was performed based on morphological and morphometrical characters and also rRNA-ITS gene sequences. Two EPNs, *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema feltiae* (Rhabditida: Steinernematidae) were identified. Out of totally 150 soil samples collected mainly from mid-southern parts of the province, 60% were positive for EPNs. *Heterorhabditis bacteriphora* showed the highest frequency in this region, remarkably 59.3% of soil samples contained this species. Incidences of *H. bacteriphora* in grasslands, woodlands and alfalfa fields were 57.3, 14.6 and 28.0 percent, respectively. *Steinernema feltiae* was found only in alfalfa fields with 0.66% frequency. Bacterial symbionts of *H. bacteriophora* and *S. feltiae* were also identified based on biochemical characters and *recA* gene sequencing. In this research, two species of *Photorhabdus* were isolated from *H. bacteriophora* namely *P. luminescens* subsp. *kayaii* and *P. temperata* subsp. *thracensis*. *Xenorhabdus bovienii* was identified from infective juveniles of *S. feltiae*.

Keywords: *Heterorhabditis bacteriophora, Photorhabdus luminescens*, *Steinernema feltiae, Xenorhabdus bovienii,* Phylogeny

Introduction

Entomopathogenic nematodes (EPNs) belonging to the families Heterorhabditidae and Steinernematidae are obligate pathogens of insects. These nematodes have symbiotic relationship with gram negative bacteria *Photorhabdus* and *Xenorhabdus* (Enterobacteriaceae). The infective juveniles (IJs) penetrate into the haemocoel by invading through natural openings or enter the haemocoel directly through the insect's intersegment integument (Wang and Gaugler, 1998). After penetration into the host haemocoel, IJs release their bacterial symbionts of the genera *Photorhabdus* and *Xenorhabdus* associated with *Heterorhabditis* and *Steinernema*, respectively (Martens *et al*., 2003; Ciche and Ensign, 2003). The bacteria and nematodes cooperate to overcome the host's immune response and result in killing the insect (Boemare and Akhurst, 2006). The nematodes produce at least two generations in the host body, and emerge from the cadavers as IJs that search for new hosts (Poinar, 1990; Kaya and Gaugler, 1993).

Efficacy of EPNs for controlling insect pests caused a huge attempt to find new efficient isolates. Earlier, morphological and morphometric methods

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were used for EPNs identification, however, recently DNA sequences variation in the internal transcribed spacer (ITS) regions of ribosomal DNA is extensively used as a complementary approach to morphological descriptions and also to estimate phylogenetic relationships among species in EPNs (Stock, 2002).

Phenotypic characteristics, biochemical and molecular methods are three stages for identification of unknown bacterial colonies. There are series of important biochemical characteristics, however, bioluminescens and catalase test which are physiologically very significant characters, are positive for *Photorhabdus* and negative for *Xenorhabdus* (Forst *et al*., 1997). Due to substantial differences in biochemical reactions of these bacteria (Grimont *et al*., 1984; Akhurst and Boemare, 1988), results of these experiments could not approach to precise identification. Thus, molecular consideration of isolated bacteria was necessary. Recombinase A (*recA*) can serve as molecular marker for the identification of *Xenorhabdus* and *Photorhabdus* (Ludwig and Schleifer, 1999). RecA protein and *recA* gene sequence comparisons have been used to consider phylogenetic relationships among different genera and species of prokaryotes (Eisen, 1995).

Five species of entomopathogenic nematodes have been reported from Iran including *H. bacteriophora* Poinar, 1976, *S. bicornutum* Tallosi, Peters and Ehlers, 1995, *S. carpocapsae* Weiser, 1955, *S. feltiae* (Filipjev, 1934) Wouts, Mrácek, Gerdin and Bedding, 1982, and *S. glaseri* (Steiner, 1929) Wouts, Mrácek, Gerdin and Bedding, 1982. The species are mainly described from East and West Azerbaijan, Ardabil, Tehran, and North Khorasan provinces (Parvizi, 2001; Tanha Maafi *et al*., 2006; Karimi and Kharazi-pakdel, 2007; Eivazian Kary *et al*., 2009; Nikdel *et al*., 2010; Kamali *et al*., 2013). The bacterial strains associated with *S. glaseri* and *S. carpocapsae* in Iran were characterised as *X. nematophila* and *X. poinarii* (Karimi *et al*., 2011). Taxonomic studies using biochemical and molecular analyses showed symbiotic relationship between *Photorhabdus* and *Heterorhbditis*, and *Xenorhabdus* and *Steinernema* (Euzeby and Boemare, 2000). Isolation and identification of symbiotic bacteria of different EPNs were performed through several studies that identified different species including *P. luminescens* subsp. *lumondii*, *P. temperate, X. bovienii*, *X. budapestensis*, *X. nematophila, X. poinarii* (Iraki *et al*., 2000; Hazir *et al*., 2004; Bussaman *et al.,* 2009; Agazadeh, *et al*., 2010; Jang *et al*., 2011; Karimi *et al*., 2011). The aims of this study were to: *a*: identify the entomopathogenic nematodes by morphological, morphometric and molecular data, *b*: find and introduce frequent native EPN species in Kurdistan province, *c*: characterize the symbiotic bacteria associated with EPNs based on biochemical and molecular approaches.

Materials and Methods

Soil Sampling and Isolation of EPNs

Soil samples were collected from oak forests, grasslands and alfalfa fields in mid-southern regions of Kurdistan province, Iran including: Qorveh, Dehgolan, Sanandaj, Sarv Abad and Marivan. Elevation of sampling locations was between 1106 and 2359 meters above sea level. The samples were taken during March and April 2012 at depth of 0-20 cm from an area of 100-500 m^2, each sample consisted of 5-10 subsamples of 50 g soil. Totally 150 soil samples were collected. Entomopathogenic nematodes were isolated from soil samples by using *Galleria* bait technique (Bedding and Akhurst, 1975). *Galleria mellonella* (wax moth larva) was reared on an artificial diet suggested by Bronskill (1961) at room conditions. Dead *Galleria* larvae were moved to a White trap for obtaining IJs from the cadavers (White, 1927), while a few cadavers were directly dissected to obtain adult nematodes of the first and second generations.

Morphological analysis

Infective juveniles and adult nematodes were fixed in TAF (triethanolamine 2 ml, formaldehyde 7 ml, distilled water 91 ml) and transferred to dehydrated glycerin according to De Grisse (1969). Morphological characters that are commonly used in EPNs diagnosis included: post anal swelling, vulval lips, presence of mucro and epiptygma and testis reflexion. Also,

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morphometric features *i.e.* body length, anterior end to excretory pore, tail length, pharynx length, maximum body width, D% and E% ratios, spicule length, gubernaculum length and SW ratio were measured using a camera Lucida installed on an Olympus BH-2 microscope. The morphological and morphometric characters were analyzed according to Stock and Hunt (2005).

DNA extraction, PCR and sequencing

Freshly harvested infective juveniles were used for DNA extraction. Protocols of DNA extraction, PCR and sequencing were described by Tanha Maafi *et al.* (2003). The ITS regions of rRNA gene were amplified using universal primers, forward TW81 5′-GTTTCCGTAGGTGAACCTGC-3′ and reverse AB28 5′-ATATGCTTAAGTTCAGCGG GT-3′ (Joyce *et al.*, 1994). The PCR products were directly sequenced in both directions at Bioneer Company (South Korea). The obtained sequences along with their chromatograms were checked visually and aligned manually by using BioEdit program. The sequences were deposited in GenBank under accession numbers KR152294, KT070192 and KT070191. The sequences of ITS-rRNA of different isolates of *H. bacteriophora* and *S. feltiae* were used for phylogenetic analyses (Table 1). The newly obtained sequences and the sequences from GenBank database were aligned using Clustal X 1.83 (Thomson *et al.*, 1997) with default parameters. Sequence alignments were manually edited using BioEdit (Hall *et al*., 1999). Outgroup taxa for each data set were chosen according to previous published data (Spiridonov *et al*., 2004). Phylogenetic analysis of the sequence data sets was performed with Bayesian inference (BI) using MrBayes 3.2 (Huelsenbeck and Ronquist, 2001) under the HKY + G model, for each gene was initiated with a random starting tree and was run with four chains for 1.0×10^6 generations. The Markov chains were sampled at intervals of 100 generations. Posterior probabilities (PP) are given on appropriate clades.

Isolation of bacteria

Bacteria associated with EPNs were directly isolated from IJs. About 100 IJs were surface sterilized with sodium hypochlorite 1% for five minutes, crushed and homogenized for one minute in 100µl of distilled water in micro-tube. Then, one milliliter of suspension was added and smeared onto nutrient bromo-thymol blue tri-phenyl-tetrazolium chloride agar (NBTA) medium (Lacey, 1997). After 48-72 h, all morphologically distinct colonies were purified through serially sub-culturing until uniform colonies in sense of size and morphology were obtained. The colonies were tested for pathogenicity on the last instar larvae of *G. mellonella*. To approach this, the isolates were inoculated in liquid LB medium and incubated for 24 hours at 28 ± 2 ºC. 10 µl of 10^4 cells of each isolate was injected into the last instar larvae of wax moth through proleg by a Hamilton syringe. The pathogenic isolates that caused mortality on wax moth larvae were identified by biochemical and molecular methods.

Identification of bacteria

Pathogenic strains on larvae of *G. mellonella* were characterized by gram reaction, anaerobic growth, catalase test, gelatin and aesculin hydrolysis, DNase, arginine dehydrolysis, lecithinase and nitrate reduction activity, growth on salicin and Simmon citrate, indole production and H_2S production from cysteine according to Akhurst and Boemare (1983).

According to phenotypic results some strains were selected to confirm by molecular identification. Total genomic DNA of the bacterial strains was extracted by alkaline lysis method (Rademaker and De Bruijn, 1997). For amplification of *recA* gene, specific Rec A primers synthesized by Eurofins MWG Operon Corporation, Germany were used based on the sequence of *recombinase* A (*rec A*) in *Erwinia* species (Waleron *et al*., 2002). The RecA-F and RecA-R sequences were 5'-GGTAAAGGGT CTATCATGCG-3' and 5'-CCTTCACCATACAT AATTTGGA-3', respectively. DNA amplification was done according to a conventional method described by Waleron *et al*. (2002).

The bacterial PCR products were sequenced directly in both directions at Macrogen, (South Korea). The obtained sequences were checked as mentioned in previous section and the checked sequences were submitted to GenBank data base

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under accessions KT070193- KT070198, KT070200-201, KR491942. Five sequences from GenBank data base along with ten sequences from the present study were used for construction of phylogenetic tree. Multiple sequence alignment of selected data was performed using MEGA5 software (Tamura *et al*., 2011). The clustering stability of the NJ tree was evaluated by bootstrap analysis of 500 data sets (Felsenstein, 1985).

Results

EPNs Identification

Heterorhabditis bacteriophora and *S. feltiae* were identified based on morphological and morphometrical characters of third-stage infective juveniles and adults (Table 2) which were generally in agreement with those published for these species (Nguyen and Smart, 1996; Stock and Hunt, 2005).

Table 1 Species and isolates of *Heterorhabditis bacteriophora*, *Steinernema feltiae* and species and subspecies of *Photorhabdus temperata*, *Photorhabdus luminescens* and *Xenorhabdus bovienii* used in phylogenetic tree construction in this study.

Species	Locality	Accession number	Reference
H. bacteriophora	Washington, USA	KT443980.1	Fu *et al*. (2015)
H. bacteriophora	Switzerland	KJ938576.1	Fesselet *et al*. (2014)
H. bacteriophora	Iran	KC675180.1	Kamali and Karimi (2013)
H. bacteriophora	Bulgaria	JX993984.1	Petrova *et al*. (2012)
H. bacteriophora	Florida, USA	JX403718.1	Akyazi *et al*.,2012)
H. bacteriophora	Mashhad, Khorasan, Iran	JX164230.1	Hassani-Kakhki *et al*. (2012)
H. bacteriophora	Marivan, Kurdistan province, Iran	KR152294	Present Study
H. bacteriophora	Qorveh, Kurdistan province, Iran	KT070192	Present Study
H. bacteriophora	West Azarbaijan, Iran	EU598228	Eivazian Kary *et al*. (2009)
H. bacteriophora	Ardabil, Iran	Eu598231	Eivazian Kary *et al*. (2009)
H. bacteriophora	East Azarbaijan, Iran	Eu598232	Eivazian Kary *et al*. (2009)
H. bacteriophora	East Azarbaijan, Iran	EU516355	Eivazian Kary *et al*. (2009)
H. bacteriophora	East Azarbaijan, Iran	EU598222	Eivazian Kary *et al*. (2009)
H. bacteriophora	Tehran, Iran	EU163272	Karimi *et al*. (2007)
S. feltiae	Mashhad, Khorasan Razavi, Iran	JN098449.1	Safari *et al*. (2011)
S. feltiae	Mashhad, Khorasan Razavi, Iran	JN098450.1	Safari *et al*. (2011)
S. feltiae	Kyiv, Ukraine	KF939329.1	Yakovlev *et al*. (2014)
S. feltiae	Hokkaido, Erimo, Japan	AB243439.1	Kuwata and Yoshiga, 2007)
S. feltiae	Qorveh, Kurdistan province, Iran	KT070191	Present Study
S. feltiae	East Azerbaijan, Iran	EU598240	Eivazian Kary *et al*. (2008)
S. feltiae	Armenia	AY171256	Spiridoniv *et al*. (2013)
S. feltiae	Karaj, Iran	FJ657532	Karimi *et al*. (2009)
S. feltiae	Tehran, Iran	FJ770381	Karimi *et al*. (2009)
P. temperate subsp *thracensis*	France	FJ862016.1	Tailliez *et al*. (2010)
P. temperate subsp *thracensis*	Kurdistan province, Iran	KT070201	Present Study
P. temperate subsp *thracensis*	Kurdistan province, Iran	KT070200	Present Study
P. temperate subsp *thracensis*	Kurdistan province, Iran	KT070194	Present Study
P. temperate subsp *thracensis*	Kurdistan province, Iran	KT070199	Present Study
P. luminescens subsp *kayayii*	France	FJ861996.1	Tailliez *et al*. (2010)
P. luminescens subsp *kayayii*	Kurdistan province, Iran	KT070193	Present Study
P. luminescens subsp *kayayii*	Kurdistan province, Iran	KR491942	Present Study
X. bovienii	Australia	U87924.1	Pinyon *et al*. (2000)
X. bovienii	Lebanon	LN835358.1	Noujeim *et al*. (2015)
X. bovienii	Australia	FJ823426	Tailliez *et al*. (2010)
X. bovienii	Kurdistan province, Iran	KT070195	Present Study
X. bovienii	Kurdistan province, Iran	KT070196	Present Study
X. bovienii	Kurdistan province, Iran	KT070197	Present Study
X. bovienii	Kurdistan province, Iran	KT070198	Present Study

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In terms of EPNs recovery 60% of collected soil samples were positive. *H. bacteriophora* was recovered from 59.33% of soil samples while only 0.66 % of samples contained *S. feltiae.* Grasslands habitat showed higher recovery frequency than other habitats. Incidence of *H. bacteriphora* in grasslands, oak forests and alfalfa fields were 57.30, 14.61 and 28.08%, respectively. *S. feltiae* was only found in an alfalfa field in Qorveh (Fig. 1).

The amplification of rRNA-ITS regions plus 5.8S gene yielded a single fragment of approximately 800 bp and 750 bp for *H. bacteriophora* and *S. feltiae*, respectively based on gel electrophoresis. BLAST search from the NCBI showed 100% homology in the ITS sequences of the isolates of *H. bacteriophora*, from Marivan (KR152294) and Qorveh (KT070192) with isolates of Mashhad, Khorasan (JX164230, KC675180), USA, Switzerland, Bulgaria (KT443980, KJ938576 JX993984, JX403718). *Steinernema feltiae* isolated from Qorveh, Kurdistan (KT070191) showed 99% similarity with those sequences of *S. feltiae* deposited in GenBank *i.e*. JN098449, JN098450 from Mashhad, Iran, AB243439 from Japan and KF939329 from Ukraine. In the phylogenetic tree (Fig. 2) all the isolates of *H. bacteriophora* used for tree construction generated one clade, all of them with good posterior probability values, the newly sequenced isolates of *H. bacteriophora* from Kurdistan showed high homology with the other isolates. In the phylogenetic tree constructed from the ITS-rRNA gene sequences of the isolates of *S. feltiae* one clade was formed. The isolate from Qorveh, Kurdistan formed a sister group with the isolates from Khorasan, Iran, and the rest of *S. feltiae*.

Table 2 Morphometric and morphologic characters of entomopathogenic nematodes isolated from Kurdistan Province.

Characters	*Steinernema feltiae*[1]			*Heterorhabditis bacteriophora*[1]		
	IJ	Male	Female	IJ	Male	Female
N	15	5	5	15	5	5
Total body Length	862.14 ± 47.42 (814.72 - 909.56)			619.37 ± 29.66 (589.71 - 649.03)		
Excretory pore (EP)	60.35 ± 4.25 (56.10 - 64.60)	75 ± 6 (69 - 81)		102.69 (99.71 - 105.67)		90 ± 2.00 (88 - 92)
Tail length (TL)	79.14 ± 6.90 (72.24 - 86.04)			94.12 ± 5.70 (88.42 - 99.82)		
Oesophagus Length	123.50 ± 8.64 (114.86 - 132.14)	152 ± 13.73 (138.27 - 165.73)		125.94 ± 5.49 (125.50 - 131.43)		117 ± 2.16 (115.84 - 119.16)
Maximum Body width	26 ± 2.25 (23.75 - 28.25)			25±1.41 (23.59 - 26.41)		
D% (EP/OesophaguS length×100)	48.973 (45.79 - 52.15)	49.61 ±5.88 (43.73 - 55.49)		81.65 ± 3.51 (78.14 - 85.16)		77.19 ± 1.75 (75.44 - 78.94)
E% (EP/TL × 100)	76.52 ± 5.27 (71.25 - 81.79)			109.42 ± 6.35 (103.07 - 115.77)		
Spicule length (Sp L)		71.30 ± 2.06 (69.24 - 73.36)			39.6 ± 1.14 (38.46 - 40.74)	
Gubernaculum Length		41.50 ± 1.29 (40.29 - 42.79)			22.40 ± 2.07 (20.33 - 24.47)	
Testis reflexion					76.60 ± 2.79 (73.81 - 79.39)	
SW (SPL/cloacal Body width)		1.30 ± 026 (1.04 - 1.56)				Present
Post - anal Swelling						Present
Vulval lips			Protruding			
Mucro			Present			
Epiptygma			Present			

[1] Measurements in µm.

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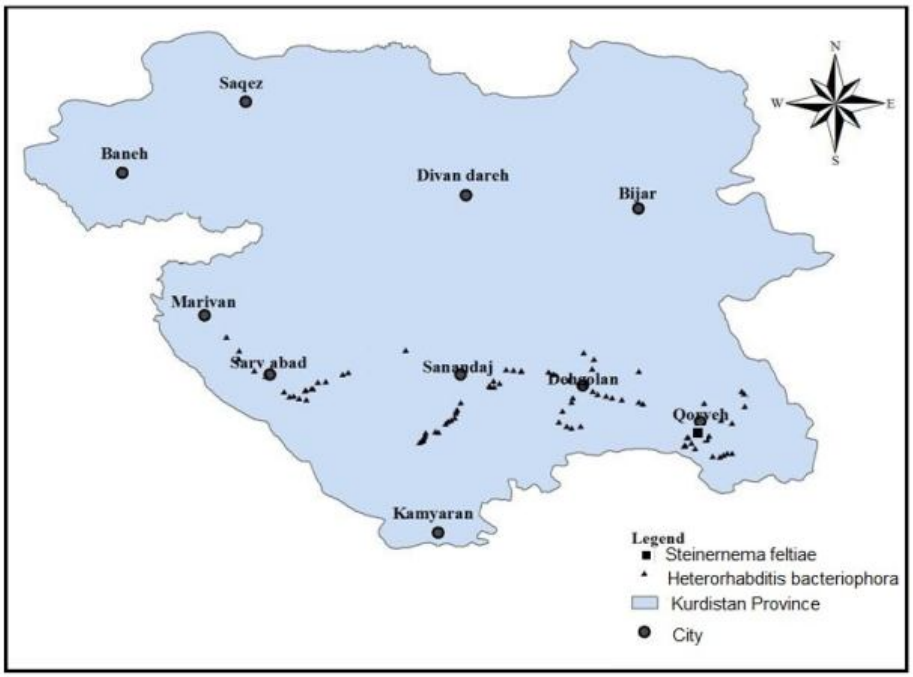

Figure 1 Distribution of entomopathogenic nematodes in Kurdistan province.

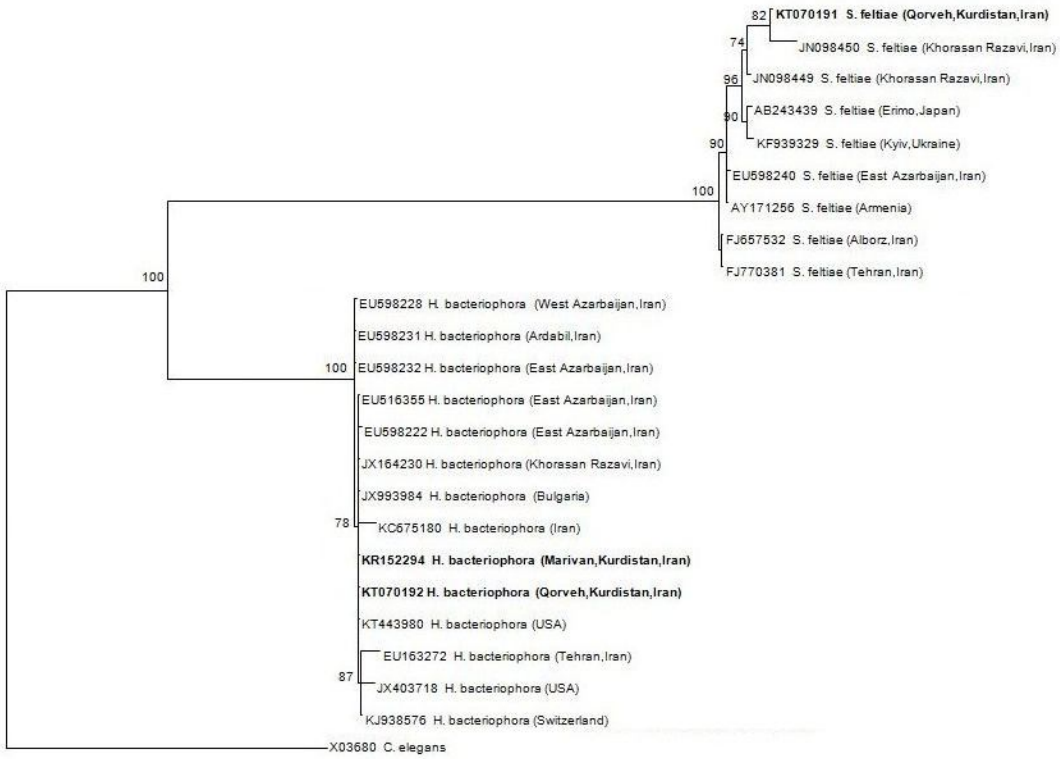

Figure 2 Bayesian phylogenetic tree inferred from ITS-rRNA gene sequences of some *Heterorhabditis bacteriophora* and *Steinernema feltiae*. Posterior probabilities more than 70% are given for appropriate clades. Name and accession number of sequences of current study are in bold.

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Bacterial Identification

All bacterial strains showed the common phenotypic characters of the *Xenorhabdus* and *Photorhabdus* genera including granular and translucent forms. Results of biochemical tests are showed in table 3, indicating 22 strains having characteristics close to *Photorhabdus*, whereas, 14 strains from *S. feltiae* are related to *Xenorhabdus*. The catalase test was negative for *Xenorhabdus*, however, it was positive for *Photorhabdus* as it was expected. Eight strains of two genera were subjected to molecular identification to confirm the biochemical diagnosis.

Table 3 Results of biochemical experiments of isolated symbiosis bacteria.

Test	*Xenorhabdus* (14 strains)	*Photorhabdus* (22 strains)
Gram	-	-
Anaerobic growth	+	+
Catalase	-	+
Gelatin hydrolysis	+	+
Aesculin hydrolysis	-	±
Salicin	±	±
Lecitinase activity	-	-
Nitrate reduction	-	-
DNA`s activity	+	-
Growth on simmon citrate	+	±
Indol production	-	-
H_2S from cysteine	+	-
Arginine dehydrolysis	-	-

+: 80% or more strains positive, -: 80% or more strains negative, ±: variable.

The amplification of *recA* gene produced a fragment of 730 bp. Genetic sequence analysis in a nucleotide blast (BLASTn) search of strains KT070193 (PLIRI7) and KR491942 (Kurd1) revealed 100% similarity to *Photorhabdus luminescens* subsp. *kayaii* (FJ861996.1) and strains KT070201 (PTIRI9), KT070200 (PTIRI8), KT070194 (PTIRI6) and KT070199 (PTIRI10) to *Photorhabdus temperata* subsp. *thracensis* (FJ862016.1). The *rec*A sequences of three isolates of *Xenorhabdus* (KT070195 (XBIRI5), KT070196 (XBIRI4), KT070197 (XBIRI3) and KT070198 (XBIRI2)) showed 99% homology with those of *Xenorhabdus bovienii* presented in the database (U87924.1, LN835358, FJ823426).

Figure 3 shows the phylogentic tree constructed using sequences of previously isolated strains and from the new isolates of this survey. Groups 1, 2 and 3 include all *P. temperate* subsp. *thracensis, P. luminescens* subsp. *Kayaii* and *X. bovienii* strains, respectively. Groups 1 and 2 representing sister group that their differences may be enough to separate them as two subspecies. Group 3 shows an out-group to groups 1 and 2 that illustrate high genetically differences to two other groups.

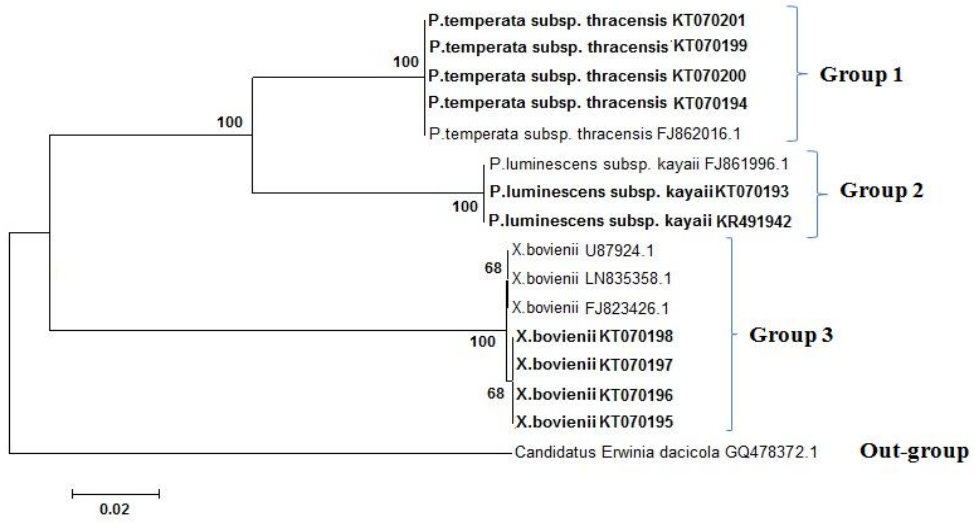

Figure 3 Neighbor joining Phylogenetic tree (Saitou, N. and Nei, 1987) showing affiliation of the symbiotic bacteria of entomopathogenic nematodes to reference data on the basis of *recA* gene sequence alignment. Bootstrap value (500 replicates) above 50% are shown next to the branches. Bolded strains show name and accession number of sequences of current study.

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Discussion

Two species of entomopathogenic nematodes, *H. bacteriophora* and *S. feltiae* were recovered from soil samples collected from Kurdistan province. The morphological identification was confirmed by molecular data. *H. bacteriophora* was the dominant species and showed remarkably high frequency in this region. In this study, more than half of the collected samples (60%) were positive for EPNs, with a predominance of *H. bacteriophora* over *S. feltiae*. The 60% prevalence of entomopathogenic nematodes in Kurdistan province is a new record for the natural occurrence of these nematodes in Iran. The majority of the positive samples were isolated from grasslands habitat, in contrast to forests where oak species constitute most of the woodlands. This rate is much higher than those reported 3% in Arasbaran (Nikdel *et al*., 2010), 3.2% in North West (Eivazian Kary *et al*., 2009), 1% and 2% in North Khorasan (Kamali *et al*., 2013). A study conducted in California showed the recovered isolates from woodlands coniferous and oak forests had the majority of the positive samples, 67.60 percent of samples were positive for presence of EPNs, in some regions all samples were positive (Stock *et al*., 1999).

This study showed entomopathogenic nematodes particularly *H. bacteriophora* are widespread in Kurdistan Province. The soil samples were principally taken from natural and virgin areas with enough humidity and vegetation, which provides more stable conditions for growth and establishment of EPN populations (Campos-Herrera *et al*., 2008).

High frequency of occurrence of EPNs in Kurdistan province is likely due to favorable environmental conditions in this region. Humidity and temperature have a significant effect on survival rates of EPNs. Kurdistan region has a temperate to cold climate with mean annual minimum and maximum temperatures of 11 to 14 °C. Meteorology information demonstrates that absolute maximum temperature and absolute minimum temperature in sampling locations were 35 to 42 and -10 to -24, respectively in recent years. The mean annual precipitation of this region ranges from 347 mm in Qorveh to 728 mm in Marivan (Meteorology organization of Kurdistan, 2015). These conditions could reflect the abundance of EPNs and adaptability of particularly *H. bacteriophora* to the natural environmental conditions and habitats of this Province. Temperatures above 40 °C and below 8 °C are lethal for most EPNs (Griffin, 1993; Grewal *et al*., 1994). Extremely high soil temperatures are rare in regions like Kurdistan province, but temperatures below 8 °C are common in this area and can be considered as a limiting factor.

The EPN species share mutual relationships with a single bacterial species; however, some species especially *Xenorhabdus* spp. are associated with more than one EPN species. In this study, two subspecies, *P. temperata* subsp. *thracensis* and *P. luminescens* subsp. *kayaii* were isolated from *H. bacteriophora*. *Xenorhabdus bovienii* was isolated from *S. feltiae*. Two isolates of *Xenorhabdus*, *X. nematophila* and *X. poinarii* were isolated from *S. glaseri* and *S. carpocapsae* collected from Tehran province, Iran. The bacterial isolates were characterized by their phenetic characters and sequences of 16S ribosomal RNA gene (Karimi *et al*., 2011). Four isolates of *P. luminescens* subsp. *lumondii*, *X. bovienii*, *X. nematophilus* and *X. budapestensis* were reported and identified by 16S-rRNA gene sequences (Agazadeh *et al*., 2010). Two species of *Photorhabdus* were identified and recorded based on rec*A* gene for the first time from Iran. *P. temperata* subsp. *thracensis* and *P. luminescens* subsp. *kayaii* were reported from *H. bacteriophora* from Turkey and Lebanon (Hazir *et al*. 2004; Noujeim *et al.*, 2011). The isolation of these symbiotic bacteria for completing their phylogeny and clarifying the ambiguous aspects of their characteristics is inevitable. Due to variation of subspecies, the obtained data from this study viz. chemical characteristics and sequences can be very helpful in the future

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research programs. Studies on signals between bacteria and nematodes and probability of substitution of robust bacteria for improving the lethal efficacy can be considered to develop the ambiguous aspects of their relationships. Surveying on factors that cause the specificity in nematode-bacteria interactions will provide information on how to manipulate this symbiotic relationship to accelerate their pathogenicity and broaden the insect host range.

The nematode isolates recovered from this survey can contribute to the biological control programs using native isolates in Kurdistan and adjacent provinces with similar climatic conditions.

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جداسازی و شناسایی نماتدهای بیمارگر حشرات و باکتری‌های هم‌زیست آنها از استان کردستان ایران

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چکیده: نماتدهای بیمارگر حشرات به‌عنوان عوامل بیولوژیکی در کنترل آفات کشاورزی مورد استفاده قرار می‌گیرند. این مطالعه به‌منظور شناسایی و تعیین فراوانی نماتدهای بیمارگر حشرات در استان کردستان و شناسایی و تعیین خصوصیات باکتری‌های هم‌زیست آنها انجام شد. شناسایی نماتدها براساس ویژگی‌های ریخت‌شناسی، ریخت‌سنجی و توالی نواحی ITS ژن rRNA انجام گرفت. دو گونه‌ی *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) و *Steinernema feltiae* (Rhabditida: Steinernematidae) شناسایی شدند. از تعداد ۱۵۰ نمونه‌ی خاک جمع‌آوری شده از نیمه‌ی جنوبی استان کردستان ۶۰ درصد آنها ازنظر حضور نماتدهای بیمارگر حشرات مثبت بودند. گونه‌ی *H. bacteriophora* بیش‌ترین فراوانی را در این منطقه داشت به‌طوری‌که ۵۹/۳ درصد نمونه‌های خاک دارای این گونه بودند. حضور گونه‌ی *H. bacteriophora* در علف‌زارها، مناطق جنگلی و مزارع یونجه به‌ترتیب ۵۷/۳، ۱۴/۶ و ۲۸ درصد بود. گونه‌ی *S. feltiae* فقط در مزرعه یونجه با فراوانی ۰/۶۶ درصد یافت شد. شناسایی باکتری‌های هم‌زیست گونه‌های *H. bacteriophora* و *S. feltiae* بر پایه‌ی خصوصیات بیوشیمیایی و توالی‌یابی ژن *recA* انجام شد. در این مطالعه دو گونه از جنس *Photorhabdus* با نام‌های *P. luminescens* subsp. *kayaii* و *P. temperata* subsp. *thracensis* از *H. bacteriophora* جداسازی و شناسایی گردید. باکتری *Xenorhabdus bovienii* نیز از لاروهای بیماری‌زای *S. feltiae* تشخیص داده شد.

واژگـان کلیـدی: *Heterorhabditis bacteriophora*، *Photorhabdus luminescens*، *Steinernema feltiae*، *Xenorhabdus bovienii*، فیلوژنی

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