

Identification of new Hungarian *Heterorhabditis* isolates and their bacterial symbionts

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Újfehértó



Subject

Most harmful insect pest: *Melolontha melolontha*



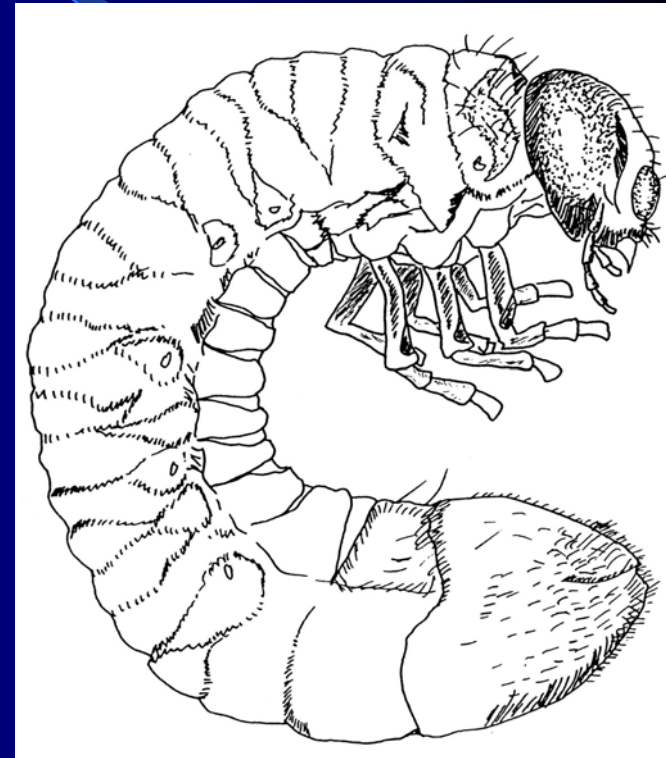
Subject

Chemical control of adults: solved

Integrated Fruit Production (IFP): no possibility to use pesticides in the soil

→ there is not chemical control of grubs of *Melolontha melolontha*

→ the only possibility is the biological control



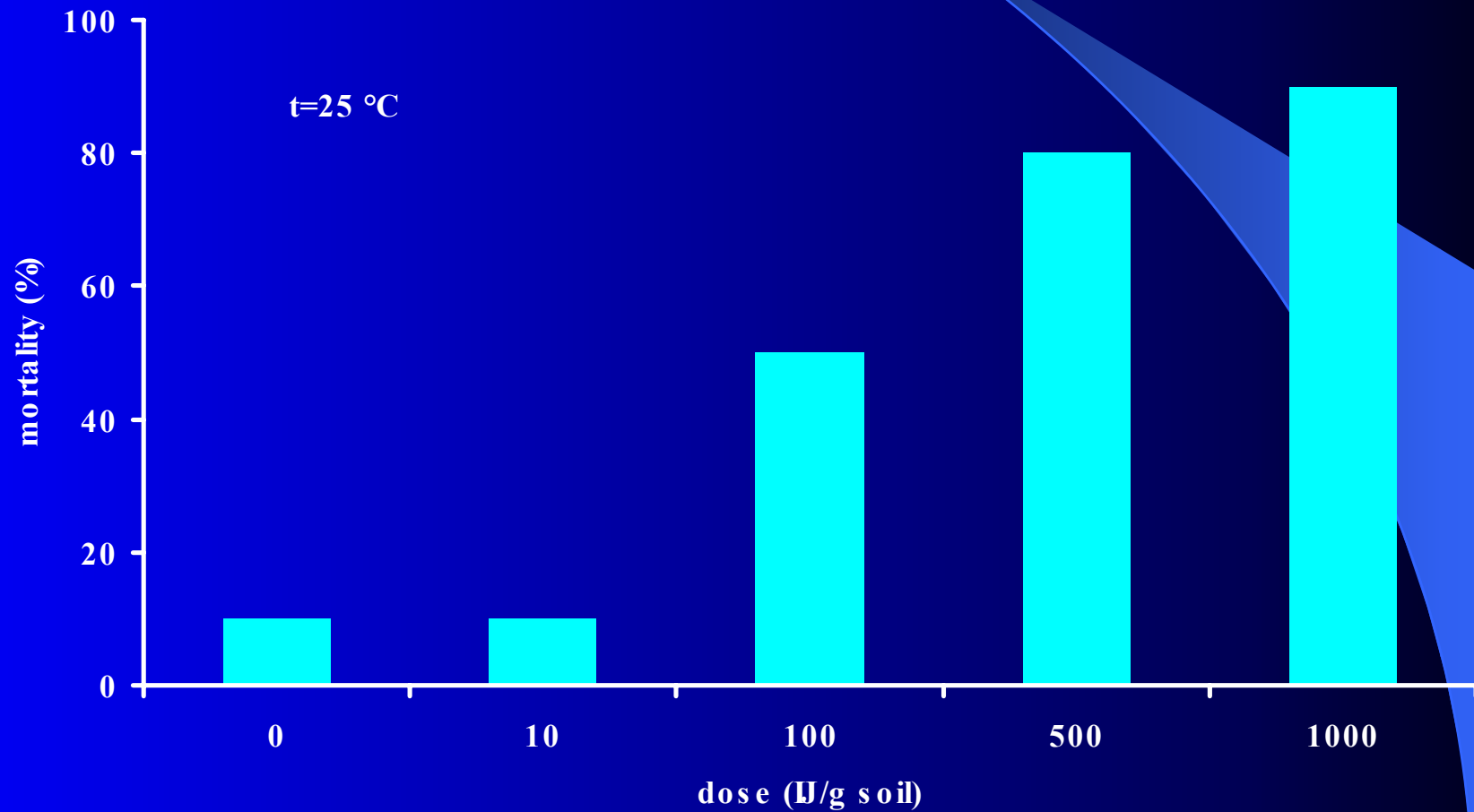
The Hungarian EPN fauna

- Only few faunistic studies
 - H. bacteriophora*, *S. feltiae*
 - Mracek and Jenser (1988)
 - H. downesi* ('Irish type')
 - Griffin et al. (1999)
- Lack of systematic and reliable information about the Hungarian entomopathogenic nematode fauna (species? host spectrum??)

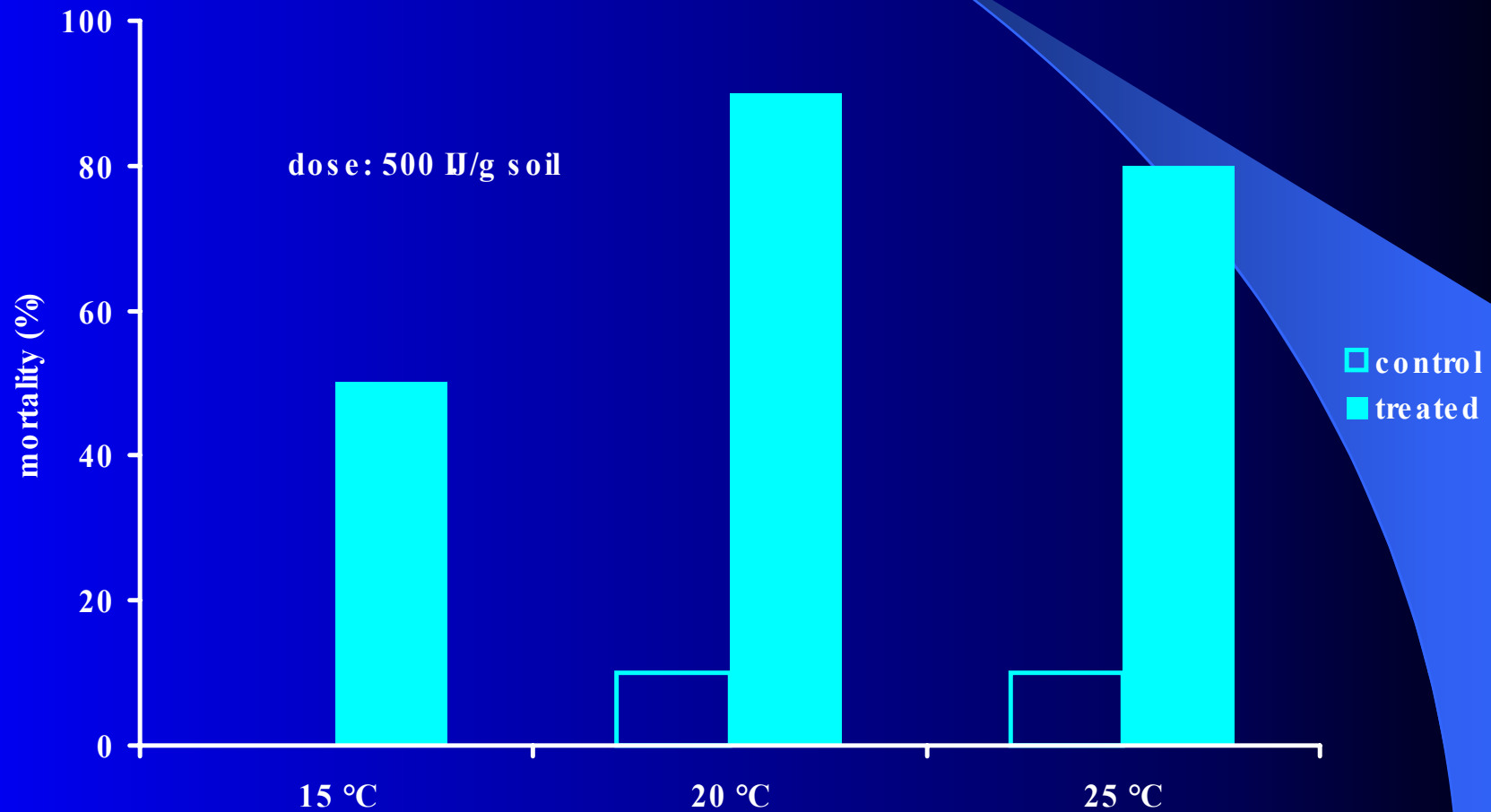
Isolation programme

- Isolation of EPNs from soils of natural habitats of *Melolontha* (oak forests, edge of other deciduous forests) near to the main fruit producing area by *Galleria* trap
- The work has started in summer of 2003
- 450 samples >> 60 unidentified isolates (7 *Heterorhabditis*)
- 4 candidates against *Melolontha*
- 2004: one effective strain (*Heterorhabditis* sp. '267')
- 2005: sampling in other parts of the country (a new research project)

Effectivity of *Heterorhabditis* sp. '267' in different doses against grubs of cockchafer



Effectivity of *Heterorhabditis* sp. '267' against grubs of cockchafer at different temperature



The aim of the present study:

- to identify the *Heterorhabditis* sp. '267' and other newly isolated *Heterorhabditis* strains by morphological and molecular biological approach,
- to isolate and identify the bacterial symbionts of studied strains.

Methods

- Morphological characteristics of IJ
- DNA sequence of ITS1
- PCR-RFLP of *gyrB* gene of symbiotic bacteria

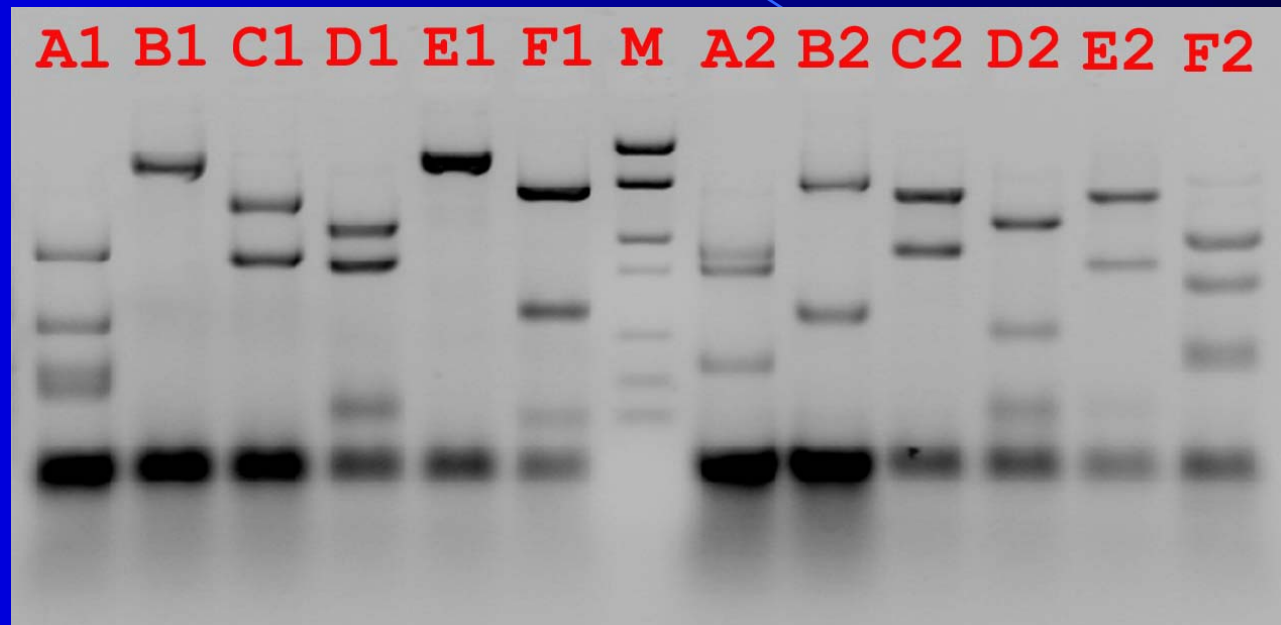
Morphology

- Three species: *H. bacteriophora* (1 isolate), *H. megidis* (4 isolates) and *H. downesi* (2 isolates including '267')
- Two types of *H. megidis*:
 - „long” IJ: length of nematodes is 815 μm (770 – 920 μm), while the typical value is 785 μm (736-800 μm)
 - „normal” strain (758 – 783 μm)

Nematode strains and the GenBank accession number of ITS sequence used to phylogenetic analysis

species	strain	GenBank accession No.	country of origin
<i>H. bacteriophora</i>	Brecon	AF029708	Australia
<i>H. bacteriophora</i>	NJ	AY170328	China
<i>H. baujardi</i>		AF548768	Vietnam
<i>H. dowensi</i>	K122	AF029712	Ireland
<i>H. indicus</i>	D1	AY170329	Australia
<i>H. marelatus</i>	OH10	AF029713	Oregon, USA
<i>H. megidis</i>	HO1	AF029711	Ohio, USA
<i>H. megidis</i>	Andong	AY293284	Korea
<i>H. zealandica</i>	X1	AY170330	China
<i>H. zealandica</i>	NHZ3	AF029705	New-Zealand
<i>H. hawaiiensis</i>	KH3	AF029707	?
<i>H. argentiensis</i>	Rafaela	AF029706	Argentina
<i>H. marelatus</i>	OH10	AF029713	USA
<i>H. hepialus</i>		AF029709	?
<i>S. carpocapsae</i>	ALL	AF036947	USA

PCR-RFLP of ITS1 and 2



M – molecular size marker pUC (*DpnI*), A – *AluI*; B – *BsuRI*; C – *Hin6I*; D – *HinfI*; E – *MspI*; F – *RsaI*. 1 - 'Uft28' isolate, 2 - '111' isolate

Phylogenetic relations of newly isolated strains

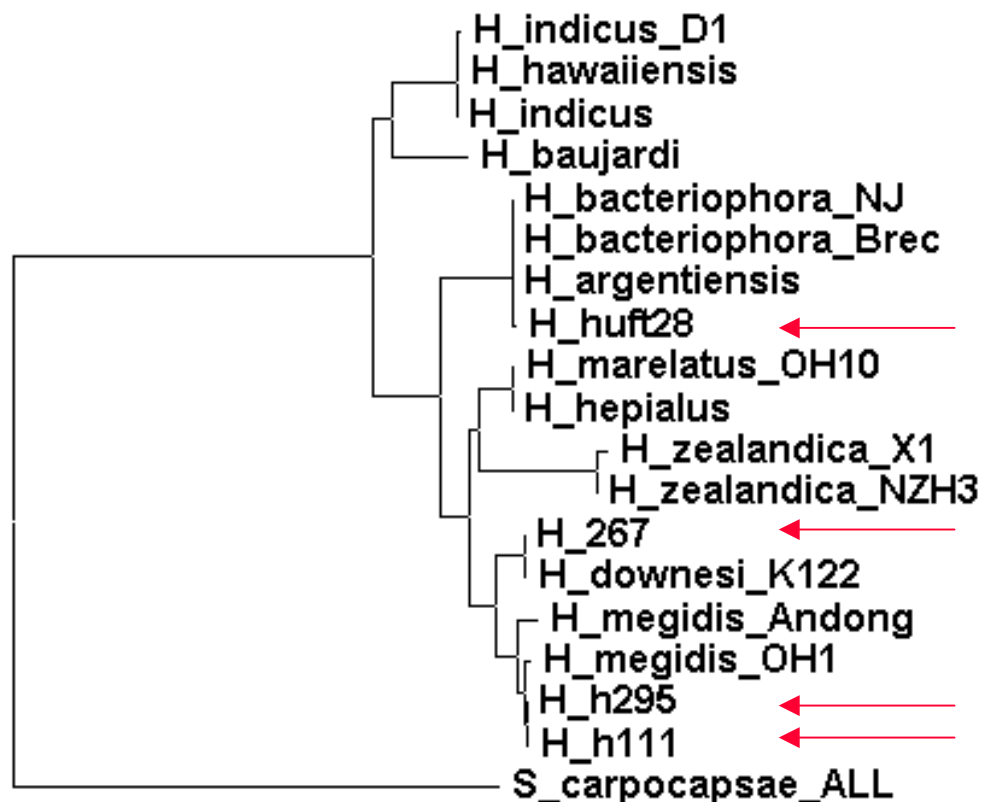
19 species , 267 sites (global gap removal)

Neighbor Joining Method

Jukes and Cantor distance

500 bootstrap replicates

0.214

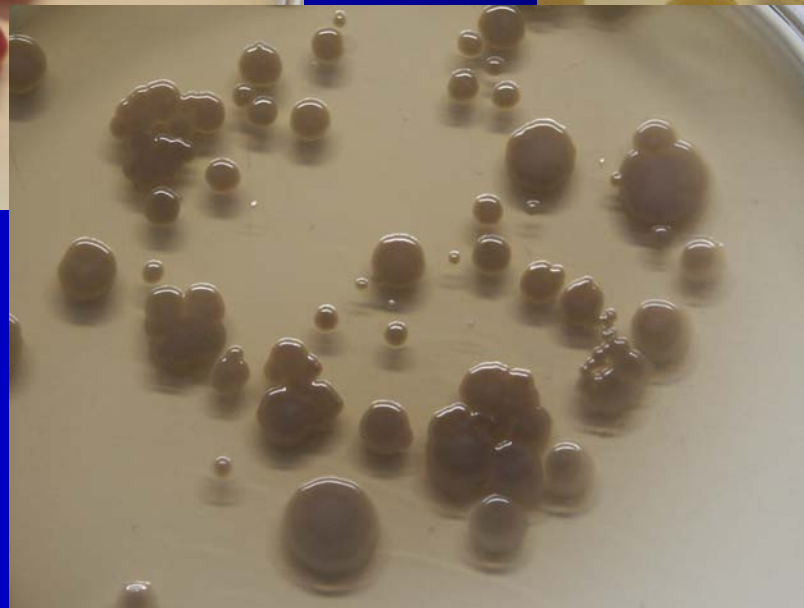
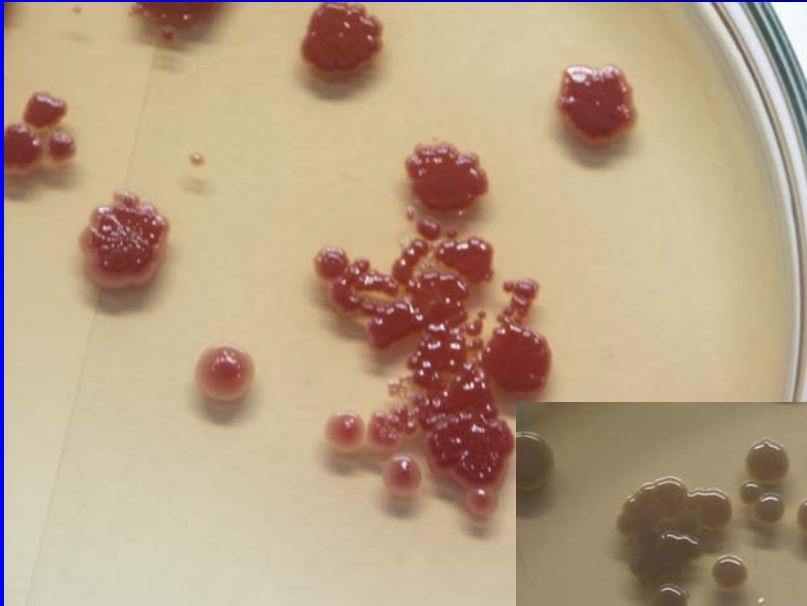


There was no differences between 'long' and 'normal' strains of *H. megidis* in ITS1 sequence.

Clustal_X
Thompson et al.
(1997)

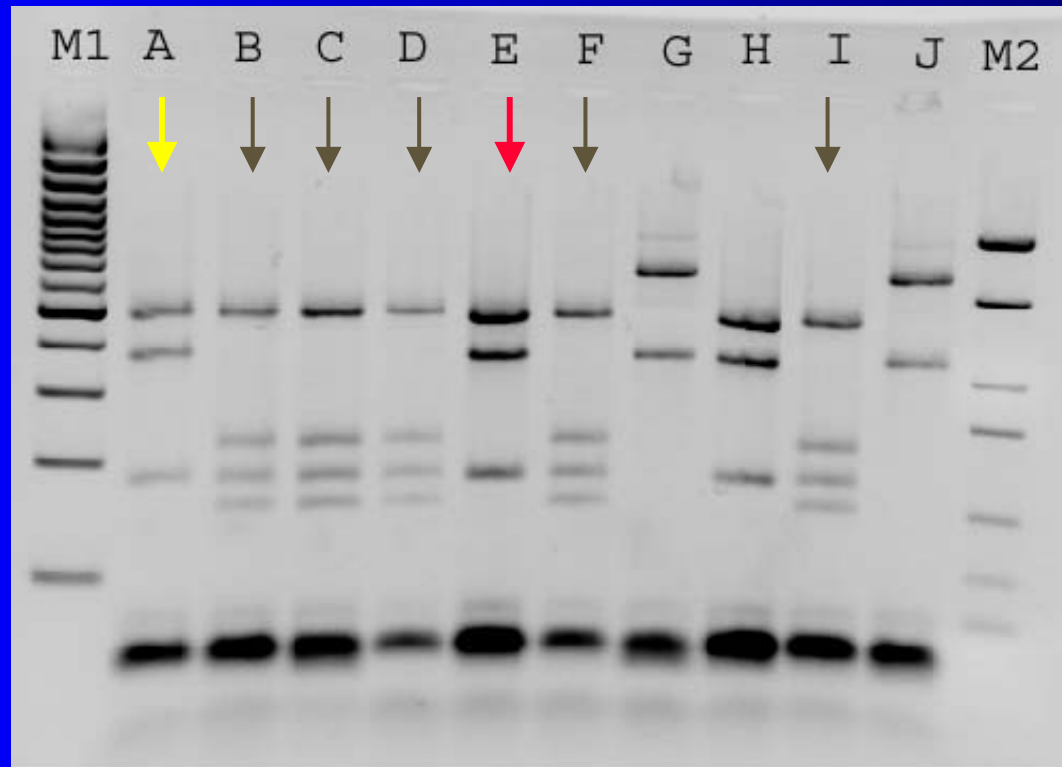
Phylo_win
Galtier et al. (1996)

Three types of *Photorhabdus* bacteria newly isolated from *H. megidis* and *H. downesi*



Species/strain	Host	GenBank accession No.	origin
<i>Photorhabdus temperata</i>			
HF85	<i>Heterorhabditis megidis</i>	AY278502	Netherland
XINach	<i>Heterorhabditis megidis</i>	AY278517	Russian
XILit	<i>Heterorhabditis megidis</i>	AY278516	Litvania
HL81	<i>Heterorhabditis megidis</i>	AY278504	Netherland
HW79	<i>Heterorhabditis megidis</i>	AY278507	Netherland
C1	<i>Heterorhabditis bacteriophora</i>	AY278497	USA
Habana	<i>Heterorhabditis sp.</i>	AY278503	Cuba
Meg	<i>Heterorhabditis megidis</i>	AY278512	USA
NZH3	<i>Heterorhabditis zealandica</i>	AY278513	New Zealand
<i>Photorhabdus luminescens</i>			
C8404	<i>Heterorhabditis sp.</i>	AY278498	China
HV16	<i>Heterorhabditis bacteriophora</i>	AY278506	Australia
<i>Photorhabdus luminescens ssp. akhurstii</i>			
K81	<i>Heterorhabditis sp.</i>	AY278510	Guadeloupe
Tetuan	<i>Heterorhabditis sp.</i>	AY278515	Cuba
D1	<i>Heterorhabditis indicus</i>	AY278499	Australia
<i>Photorhabdus luminescens ssp. laumondii</i>			
HP88	<i>Heterorhabditis bacteriophora</i>	AY278508	USA
K80	<i>Heterorhabditis sp.</i>	AY278509	Argentina
<i>Photorhabdus luminescens ssp. luminescens</i>			
Hb	<i>Heterorhabditis bacteriophora</i>	AY278501	Australia
Hm	<i>Heterorhabditis sp.</i>	AY278205	USA

AluI pattern of *gyrB* gene



MetaPhor agarose gel

M1: GeneRuler 100bp
DNA Ladder Plus

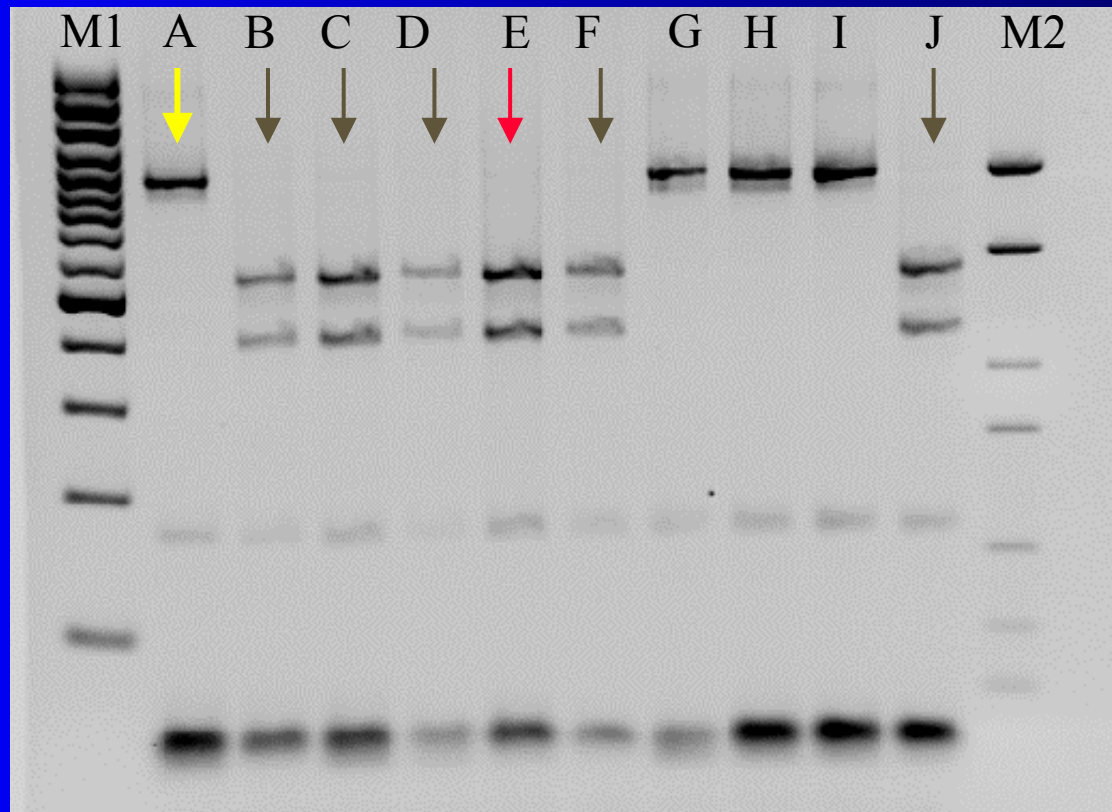
M2: pUC18 DNA *DpnI*
digest

A – F and I: newly
isolated *P. temperata*
strains

G and J: *P. luminescens*

H: *P. temperata* K122

*Hinf*I pattern of *gyrB* gene



MetaPhor agarose gel

M1: GeneRuler 100bp
DNA Ladder Plus

M2: pUC18 DNA *Dpn*I
digest

A – F and J: newly
isolated *P. temperata*
strains

G and I: *P. luminescens*

H: *P. temperata* K122

Similarity matrix of bacterial isolates

	111	121	267	269	295	297	Uft28	K122
111	8							
121	2	8						
267	2	8	8					
269	2	8	8	8				
295	6	2	2	2	8			
297	2	2	8	8	2	8		
Uft28	3	3	2	3	1	2	8	
K122	8	2	2	2	6	2	1	8

Note: highest (8) the value, when RFLP pattern is the same for all the studied restriction endonuclease in both isolates

Conclusion

Nematode	Strain	Bacteria
<i>H. megidis</i>	111	Yellow
<i>H. megidis</i>	121	Brown-grey
<i>H. downesi</i>	267	Brown-grey
<i>H. downesi</i>	269	Brown-grey
<i>H. megidis</i>	295	Red
<i>H. megidis</i>	297	Brown-grey
<i>H. bacteriophora</i>	Uft28	<i>P. luminescens</i>

The relationship between bacterial and nematode strains

- There are three different types of *P. temperata* in a relatively small sampling area
- *H. megidis* has three different bacterial symbionts, contrary, bacteria with brown-grey colonies has two hosts (*H. megidis* and *H. downesi*)

Acknowledgement

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