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ROVARPATOGÉN BAKTÉRIUMOK ANTIBIOTIKUM TERMELÉSE ÉS SZIMBIOTIKUS PARTNER-SPECIFITÁSÁNAK GNOTOBIOLÓGIAI ANALÍZISE

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I. INTRODUCTION

Introduction, AIMS, Objective, Rationale:

The entomopathogenic nematode-symbiotic *Photorhabdus* and *Xenorhabdus* bacteria colonize the intestines of the infective soil-dwelling (dauer juvenile, IJ) stage of entomophagous nematodes, *Heterorhabditis* and *Steinernema*, respectively. These nematodes infect susceptible insects and release the bacteria into the insect blood. The bacteria kill the insect and convert the cadaver into a food source suitable for nematode growth and development. They also produce large spectrum antimicrobials and keep the cadaver monoxenic in soil conditions. After several rounds of reproduction the guts of young nematodes growing to the alternative developmental variant IJ are recolonized by the bacteria before emerging from the insect cadaver into the soil to search for a new host. *Photorhabdus* and *Xenorhabdus* bacteria therefore engage in both pathogenic and mutualistic interactions with different invertebrate hosts as obligate components of their life cycle. According to our current knowledge the molecular mechanisms utilized by *Photorhabdus* and *Xenorhabdus* to control their host-dependent interactions there is a trade-off between pathogenicity and mutualism in both these species of bacteria suggesting that the transition between these interactions must be under regulatory control. Despite the superficial similarity between the life cycles of these bacteria, it is now apparent that the molecular components of the regulatory networks controlling pathogenicity and mutualism in *Photorhabdus* and *Xenorhabdus* are very different (Goodrich-Blair and Clarke, 2007). Our study is restricted to the *Photorhabdus / Heterorhabditis* mutualistic associations.

As for motile, gram negative *Photorhabdus*, this is the only terrestrial bacteria known to exhibit bioluminescence (Gerrard et al., 2003). The production of light in *Photorhabdus* may not have any real function and instead represents a trait present in an aquatic ancestor that is now being lost on colonization of a terrestrial environment via the nematode vector or is a remnant of a horizontal gene transfer event that has not had sufficient evolutionary time to disappear (Peat and Adams, 2008).

One of the most unique characteristics of *Photorhabdus* is its symbiotic capabilities. The obligate symbiotic relationships of *Photorhabdus* and *Heterorhabditis* has gained much attention due to their ability to act together to kill their insect host (Peat et al., 2010). Although *Photorhabdus* has a capability of killing insects alone, the mutualistic association with nematodes from the family Heterorhabditidae provides the only natural option to enter the insect haemocoel. Therefore, the bacteria have a complex life cycle that involves temporally separated pathogenic and mutualistic associations with two different invertebrate hosts. This tripartite *Photorhabdus*-insect-nematode association provides researchers with a unique opportunity to characterize the prokaryotic contribution to two different symbioses, i.e. pathogenicity and mutualism while also studying the role of the host in determining the outcome of association with the bacteria (Clarke, 2008).

Technically, upon locating a suitable insect host, *Heterorhabditis* penetrates through natural openings (mouth, anus, spiracles) (Boemare, 2002), or directly into the haemocoel of the larval insect via the integument (Akhurst and Dunphy, 1993; Forst et al., 1997; Poinar, 1990), subsequently releasing bacteria into the hemolymph (Forst et al., 1997). Once in the hemolymph, *Photorhabdus* begins multiplying, simultaneously releasing toxins virulent enough to kill the insect within 24 h (Ciche and Ensign, 2003; Forst et al., 1997). All *Photorhabdus* strains are considered highly entomopathogenic, with an LD50 of <100 cells per insect (Boemare, 2002). Following death of the insect and consumption of all available nutrients, *Photorhabdus* and *Heterorhabditis* re-assimilate, leaving the dead insect in search of another insect host (Forst and Nealson, 1996).

Initially classified as *Xenorhabdus luminescens*, the genus *Photorhabdus* would later be proposed by Boemare et al. (1993) based on the examination of phenotypic characters and DNA relatedness studies. Specific and sub-specific taxonomic designations within the genus *Photorhabdus* are based on phenotypic data including morphological, biochemical and physiologic characters were suggested by Akhurst et al (1996). Akhurst et al. (1996) concluded that phenotypic data alone could only separate two groups of *Photorhabdus*, the symbionts and the clinical strains

Historically, 16S rRNA gene has been the marker of choice when classifying/naming *Photorhabdus* species and subspecies. It has been suggested that *Photorhabdus* species may be subjected to a higher evolutionary rate than that of its sister taxon *Xenorhabdus* (Rainey et al., 1995) based on analysis of 16S rRNA gene data. This hypothesis was tested and proved in the laboratory of Prof. E. Stackebrandt (DSMZ, Braunschweig, Germany) by Szállás et al., (1997), who compared the sequences of the 16S rRNA gene of 40 strains of bacterial symbionts isolated from the nematodes *Heterorhabditis* spp. and seven bacterial symbionts of the nematodes *Steinernema* spp. which were isolated from different geographical areas, as well as the type strain *of Xenorhabdus japonica,* were determined and compared to each other and to the sequences of several reference strains of members of the *Enterobacteriaceae.* The data confirmed the separate status of the two genera of symbionts of entomopathogenic rhabditid nematodes. The symbionts of *Heterorhabditis* spp. clustered with the type strain of *Photorhabdus luminescens,* while the symbionts of *Steinernema* spp. grouped with *Xenorhabdus* species. *X. japonica* clustered with the other *Xenorhabdus* species. Phylogenetic analysis of 15 almost complete 16s ribosomal DNA (rDNA) sequences of the *Heterorhabditis* symbionts indicated that there were several subclusters. The properties correlated with these subclusters were not then yet apparent, although there may be some geographical and ecological correlations. For example, among the nematode-symbiotic bacteria, the members of subclusters I and III are from south-eastern and Midwestern North America, respectively, while the members of subclusters II and IV are primarily from Europe and Australia, respectively. The non-symbiotic strains of *P. luminescens* form a highly homologous subcluster by themselves. The results of DNA-DNA hybridization studies performed with a few selected strains of five of the 16s rDNA subclusters supported the existence of several genospecies within *P. luminescens*. The comparative sequence analysis was later expanded to other *Photorhabdus strains*. These studies also included PhastSystem PAGE RFLP and automated RiboPrint phenotypes of *Photorhabdus strains* (Szállás et al., 2001). Liu et al. (1997) adopted this approach and developed a phylogeny of *Photorhabdus* and another closely related bacterial endo-symbiont of nematodes, *Xenorhabdus*. The study used only 13 *Photorhabdus* isolates, most of which had no species designation. Based upon maximum likelihood analysis of a portion of 16S rRNA gene, Liu et al. showed four well supported major clades within the one

recognized clade, supporting the possibility that more than one species of *Photorhabdus* exists.

Through a polyphasic approach utilizing 16S rRNA gene phylogenetic inference, phenotypic characterization, and DNA–DNA hybridization data, Fischer-Le Saux et al. (1999) proposed the existence of three separate species of *Photorhabdus: P. luminescens, P. temperata*, and *P. asymbiotica*. Furthermore, the study went on to propose the existence of three subspecies within *P. luminescens*. A second polyphasic approach utilizing phenotypic characterization, DNA–DNA hybridization, and two molecular markers, *gyr*B and 16S rRNA genes proposed the separation of *P. asymbiotica* into two subspecies (Akhurst et al., 2004), and a multilocus sequence typing analysis of *rec*A, *gyr*B, *dna*N, *glt*X (Tailliez et al., 2009)**.**

I am interested in reconstructing the evolutionary history of the *Photorhabdus* – *Heterorhabditis* symbiotic associations. Our gnotobiological approach (a term of Prof. Noel Boemare, Montpellier, France) based on culturing different strains of *Heterorhabditis* sp. On each other's *Photorhabdus* symbionts. In order to be able to do that we had to elaborate a new solid, entomopathogenic nematode growth media (ENGM) amenable to grow both the symbiotic partners in optimum conditions. Unlike to other this media conventionally used ENGM lets nematodes visible for stereomicroscopic observation throughout their life.

All current data concerning outline the life cycle of *Photorhabdus* and description of recent important advances in our understanding of the symbiology of *Photorhabdus* are reviewed by Clarke (2008). To evaluate hypotheses of co-speciation between *Photorhabdus* and *Heterorhabditis* thoroughly, the use of the recently published (Peat et al., 2010) robust phylogenetic hypothesis of the genus is required. I also intended to characterize the bacterial symbionts for their antibiotics resistance, production, phase variation and other phenotypes with special attention of potential use.

Recently Peat et al (2010) examined phylogenetic relationships among *Photorhabdus* taxa utilizing total evidence Bayesian, likelihood, and parsimony based analyses of three

genes (loci), including 16S rRNA, *gyr*B, *and gln*A respectively, and constructed a robust evolutionary hypothesis for the genus *Photorhabdus*. They used this phylogeny to evaluate existing specific and sub-specific taxonomic statements within the genus, identify previously undescribed *Photorhabdus* strains; test the utility of genes 16S rRNA, *gyr*B, and *gln*A in resolving various levels of relationships within the genus. Here we use this robust phylogenetic framework for the bacterial genus *Photorhabdus* to investigate the c-evolution processes leading to taxon-specific obligate symbiosis between *Photorhabdus* bacterium and *Heterorhabditis* nematode strains. We intended to test whether the generally accepted co-speciation hypothesis provided an unambiguous explanation. We wanted to trace the evolution of symbiosis partner-specificity through genus *Photorhabdus* to determine symbiotic partner specificity and range existing across the evolution of basal to the more derived clades.

II. MATERIALS, METHODS, EXPERIMENTAL STRATEGY:

DNA extraction

Bacteria were isolated from surface sterilizes IJ as previously described (Szállás et al., 1997). DNA was extracted from a single colony, dispersed into 400 µl salina-EDTA buffer in a 1.5 ml microfuge tube. 10 µl lysosime (10 mg/ml) (Sigma) was added and incubated at 37 oC for 30'. 5 µl of proteinase-K (15 mg/ml) (Boehringer, Mannheim), 10 µl of sodium dodecyl sulfate (SDS) (25 % {w/v} (Sigma) were added to the tube, and incubated at 60 ^{o}C for 30'. 400 µl phenol (Sigma) as added to the lysate and vortexed, then centrifuged at 14,000 rpm for 10'. The upper aqueous phase was transferred to a new 1.5 ml tube. The DNA was recovered from the aqueous phase, using the Pre-A-Gene kit (BioRad, Hercules Ca).

PCR amplification of the 16S-23S rDNA intergenic spacer (IGS) region:

Preparation of 25 µl volume of PCR mixture was used: 23. µl PCR SuperMix (Cat. No. 10572-014, Gibco); 0.5 µl 1241 forward primer (from 16S rRNA) locus; (0.5 µg / µl); 0.5 µl 241 reverse primer (from 23S rRNA) locus (0.5 µg / µl); 1 µl genomic DNA. PCR amplification was carried out under the following conditions: 1 cycle at 94 ^{o}C for 30'; 72 ^{o}C for 1' and a final cycle for 10' at 72 ^{o}C. Amplification of the 16S-23S rDNA intergenic spacer region was performed using primers 1241F: 5' TACACACGTGCTACAATG 3'

(position 1224-1242) of 16S rDNA and 241R: 5' (G/T)TTCGCTCGCC(A/G)CTAC 3'(position 241-255 of 23S rDNA). The PCR product was checked in 1% agarose for purity in a 15'run.

Sequencing, sub-clustering *Photorhabdus* strains and distinction of within taxon (subcluster) on the basis of *Alu*I restriction pattern

The almost complete (>1400 nucleotides) sequence of 16S rDNA was determined and the phylogenetic analysis based on the comparative analysis of the full sequences of 16S rDNA was carried out as described previously (Szállás et al., 1997). We found that practically each of our "subclusters" corresponded to a taxon identified by Peat et al (2010).

The *Alu*I restriction pattern of the IGS region of the 16S – 23S rDNA operon in different *Photorhabdus* strains were first established by H. Pamjav by using PhastSystem PAGE PCR RFLP analysis (Pamjav, 2000; Szállás et al. 2001). The *Alu*I restriction pattern established later by simpler agarose gel electrophoresis techniques did not give so excellent resolution but was suitable to distinguish *Photorhabdus* strains of the same subcluster (taxon) from each other. Prior to *Alu*I restriction analysis the PCR products were purified and its purity was checked on 1% agarose gel. The *Alu*I was made overnight similar at 37°C. The final volume (25 µl /sample) contained 10 µl of purified PCR product; 12 µl of dH_2O; 2, 5 µl of buffer and 0.5 µl enzyme. The digestion was stopped on ice. The digested samples were run on Etidium-bromid containing agarose-gels (of 1.5 As running buffer TBE and TAE were used alternatively. Routinely 10µl of sample was mixed 6µl of stain, before electrophoresis. The control DNA ladder included fragments of 110-1116 D. The electrophoresis was carried out at 80 V for 90' in each case. The upper buffer consisted of 1, 5 ml AA: BIS; 2, 5 ml TBE; 100µl of 10% TEMED; 5.8 ml dH_20 and 200µl of 10% persulfate. The lower buffer consisted of 2, 5 ml AA: BIS 29: 1; 1. 0 ml 10 X TBE; 300 µl of 10% TEMED and 10 ml dH_20.

Taxon sampling, origin of the strains

Altogether 51 *Photorhabdus* strains were examined for their symbiotic partner specificity. (Many of them are kept deposited in the Georgikon EPN/EPB Stock Center in Keszthely, Hungary). Four of the studied strains (ATTC strains of *P. asymbiotica ssp. asymbiotica*) do

not have any known natural nematode symbiont. The nematode symbionts of 10 strains (DSM 3368^T, Meg 1, Meg 2 (from E. Stackebrandt); Hm1,WX6, WX8, WX9 Hyper, WX11, WX 12, WX 13) (from K.H. Nealson) had been lost before they arrived to our laboratory. We isolated 14 strains (HP88, ACOWS, Arap Vin 15H, AZ29, AZ35, AZ36, AZ37, AZ39, BRECON) from *H. bacteriophora*; 2 strains (Hepialius, OR-10) from *H. marelata*; 5 strains (OHI, H4, HL81, HE87.3, JUN) from *H. megidis*; two strains from *H. downesii* (HIT); 4 strains (IS5, EG2, IND, Hawaiiensis,) from *H. indica*; and one strain (NZH3) of *H. zealandica* in our laboratory (E. Szállás and A. Lucskai) and the full sequences of their 16S rDNA were also sequenced by us (Szállás et al., 1997; 2001). Other *Photorhabdus* strains (HB, Heliothidis, K122, HSH2, CJG, Arap VIN 19H, TT01, NC19, V6-1, Q-614) were respectively provided by fellow researchers: N. E. Boemare, D. Clarke, J. Gunther, R.-U. Ehlers, B. Adams, J. Ensign, L. E. Stackebrandt. Off the 51 strains were also used in the study of Peat et al (2010). Altogether 36 strains were used both in this study and that of Peat et al. (2010). Altogether 15 strains (DSM3368, Acows, Arap Vin 15H, AZ35, AZ37, AZ 39, V6-1, Q614, A1, OR-1, Meg2, HE87.3, CJG and NZH3 was studied only this analysis. All available important data (accession numbers, natural symbionts, etc.) are given in Table 1.

Culture media

Photorhabdus strains were cultures in conventional solid (Luria-Broth Agar, LBA) plates or in liquid (LB) (Ausubel et al., 1999). LBTA indicator media contained 1 ml of 80 mg/ml bromthymol blue (BTB) and 1 ml of 40 mg/ml TTC (see Szállás et al., 1997). Heterorhabditid nematodes were grown on ENGM plate, a media developed for growing and propagating entomopathogenic nematodes on nematode-symbiotic entomopathogenic bacteria. The recipe for ENGM is as follows: 2.5 g DIFCO bacto-peptone; 4 g DIFCO beef extract; 15 g agar; 1 liter of deionized water. After autoclaving: 2 ml of 0.5M $MgSO_4$; 1 ml of 1 M $CaCl_2$; 5 ml linseed oil; 1 ml of oversaturated (>5 mg/ml) cholesterol (dissolved in EtOH).

Methodology of gnotobiological analysis

In order to determine the limits of the symbiotic capabilities of the EPN and the EPB partners and see, the symbiotic partner range and micro taxonomy of genus *Heterorhabditis* and *Photorhabdus* were correlated (gnotobiological analysis). Each *Heterorhabditis* strain was tested on each of the other strains a follows: either freshly hatched axenic first stage (J1) juveniles or more frequently surface sterilized (IJ) nematodes were transferred to ENGM plates seeded either with their own natural symbiont or *Photorhabdus* symbiont from another *Heterorhabditis* strain. The growth, development, fertility, and reproduction of the nematodes were monitored. The score was based on IJ formation and bacterial retention in their guts. If they grew to fertile adults producing progeny and IJs amongst them, the nematodes were scored as accepting the new bacterium as a food source. To determine if the new bacteria were retained, the bacteria were isolated directly from the next generation IJs according to Lucskai (Pamjav, 2000). The isolated bacteria were cultured on indicator (LBTA) plates; and identified by using colony morphology and /or *Alu*I restriction pattern of the IGS of the 16S – 23S rRNA operon and compared with both the original and with the new symbiont. The IJs with their new symbionts were also tested for capability of infecting 4^{th} instar *Galleria mellonella* wax moth larvae, and of propagating in them.

RESULTS AND DISCUSSION:

1. ARE THE *HETERORHABDITIS / PHOTORHABDUS* SYMBIOTIC RELATIONS EXPLAINED BY CO-SPECIATION?

The main goal of this work was to clarify the relations between existing symbiotic relations and evolution of the symbiotic partners. *Photorhabdus* and *Xenorhabdus* bacteria engage in both pathogenic and mutualistic interactions with two different invertebrate (nematode, insect) hosts as obligate components of their life cycle. This tripartite association, along with their ability to kill insects, has aroused interest in the evolutionary relationships of the symbiotic partners. At present three species are recognized within the genus *Photorhabdus*: *P. temperata*; *P. luminescens*; and *P. asymbiotica*. Each includes subspecies, which, apart from *P. asymbiotica ssp. asymbiotica* and *P. asymbiotica ssp. australis*, are obligate endosymbionts of heterorhabditid nematodes. The evolutionary aspects of the obligate, taxon-specific *Heterorhabditis / Photorhabdus* symbioses were studied on a new agar media (ENGM) experimentally.

My gnotobiological analysis delineated partner specificity of different *Photorhabdus* and *Heterorhabditis* taxa. We introduce the new term "symbion" for those EPN/EPB associations, within which the symbiotic partners could mutually been exchanged. *P. temperata ssp. kleini* are symbionts of *H. marelatus* and North American *H. megidis* (USA) strains (Symbion I). *P. temperata ssp. temperata* are symbionts of North Western European *H. megidis* (NWE) forming Symbion II. The exclusive natural symbiotic partner of each known strain of *P. temperata ssp. thracensis*, *P. luminescens* ssp. *laumondii* and *P. luminescens* ssp. ssp. *luminescens* is a *H. bacteriophora* forming (Symbion III). *P. luminescens* ssp. *akhurstii* are symbionts of *H. indica* but the symbionts of *H. indica* strains cannot be exchanged. Two known strains (HIT, JUN) of *P. asymbiotica ssp. stackebrandtii* are symbionts of *H. downesii* (HIT) and a North-Western European *H. megidis* (JUN), but the *H. downesii* HIT and K122 symbiotic complexes do not form Symbion either. Hardly any overlap was found between symbions. Data are discussed at the light of the co-speciation hypothesis.

HOW THE PHASE VARIATIONS AND SYMBIOIS RELATED?

My data confirmed that both the symbiotic capabilities as well as the antibiotics production are „primer-specific" characters. My experiments also proved unambiguously, that the secondary forms could not even utilized by the nematodes as food source.

IS THE ANTIBIOTICS RESISTANCE TAXON SPECIFIC?

My data indicate that there were no significant differences between strains concerning their sensitivity or resistance toward different antibiotics of clinical use.

DO THE COMPOUNDS OF ANTIMICROBIAL ACTIVITY HAVE A POTENTIAL FOR MEDICAL AND/OR AGRICULTURAL POTENTIAL?

Broad spectral antibiotics produced by symbiotic bacteria (EPB) of entomopathogenic 3 nematodes keep monoxenic conditions in insect cadavers in soil. Comparison of the antimicrobial potential of our EPB stock collection resulted in selection of two strains of outstanding antimicrobial potential (Bachmann et el., 2006; Furgani et al, 2008). We found that produce antibiotics effective against the fire blight bacterium *Erwinia amylovora,* including streptomycin resistant strains, and were as effective in phytotron experiments as kasugamycin or streptomycin. *Xenorhabdus budapestensis* and X. *szentirmaii* antibiotics inhibited colony formation and mycelial growth of *Phytophthora nicotianae.* From X. *budapestensis,* an arginin-rich fraction (bicornutin) was adsorbed by Amberlite R XAD 1180, and eluted with methanol - IN HCI (99: 1). Bicornutin inactivated zoospores, and inhibited germination and colony formation of (bicornutin-A, MW= 826), separated by HPLC and thin-layer chromatography, was identified as a novel hexa-peptide: RLRRRX. I concluded that *Xenorhabdus budapestensis* produces strong cytotoxic antibiotics. Active molecules can be isolated, identified and patented, but their full antimicrobial potential may be multiplied by synergic interaction of different molecules. The significance and impact of the study: can be summarized as follows: Antibiotics of two new *Xenorhabdus* species

might be control agents of diseases caused by *E. amylovora*, P. *nicotianae* and other related pathogens of great importance to agriculture. I would like to continue my research work on this area. This study evaluated antibiotics produced by EPB for their potential to control plant pathogenic bacteria and oomycetes (Böszörményi et al. 2009).

DATA CONCERNING *XENORHABDUS - XENORHABDUS* INTERACTION

Insect-nematode-bacterium mutualistic associations provide attractive systems for discovery of inter kingdom signal compounds and antibiotics. A better understanding of the biological meaning of the inter-specific diversity of compounds with antimicrobial activity of the *Steinernema*-symbiont *Xenorhabdus* bacteria may provide options for simultaneous applications in pathogen control. Antibacterial activities of representative strains of *Xenorhabdus budapestensis, Xenorhabdus szentirmaii, Xenorhabdus innexi, Xenorhabdus ehlersii*, *Xenorhabdus nematophila, Xenorhabdus bovienii* and *Xenorhabdus cabanillasii* were tested on non-related (*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*) bacteria and on each other by previously published bioassays. All active compounds were adsorbed by AmberliteR XAD1180. Chemical and thermal stability of antibacterial factors were determined.

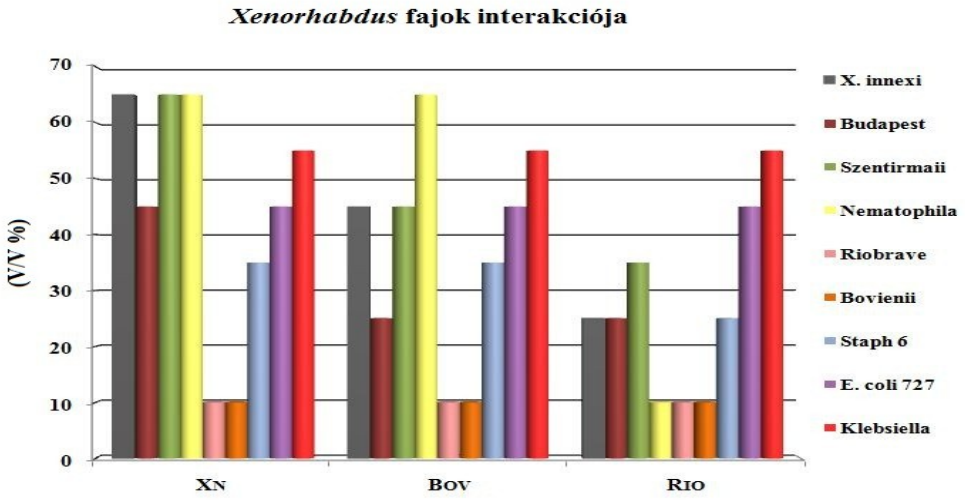

Fig 1: Interactions of *Xenorhabdus* species.

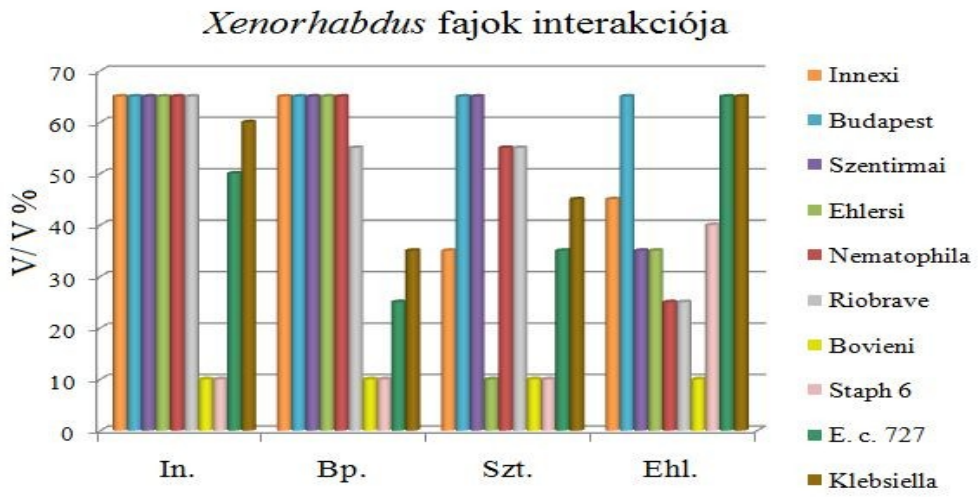

Fig 2: Interactions of *Xenorhabdus* species

Antibiotic factors produced by different *Xenorhabdus* species against each other differ from those used against other competing bacterial genera. Anti-*Xenorhabdus* activity of the cell-free medium and sensitivity of the cells of other *Xenorhabdus* strains negatively correlated in *X. innexi* and *X. bovienii*. Some activity remained unchanged during high pressure and 121°C for 10 min. The first comparative analysis of the intraspecific antibacterial activities of *Xenorhabdus* species demonstrated that some *Xenorhabdus* species with strong antibacterial activity could be co-cultured and they might be used simultaneously for pathogen control. (Fodor et al., 2010b; Fig. 1. & 2.)

SUMMARY

The objective of my PhD Dissertation the tripartite mutualistic system including: (1) entomopathogenic bacteria (EPB) as an insect pathogen and nematode symbiont; (2) entomopathogenic nematode (EPN) as insect predator and symbiotic partner of the nematode. I am personally interested in he details of the EPN-EPB symbiosis, with special attention to the partner specificity as well as the antibiotics produced by EPB in order to keep the colonized insect cadaver monoxenic in polyxenic soil conditions. In he introductory part the taxonomy of both EPN and EPB species, as well as the evolutionarily conserved symbiotic partner relations are summarized. The aim of this study is the better understanding this symbiotic relation and gets more information of EPB antibiotics of a potential use. The evolutionarily conserved EPN/EPB symbiotic relations are believed are

results of co-speciation. The basic argument of the co-speciation hypotheses is that each *Heterorhabditis* uses exclusively *Photorhabdus* as symbiont and each *Steinernema* uses exclusively *Xenorhabdus* as symbiont. Whether the co-speciation is also a trend within the two nematode genera: This should be tested. The main goal of this Dissertation is to test the co-speciation hypotheses in Heterorhabditis */Photorhabdus* relation. I intend to determine the limits of symbiotic partner-specificity through gnotobiológiai analyses. We intend to determine the limits of the symbiotic capabilities of both the EPN and the EPB partners and see, whether any correlation between the symbiotic partner range and micro taxonomy of genus *Heterorhabditis* and *Photorhabdus* could be found (gnotobiological analysis). We have adopted and established the technique of direct isolation EPB symbiont from a single dauer larva originally elaborated by. A. Lucskai. The nematodes of each strain were tested on each other's symbionts for growth, development, fertility, dauer larva formation and retention of EPB in their guts. The results suggest handling the "co-speciation theory" with some reservation. We concluded, that establishing symbiotic associations must have been prelude by genetic mutation (s) of one or both potential symbiotic partner and then isolated and fixed. At the start the taxonomic identity of the EPB strain is not a matter. These conclusions are supported by data concerning incompatibilities of utilize each others' symbionts by different con-specific EPN strains, such as those of European and American *H. megidis*; or Irish and Hungarian strains of *H. downesii*. Congenic EPB strains, symbionts of non-con-specific EPN strains cannot either be utilized by each other.

My dissertation I attempted to summarize what we knew antibiotics produced by EPB for their potential to control plant pathogenic bacteria and oomycetes. Two bioassays were adapted for EPB antibiotics: an overlay method on agar plates and serially diluted, cell-free, *Xenorhabdus* and *Photorhabdus* cultures. Antimicrobial activities of the type strains of *X. nematophila*, *X. budapestensis* and *X. szentirmaii* were tested on mastitis isolates of *S. aureus*, *E. coli*, *K. pneumoniae E. amylovora* with both bioassays. The best result (antibiotics activity equivalent to 100-200 ppm streptomycin sulfate) was provided by the strains of *X. budapestensis*. There was a negative correlation demonstrated between producing anti-*Xenorhabdus* activity and resistance to *Xenorhabdus* activity.

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PUBLICATIONS IN PER REVIEW JOURNALS:

1, Böszörményi, E., Érsek, T., Fodor, A., Fodor, A. M., Földes, L. Sz., Hevesi, M., Hogan, J. S., Katona, Z., Klein, M. G., Kormány, A., Pekár, S., Szentirmai, A., Sztaricskai, F., and Taylor, **R. A. J.,** Isolation and activity of *Xenorhabdus* antimicrobial compounds against the plant pathogens *Erwinia* compounds against the plant pathogens *Erwinia amylovora* and *Phytophthora nicotianae Journal of Applied Microbiology* 107 **(2009):**746-759.

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PRESENTATIONS IN MEETINGS AND CONGRESSES:

1, Böszörményi E., Lengyel K., Pamjav H., Szállás E., Fodor A.**,**Gnotobiological analysis of *Heterorhabditis/Photorhabdus* complexes. 13th International Congress of Hungarian Society for Microbiology **(1999)** Budapest. Book of Abstracts p:14.

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