European cooperation in the field of scientific and technical research

Agriculture and biotechnology

Developments in entomopathogenic nematode/bacterial research

Proceedings of the workshop held at the National University of Ireland Maynooth 13 to 15 April 2000

COST Action 819

EUR 19696

Interested in European research?

RTD info is our quarterly magazine keeping you in touch with main developments (results, programmes, events, etc.). It is available in English, French and German. A free sample copy or free subscription can be obtained from:

Directorate-General for Research
Information and Communication Unit
European Commission
Rue de la Loi/Wetstraat 200
B-1049 Brussels
Fax (32-2) 29-58220
E-mail: research@cec.eu.int
Internet: http://europa.eu.int/comm/research/rtdinfo.html

EUROPEAN COMMISSION
Directorate-General for Research
European Research Area: structural aspects — COST

Contact: Mr Roelof Mulder
Address: European Commission, rue de la Loi/Wetstraat 200 (B7 3/9),
B-1049 Brussels — Tel. (32-2) 29-65507; fax (32-2) 29-64289
E-mail: roelof.mulder@cec.eu.int

E
D
E

C
A
B
E

European Commission

COST Action 819

Developments in entomopathogenic nematode/bacterial research

Proceedings of the workshop held at the National University of Ireland Maynooth 13 to 15 April 2000

Edited by
Christine T. Griffin
Ann M. Burnell
Martin J. Downes and
Roelof Mulder

Directorate-General for Research

2001 EUR 19696

A great deal of additional information on the European Union is available on the Internet.
It can be accessed through the Europa server (http://europa.eu.int).

Cataloguing data can be found at the end of this publication.

Luxembourg: Office for Official Publications of the European Communities, 2001

ISBN 92-894-0467-1

Printed in Belgium

PRINTED ON WHITE CHLORINE-FREE PAPER

SPECIFIC ADHERENCE OF SPORANGIA OF A *PAENIBACILLUS* SP. BACTERIUM TO *HETERORHABDITIS* SPP. NEMATODES. HITCHING A RIDE TO LUNCH?

Michael Roy Enright[1, 2], Michelle M. Finnegan[1], James O. McInerney[1], Matthew O'Laighleis[1] and Christine T. Griffin[1, 2]

[1] Department of Biology, National University of Ireland, Maynooth, Co. Kildare, Ireland.
[2] Institute of Bioengineering and Agroecology, National University of Ireland, Maynooth, Co. Kildare, Ireland

INTRODUCTION

The fact that entomopathogenic nematodes of the genera *Heterorhabditis* and *Steinernema* are normally found in mutualistic association with the bacteria *Photorhabdus* spp. and *Xenorhabdus* spp , respectively, has long been universally accepted However, the extent and nature of their interaction with bacteria other than these, under natural conditions, is less well known. There have been a number of reports of other bacteria being isolated from entomopathogenic nematodes, particularly from *Steinernema* spp. (reviewed by Boemare *et al*, 1998a). Jackson *et al* (1995) reported the occurrence of *Providencia rettgeri* with a number of strains of *Heterorhabditis* spp. originating from different geographical regions. Boemare *et al* (1998b) point out that ecologists will tend to harvest nematodes resulting from "successful" parasitisms, i e. those where the cadaver is not more rapidly putrefied by the presence of co-associated bacteria other that the natural symbiont. With this in mind it is conceivable that we may under-estimate the frequency of association of these nematodes with other bacteria

A NEW *PAENIBACILLUS* SP. FOUND ASSOCIATED WITH *HETERORHABDITIS MEGIDIS*, STRAIN EU17

Heterorhabditis megidis develops within the insect cadaver with its bacterial symbiont *Photorhabdus temperata* that kills and breaks down the host tissues. It also reduces proliferation of other microbes by the production of antibiotics. We have isolated a *Heterorhabditis megidis* strain, EU17 from Estonia, found associated with a *Paenibacillus* sp Based on phenotypic, chemotaxonomic and 16S rRNA gene sequencing this bacterial strain, NEM 1, represents a new species, proposed as *Paenibacillus estoniaensis* (Enright *et al.*, submitted). A phylogenetic tree including the proposed species is shown in Figure 1. The genus *Paenibacillus* is one of several genera that were created following a number of 16S rRNA sequence analysis studies which identified at least 10 phylogenetic groups within the genus *Bacillus* as it then stood (Shida *et al*, 1997). The genus *Paenibacillus* contains a number of species that have been shown to be associated with dead insects in one capacity or another. These include *Paenibacillus popilliae*, *P lentimorbus*, *P larvae*, *P. pulvifaciens*, *P. alvei* (Pettersson *et al*, 1999) and *P apiarus* (Nakamura *et al*, 1996). However, only *P*

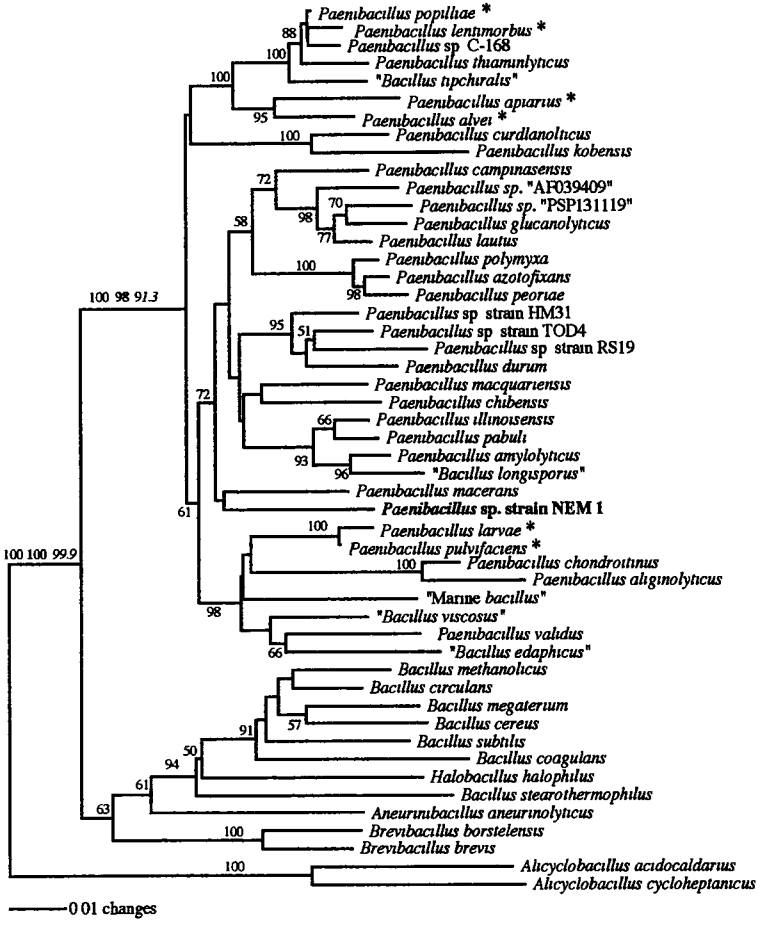

Fig 1. Phylogenetic tree, derived from an alignment, comprising of 16S rRNA gene sequences from *Paenibacillus* species and selected members of closely related genera, based on neighbour joining method. Bootstrap values for distance analysis (>50% only) are shown in plain text, while bootstrap values for parsimony analysis and quartet puzzling values for maximum likelihood (both for 2 branches only) are shown in bold and italics respectively.

* denotes *Paenibacillus* sp. know to be in some capacity associated with dead insects.

popilliae, *P lentimorbus* and *P larvae* are described as being obligate insect pathogens, with *P popilliae* and *P lentimorbus* causing milky disease types A and B, respectively, in Japanese beetles, and *P larvae* causing American foulbrood in honey bee larvae.

This *Heterorhabditis* - *Paenibacillus* association has been maintained through *in vivo* laboratory culturing since its isolation in 1994 The spindle shaped sporangia of the bacterium adhere to the sheath of the infective juveniles (Fig. 2) as they leave the cadaver, enabling them to be carried to new hosts NEM 1 then proliferates within the insect cadaver. Shortly before the infective juveniles begin to emerge from the cadaver, sporangia begin to accumulate in large numbers and, hence, emerging infective juveniles become encumbered.

SPECIFICITY OF THE ASSOCIATION

Sporangia-bearing nematodes form "clumps" (Fig. 3) in water. Clumping is inhibited by N-acetylneuraminic acid (sialic acid) but not by other sugars. This suggests that a specific carbohydrate-lectin interaction is involved in adherence Sporangia of NEM 1 adhered to all *Heterorhabditis* spp tested but did not adhere to *Steinernema* spp., *Phasmarhabditis hermaphrodita, Caenorhabditis elegans*, *Aphelenchus avenae*, potato cyst nematode or *Meloidogyne* sp (Enright, unpublished) Nor did sporangia of a number of other *Paenibacillus* spp. tested adhere to *H megidis* Amongst these were a number of undescribed strains isolated by Elo *et al* (2000) and an undescribed strain isolated from Northern Ireland (isolated by Dr. G. McMullan, University of Ulster) These were the most closely related strains in the GenBank database to NEM 1

CONCLUSION

An association between *Heterorhabditis* and *Paenibacillus* may be of fairly widespread occurrence. Marti and Timper (1999), in an abstract, report a similar association between a number of *Heterorhabditis* sp. isolates from Georgia, USA, and an unidentified *Bacillus* species. The sporangia they report are of very similar description to those of NEM 1. We have also observed similar sporangia adhering to a strain of *Heterorhabditis indica* isolated from India (since lost from culture). Thus, a similar nematode-bacterial association, involving at least two species of *Heterorhabditis*, is reported from three continents, though the bacterium in the American and Asian association has not been identified as a *Paenibacillus*.

There is no sign of any *Paenibacillus*-induced pathology in juvenile or adult stages of *Heterorhabditis*, and there are no obvious effects on cadaver condition when using *Galleria mellonella*. There is certainly a phoretic element to the relationship between *Paenibacillus* sp. NEM1 and *H megidis*, as is also suggested by Marti and Timper (1999) for the relationship that they describe However, it is not possible to say how important nematode phoresis is in dispersal of the bacterium. Various aspects of the *Paenibacillus* sp. interaction with *Heterorhabditis*, its symbiont, and the insect host and are currently being investigated.

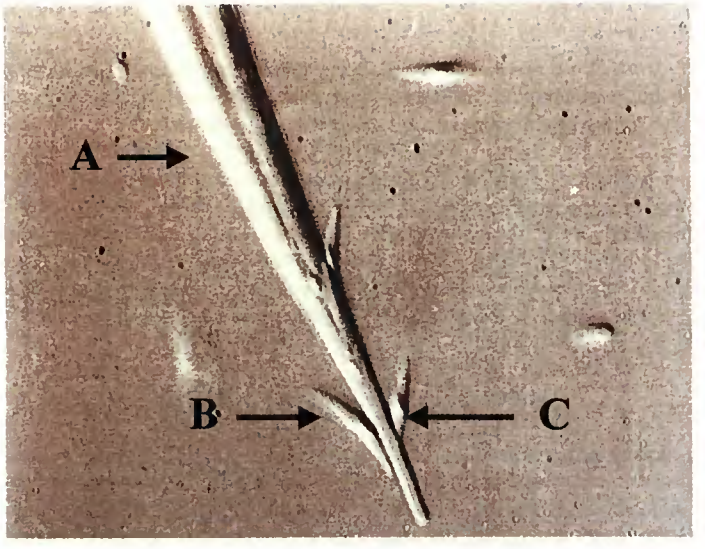

Fig 2: Tail end of a *Heterorhabditis megidis* IJ (A) with adhering sporangia of *Paenibacillus* sp. strain NEM 1 (B). Sporangia are spindle shaped and contain a central, oval shaped endospore (C), which, as is typical of *Paenibacillus*, swells the sporangium.

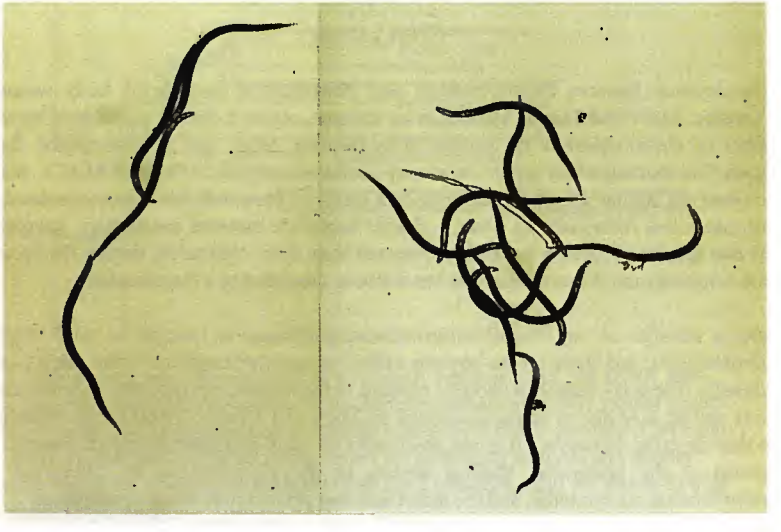

Fig 3: *Heterorhabditis megidis* infective juveniles form clumps because of adhering *Paenibacillus* sp. strain NEM 1 sporangia.

ACKNOWLEDGMENTS

We are grateful to Keith Davis (IACR-Rothamsted) for his assistance in testing for adherence to *Meloidogyne* sp. and potatoe cyst nematodes We also thank Colin Stack and John Browne (both from NUI, Maynooth) and MicroBio for providing us with various nematode species Finally we thank Seija Elo (University of Helsinki) and Geoffrey McMullan (University of Ulster) for providing us with *Paenibacillus* strains

REFERENCES

Boemare, N , Babic, I. and Fischer-Le Saux, M. (1998a) Accurate checking of bacterial cultures for the mass production, storage and delivery of EPN products. In Proceedings of the COST Action 819, 4th Working Group 2 Meeting: *Quality Control of Entomopathogenic Nematodes*, December 10-12, 1998, Merelbeke, Belgium.

Boemare, N., Bonifassi, E , Fischer-Le Saux, M. and Smart, G Jr. (1998b) The concept of monoxenic associations in EPNs limits of their natural occurrence and of gnotobiological interpretations. In *Taxonomy, phylogeny and gnotobiological studies of entomopathogenic nematode bacterium complexes* (Ed. N Boemare, P. Richardson and F Coudert). 101-103. European Commission, Brussels

Elo, S , Maunuksela, L., Salkinoja-Salonen, M , Smolander, A & Haahtela, K. (2000) Humus bacteria of Norway spruce stands· plant growth promoting properties and birch, red fescue and alder colonizing capacity. FEMS Microbiological Ecology **31**, 143-152.

Enright, M. R., McInerney, J O and Griffin, C. T. *Paenibacillus estoniaensis* sp. nov., a bacterium isolated from Estonia in association with the entomopathogenic nematode *Heterorhabditis megidis*, strain EU17. International Journal of Systematic and Evolutionary Microbiology, submitted

Jackson, T.J., Wang, H , Nugent, M J , Griffin, C T , Burnell, A. M and Dowds, B. C. A. (1995) Isolation of insect pathogenic bacteria, *Providencia rettgeri*, from *Heterorhabditis* spp Journal of Applied Bacteriology **78**, 237-244

Marti, O. G Jr. & Timper, P (1999) Phoretic relationship between a *Bacillus* sp. and the entomopathogenic nematode, *Heterorhabditis* sp. Journal of Nematology **31**, 553.

Nakamura, L. K (1996) *Paenibacillus apiarius* sp nov International Journal of Systematic Bacteriology **46**, 688-693.

Pettersson, B., Rippere, K. E , Yousten, A A. & Priest, F G. (1999) Transfer of *Bacillus lentimorbus* and *Bacillus popilliae* to the genus *Paenibacillus* with emended descriptions of *Paenibacillus lentimorbus* comb. nov. and *Paenibacillus popilliae* comb. nov. International Journal of Systematic Bacteriology **49**, 531-540.

Shida, O , Takagi, H , Kadowaki, K., Nakamura, L K & Komagata, K (1997) Transfer of *Bacillus alginolyticus*, *Bacillus chondroitinus*, *Bacillus curdlanolyticus*, *Bacillus glucanolyticus*, ***Bacillus kobensis*** and *Bacillus thiaminolyticus* to the genus *Paenibacillus* and emended description of the genus *Paenibacillus*. International Journal of Systematic Bacteriology **49**, 531-540.