

***Pasteuria* spp. as biocontrol agent against plant-parasitic nematodes :an overview**

Abstract:

Plant-parasitic nematodes are important threat to agricultural crops. Management through biological control agents like endoparasitic bacteria, *Pasteuria* spp. has shown great promise. They occur worldwide and have been reported from a wide range of environment. A comprehensive understanding of the biology of *Pasteuria* spp. and their mechanism underlying nematode disease suppression may help to develop useful biocontrol agent against plant-parasitic nematodes. This review gives an account of the morphology, biology, parasitic mechanisms of *Pasteuria* spp. and their potential uses.

Key words: Plant-parasitic nematodes, endoparasitic nematodes, biological control, *Pasteuria* spp., biology and parasitism.

1. Introduction

Plant-parasitic nematodes are the important biotic factor that causes major losses to agriculture. The management of nematodes is more difficult than that of other pests because nematodes mostly inhabit the soil and usually attack the underground parts of the plants. Although chemical nematicides are effective, easy to apply, and show rapid effects, they have begun to be withdrawn from the market due to increased environmental pollution and health hazards. Nematodes in soil are subject to infections by various soil-organisms and bacteria are numerically the most abundant organisms in soil. A variety of nematophagous bacterial groups have been isolated from soil, host-plant tissues, and nematodes possess diverse modes of action, and have broad host ranges and are considered as biological control agent. Among the nematophagous bacterial groups, *Pasteuria* are parasitic to a number of important plant parasitic nematodes. A number of bacterial species in this genus have shown great potential as biocontrol agents against plant-parasitic nematodes. They occur worldwide and have been reported from a

wide range of environments. Members of the genus *Pasteuria* are obligate, gram-positive, dichotomously branching, endospore-forming endoparasitic bacteria. *Pasteuria* spp. have the potential to offer an environmentally friendly method of nematode control.

2. History and classification

The bacterium was first described as *Pasteuriaramosa* by Metchnikoff in 1888 on water fleas of the genera *Daphnia* (Cladocera; Daphniidae). In 1906, Cobb studied the morphology of this parasite on *Dorylaimus bulbiferous* and placed among the protozoans. The first observation of a *Pasteuria* from plant parasitic nematodes, *Pratylenchus pratensis* was provided by Thorne (1940), which considered the organism a microsporidian and named it *Dubosqiapenetrans*. Electron microscope techniques have shown that the bacterium is more *Bacillus*-like than protozoan, and hence, it was renamed again, as *Bacillus penetrans*. Sayre and Starr [1] recognized the morphological similarities between *B. penetrans* and *P. ramosa* and placed the organism in the genus *Pasteuria*.

“At present, the taxonomy within the genus *Pasteuria* is based mainly on morphological and pathological characteristics, including the size and shape and ultrastructures of sporangia and endospores, life cycles and host ranges. As the *Pasteuria* has a branched filamentous vegetative thallus, so it was classified in the *Actinomycetales*” [2]. “Recent analysis of a portion of the 16S rRNA gene showed that the genus *Pasteuria* is a deeply rooted member of the *Clostridium-Bacillus-Streptococcus* branch of the Gram-positive Eubacteria” [3]. “Identification and characterization have been demonstrated by a number of molecular biological analyses using GyrB and SigE housekeeping genes and the 16S rRNA gene” [4,5]. “Additionally, all analyses revealed that *P. penetrans* is more closely related to the saprophytic *Bacillus haladurans* and *Bacillus subtilis* than to the pathogenic species *Bacillus anthracis* and *Bacillus*

cereus. Collectively, these findings strongly imply that *P. penetrans* is an ancient member of the *Bacillus* group”[6]. Some isolates are able to parasitize nematodes across genera (Table 1). Within the *P. penetrans* group, protein-encoding genes involved in sporulation appear to have sufficient polymorphism to be used for species differentiation, although they appear to be insufficient to resolve populations at the intra-species level. The 16s rRNA of *P. ramosa* from *Daphnia*, *P. penetrans* from *Meloidogyne*, *Pasteuria* strain S-1 from *Belonolaimus*, and *Pasteuria* strain NA from *Heterodera* were successfully sequenced and compared to support the current taxonomical position [7-10]. Research has demonstrated that “arms races between nematode hosts and endospore populations of *Pasteuria* can occur very rapidly”[11].

3. Mode of action

Pasteuria sp. interferes the normal sinusoidal movement of nematodes [12,13]. Around 5-10 endospores per juvenile is enough to initiate infection without reducing the ability of the nematode to invade roots and more than 15 endospores may disable the movement towards host roots. The endospore of these bacteria adheres to the cuticle of a nematode. Once gaining access, germination tube develops which pierces through the cuticle and entering nematode body cavity to establish parasitism. The bacteria form mycelia and microcolonies inside of the nematode. Multiplication of endospores in the body of nematode manifests the death of free-living juveniles, and induces a loss of fecundity in mature individuals.

4. Distribution and host range

With their worldwide distribution and reported host specificity, it appears the genus *Pasteuria* may consist of hundreds of species and subspecies, with different host, temperature, and ecological preferences. *Pasteuria* species were identified on 323 nematode species from 116

genera in 80 countries [14-18].But its occurrence and abundance seems to be variable due to variation in morphology, host range, and nematode life stage required for development.

Table 1.Nematodes and their associated*Pasteuria* spp.

Bacteria	Nematode	References
<i>Pasteuriapenetrans</i>	<i>Meloidogyne spp.</i>	[19-24]
<i>P.penetrans</i>	<i>Helicotylenchuslobus</i>	[25]
<i>Pasteuriaspp.</i>	<i>Helicotylenchusdigonicus,</i> <i>Pratylenchusthornei,</i> <i>P. neglectus,</i> <i>Tylenchorhynchuscylindricus,</i> <i>Rotylenchuscypriensis,</i> <i>Meloidogynejavanica, M.incognita</i>	[26]
<i>P.penetrans</i>	<i>M arenaria</i>	[27]
	<i>M javanica,</i>	[28]
	<i>M camelliae,</i>	
	<i>M hapla,</i>	
	<i>M mali,</i>	
	<i>M. suginamiensis</i>	
<i>P. thornei</i>	<i>Pratylenchusbrachyurus</i>	[19]
	<i>P.zeeae</i>	[29]
<i>Pasteuria sp.</i>	<i>Pratylenchusandinus</i>	[30]
<i>P.nishizawae</i>	<i>Heteroderaglycines;</i>	[31]
	<i>Globoderasp.</i>	[8,32,33]

<i>Pasteuria</i> sp.	<i>Heteroderaavenae</i>	[34]
<i>Pasteuria</i> sp.	<i>Heteroderagoettingiana</i>	[35]
<i>Pasteuria</i> sp.	<i>Hoplolaimusgaleatus</i>	[36,37]
<i>CandidatusP. usage</i>	<i>Belonolaimuslongicaudatus</i>	[14]
<i>Pasteuria</i> sp.	<i>Heteroderacajani</i>	[38]
<i>P. penetrans</i>	<i>Heteroderacajani</i>	[39]
<i>Pasteuriasp.</i>	<i>Tylenchulussemipenetrans</i>	[40-42]
<i>Pasteuria</i> sp.	<i>Trophonemaokamotoi</i>	[43]
<i>Pasteuria</i> sp.	<i>Tylenchorhynchuscylindricus</i>	[44]
	<i>T. annulatus</i>	[36]
	<i>T. maximus</i>	[45]
	<i>T. leviterminalis</i>	[46]
<i>Pasteuria</i> sp.	<i>Meloidogyne</i> sp., <i>Heteroderafici</i>	[47]
<i>Pasteuriahartimeri</i>	<i>Meloidogyneardenensis</i>	[16]
<i>CandidatusPasteuria</i>	<i>Bursilla</i> sp.	[48]
<i>aldrichii</i>		
<i>Pasteuriasp.</i>	<i>Rotylenchulusreniformis</i>	[49]

5.The life cycle of*Pasteuriapenetrans*

The *Meloidogyne* J2 stage is a nonfeeding, developmentally arrested, long-lived dispersal stage and can survive in the soil for weeks or even months on stored lipid reserves, and this is the nematode stage exposed to *P. penetrans* spores in the soil. The initial stage of the life cycle of *P.*

penetrans root-knot nematodes is the chance contact of endospores to the second (infective) stage juvenile, which occurs in the soil as the juvenile seeks a suitable host root. The life-cycle of *Pasteuria penetrans* commences when endospores attach to the cuticle of second-stage juveniles of *Meloidogyne* sp. Germination of the spores usually takes place between 6 and 12 days after spore-encumbered juveniles enter the root and begin to feed, but before they moult to the third stage. The endospores do not germinate until the J2 has entered the plant root and established a feeding site. The germ tube emerges through a central opening in the basal attachment layer of the endospore and penetrates the nematode cuticle and enters the hypodermal muscle tissue and pseudocoelom where it produces mycelial colonies. The process of penetration seems to be enzymatic. After entering the pseudocoelom of the nematode, the germ tube develops into a microcolony consisting of a dichotomously branched septate mycelium. Daughter colonies form when the intercalary cells in the microcolony lyse. The colony forms fragmentations, the terminal cells of the fragmentation enlarge and undergo sporogenesis. Eventually, quarters and doublets of developing sporangia predominate in the nematode body cavity and finally separate into a single sporangium containing an endospore. The mature endospores (10^6 spores per individual female) are released into soil when the plant root with its complement of parasitized root-knot nematode female decomposes. The life-cycle is completed when the female is destroyed and egg production is prevented. The infective spores are released into the soil where they remain dormant until they attach to J2 and the cycle is repeated. Once a spore-encumbered juvenile has invaded a root, it will establish a feeding site and apparently normal development will continue. Sometime between the establishment of a feeding site and the second nematode molt, an endospore germinates and produces rhizoids which extend throughout the developing nematode. The stressed germ tubes look like rod-shaped bacteria that attach to the endospores on nematode

cuticles. The rhizoids eventually produce bacterial rods that undergo rapid exponential growth, resulting in degeneration of the nematode's reproductive tract and inhibition of egg production. The development of *P. penetrans* is temperature dependent . The minimal growth temperature is 17°C and at 20°C, *P. penetrans* requires 120 days to complete the life cycle.

6. Diagnosis of *Pasteuria* infection in nematodes

“Nematode parasites of the *Pasteuria* group are often overlooked because their presence on or within nematodes can only be seen under a microscope at more than 100× magnification. This may be an impediment to their recognition in samples taken to a laboratory. When soil samples are processed for nematode extraction, juvenile or vermiform stages of the nematode species present may be recovered. These may have *Pasteuria* endospores attached to them if the bacterium is present in that soil, but spore attachment will only be seen if nematodes are observed under high power magnification. If a number of root-knot nematode juveniles are endospore encumbered, they may appear to aggregate into clumps: this is often a useful characteristic that can be noticed at lower magnifications. Infected female root-knot nematodes can be found in root systems but where the incidence of *P. penetrans* is low, and then the chance of detection is small. Infected females do not produce egg masses, they appear dense and cream coloured in contrast to healthy females which become partially translucent as they mature and produce egg masses” [50,62,75].

7. *Pasteuria* spore structure

Pasteuria spp. transmission occurs after the exosporium coated endospores are dispersed and activated in the nematodes environment. Once free from the exosporium, the non-motile endospores passively adhere to the cuticle of nearby susceptible hosts moving in soil. The endospores are the dominant stage of the bacterium, resistant to adverse conditions such as high

temperature or desiccation, and may remain viable for a decade or more .The endospore is generally structured in a central, multilayered core (the true endospore) with surrounding layers of epicorticalparasporalfibres and outer episporic coats. The endospore cytoplasm is surrounded by a plasma membrane and a number of concentric walls with different electron densities. These include the cortex, an electron-dense layer directly in contact with the protoplasm membrane. The cortex is responsible for properties such as durability and resistance. The inner and outer layers are surrounded by a peripheral epicortical layer. The endospores have a typically rounded or cup-like aspect. This ‘aerodynamic’ shape allows resistance to the forces produced on their surface by the moving host. The endospore structure and shape (endospore diameters) appear relatively conserved among the nematode parasitic lineages, but differences may be observed among species, concerning the inner and outer core layers and the organization of the episporicfibres. The endospore activation and induction to germinate, however, may be independent from the host metabolism. At germination, the peg extruded from the central core penetrates the host cuticle and hypodermis. The factors triggering germination may be related to the biochemical changes occurring at the host-endospore interface. Germination has been also observed in endospores adhering to already parasitized nematodes, such as juveniles of *Heterodera goettingiana* or specimens of *Tylenchorhynchuscylindricus*, that were already filled with propagules, originated by a previous parasitic cycle. After germination, the vegetative stages fill, at various extents, the host body. It originates further cells arranged in dichotomic, branched and septate mycelium-like thalli, which spread the infection inside the host by fragmentation and eventually originates a sporulation phase. In *Pasteuria hartismerii*, the endospore precursors are arranged in tetrads and then pairs or in clusters. They show an asymmetric cell division, in which the endospore matures inside the enlarged terminal cell,

whose envelope forms the exosporium, maintained until release from the host. Thus the sporulation is completed inside the host, usually after partial or total consumption of its body content, resulting in a dramatic reduction of its fecundity and reproductive capacity.

8.Host specificity

“If *Pasteuria* is to be deployed successfully as a biological control agent, an understanding of its host specificity is fundamental. The majority of this research has investigated the bacterium’s interaction with the economically important root-knot nematodes, *Meloidogyne* spp., in which endospores exhibit a high degree of host-specific adhesion, where endospores are capable of attaching to one population of root-knot nematodes but not another even from the same phylogenetic clade”[50].“Moreover, the specificity of *P. penetrans* isolates may be a response to nematode populations rather than to nematode species. The number of spores attaching to *H. cajani* and *G. pallida* were not significantly different, but there were considerably more inverted endospores on *G. pallida* than on *H. cajani*. This suggests that the receptor(s) involved in attachment to the cuticle of the two species of nematode are different. The processes associated with the initial binding of the endospores of *Pasteuria* spp. to their respective hosts have been explored by several laboratories by using biochemical and immunological methods. These studies have led to a model in which sugar moieties on the surface of the endospores may be responsible for protecting endospores from extracellular proteolytic digestion and therefore may have relevance for endospore survival in the soil. Earlier work suggests they may also have a functional role in binding to a lectin-like receptor on the cuticle of the nematode host”[51].“The fibres surrounding the *Pasteuria* spore core are thought to be responsible for the host adhesion and specificity. These fibres were shown to be beta-mercaptoethanol (BME)-soluble glycoproteins containing a high level of *N*-acetylglucosamine, distinguished by their electron

densities is thought to be involved in adhesion by interacting with a receptor on the nematode cuticle. Genome sequencing of *Pasteuria* suggests that there is an array of diverse glycosylated collagens that form a 'hair-like nap' on the surface of the endospores that are responsible for endospore specificity through a 'Velcro-like' attachment mechanism to the nematode cuticle" [52-55]. Recently, the use of transcriptome analysis combined with RNAi knockdown approaches has revealed several nematode genes, in particular, Mi-FAR1 and a mucin-like gene [56] which modulate endospore adhesion on the nematode side of the interaction. "As there is a greater density of these collagen like fibres on the concave surface of the endospore than on the convex surface, there is possibility of other attractive forces (electrostatic interactions) in the binding process" [53,57-59]. "Host specificity of spore attachment varies in robustness within *Pasteuria* spp. populations, ranging from cross-genera to race-specific" [60]. "This suggests the existence of some degree of plasticity in host recognition, and the presence of mechanisms aside from host recognition and attachment promulgate infection" [60].

9. Factors affecting efficacy of *Pasteuria* spp.

- *P. penetrans* spores are non-mobile and so their attachment to nematode is dependent on the chance contact between nematodes and spores in the soil. Spore density and distribution influences on the migrated nematodes.
- A number of *Pasteuria* cells are lost during the sporulation phase.
- The time spent in soil by the endospore and required for parasporal fibers exposure.
- The time period required for endospore activation and germination,
- The removal of propagules by wind or soil water.
- The possible feeding of other soil organisms on resting endospores.

- A high and constant level of food source (plant nutrition) may balance nematode mortality by enhancing nematode reproduction.
- Climatic conditions or temperature has been shown to influence *P. penetrans* parasitism. Temperature affects endospore attachment, germination, pathogenicity, and endospore production. The minimal developmental temperature of *P. penetrans* was determined as 17°C, with optimal growth temperature between 28°C and 35°C. An Indian isolate of *P. penetrans* that infects both *Heterodera* spp. and *Meloidogyne incognita* completed its life cycle in *M. incognita* in 49 days at 10°C to 17°C [61]. However, preheating above normal temperatures (60°C) significantly increased attachment to *M. javanica* but reduced infection of *P. penetrans* [62].
- Soil texture - the degree of porosity for attachment and infection and the presence of clay has been shown to improve retention of spores in the upper soil profile.
- Soil moisture requirement for endospores attachment and development. It is possible that oxygen depletion in wet soil inhibits respiration, resulting in an inhibition of development of both the nematode and the bacterial parasite.
- “The endospore surface has a net negative charge, which was greatest at neutral pH and was reduced with a change of pH away from neutral. Electrostatic forces between the nematode cuticle and the endospore surface oppose attachment because the charges on nematode cuticle also were negative. The attachment of the sonicated endospore was higher per J2 at pH 7 in tap water than in distilled water” [63].

10. Biocontrol potential of *Pasteuria* spp.

Numerous studies have established the causal effect of *Pasteuria* spp. in reducing plant parasitic nematode populations and increasing crop yields (Table 2).

Table 2. Bioefficacy of *Pasteuria* spp.

<i>Pasteuria</i> spp	Nematode	Crop	Efficacy	Reference
<i>P. penetrans</i>	<i>M. javanica</i> / <i>M. incognita</i>	tomato	reduced root galls	[64]
<i>P. penetrans</i> @ 100,000 endospores /g of soil	<i>M. arenaria</i>	peanuts	Root gall reduction 60% Pod gall reduction 95%	[22] [65]
<i>Pasteuria penetrans</i> @ 100,000 and 200,000 endospores/g medium	<i>Meloidogyne arenaria</i>	tomato oriental melon	reduction in root galling and egg mass numbers	[66]
<i>P. penetrans</i> @ 1.5×10^5 endospores/cm ³ to 3 $\times 10^5$ endospores/cm ³ of transplant mix applied at seeding	<i>M. incognita</i> <i>M. arenaria</i>	tomato and cucumber snapdragon	87% reduction in total nematode eggs	[67] [68]
<i>Pasteuria</i> sp.	<i>M. incognita</i>	<i>Hosta</i> spp	reduction in	[69]

@10 ⁵					
endospores/cm ³ of					
soil					
<i>P. penetrans</i> @ 1 ×	<i>M. incognita</i>	Okra	35%	[70]	
10 ⁴ / g of soil.			reduction in		
			number of		
			galls		
			41%		
			reduction in		
			number of		
			egg masses		
<i>P. penetrans</i>	<i>Heterodera</i>	spp. brinjal	Nematode	[61]	
	<i>M. incognita</i>		population		
			reduction		

11. Presence of *P. penetrans* in suppressive soil

P. penetrans has been considered as the primary microorganism responsible for soil suppressiveness to root-knot nematodes in many fields. In old vineyards were infested with *P. penetrans* having fewer root-knot nematodes than in young vineyards without the bacterium. The reproductive capacity of *M. javanica* was much lower in soil infested with *P. penetrans* than in non-infested soil [71]. *Pasteuria* sp. caused population decline of *Heterodera elachista* in monocultured upland rice. Suppressiveness may be induced by some

agronomic practices such as planting crops susceptible to root-knot nematodes in succession or by crop rotation with alternate poor hosts.

12. Mass culture and Commercialization

The obligate nature of the bacterium's life style and its host specificity has made it difficult to develop *P. penetrans* into a commercial product. For these reasons, a genomic approach has recently been used to help understand the mechanisms of parasitism of *Pasteuria* spp. and the possible exploitation of their ecological niche. Stirling and Wachtel [72] were able to "produce large numbers of spores by inoculating tomato with infected *Meloidogyne* juveniles. Dried tomato roots were then milled into a powder containing *Pasteuria* spores". "Such production system might be improved by culturing the nematode and pathogen in excised or transformed root cultures, but commercial use of the pathogen will most likely require an *in vitro* method of cultivation which is not successful" [73]. Previous research with an *in vivo* produced isolate of '*Candidatus Pasteuria* sp. *gusiae*' in field plots demonstrated a reduction of sting nematode 13 months after inoculation [14,74]. However, the number of spore produced depends on the optimum temperature and time of harvest, nematode inoculum density in the host plant, and host plant susceptibility to the nematode. Although this method may not produce the amounts needed for treating large areas it is feasible for smallholder crops, spot treatments to perennial crops, and protected crops. The isolate Pn1 of *P. nishizawae* has been largely used in USA, Canada and Brazil with the commercial name ClarivaTM (Syngenta).

13. Conclusion

Pasteuria spp. have many advantages like longevity of the endospores in soil, compatible with other biocontrol agent and resistance to various nematicides, fungicides and adverse environment [75]. Now-a-days they are also used as a model system to study coevolutionary trade-offs

between hosts and parasites .However, increased understanding of the molecular basis of the various pathogenic mechanisms of the bacteria could potentially enhance their value as effective biological control agents. It is necessary to evaluate development within the host as a requisite to assigning host parasite relationships with new species or strains of *Pasteuria* with understanding of the impacts of soil properties and management practices in the field.

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