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Pasteuria spp.: Systematics and Phylogeny of These Bacterial Parasites of Phytopathogenic Nematodes[1]

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Abstract: *Pasteuria* spp. include endospore-forming bacterial pathogens of cladoceran crustaceans and plant-parasitic nematodes. Propagation of these nematode pathogens requires attachment of soilborne endospores to nematode hosts, infection, growth, sporulation, and release of endospores to repeat the cycle of infection and propagation. The ability of these bacteria to suppress the levels of plant-parasitic nematodes in the field has made them particularly promising candidates for biocontrol of nematode diseases of plants. Genes encoding 16S ribosomal RNA have been sequenced for the cladoceran (water flea) parasite and type species, *Pasteuria ramosa,* and for *Pasteuria* spp. isolated from root-knot (*Meloidogyne arenaria* race 1 and *Meloidogyne* sp.), soybean cyst (*Heterodera glycines*), and sting (*Belonolaimus longicaudatus*) nematodes. These have provided a phylogenetic basis for their designation to a distinct clade within the family *Alicyclobacillaceae* of the gram-positive endospore-forming bacteria. Two apparent biotypes of *P. penetrans* demonstrating a host preference for different *Meloidogyne* spp. showed identical 16S rDNA sequences, suggesting host-recognition evolves within a given species. The sequences of genes encoding sporulation transcription factors, *sigE* and *sigF,* from *P. penetrans* biotype P-20 show different phylogenetic relationships to other endospore-forming bacteria, supporting their application to further discriminate *Pasteuria* spp. and biotypes. Distribution of an adhesin-associated epitope on polypeptides from different *Pasteuria* isolates provides an immunochemical approach to differentiate species and biotypes with specific host preferences. Application of bioinformatics to genomic data, as well as further characterization of the biochemical basis for host recognition, will facilitate development of *Pasteuria* spp. as benign alternatives to chemical nematicides.

Key words: biological control, cladocerans, endospore, *Meloidogyne arenaria,* nematode, *Pasteuria penetrans, Pasteuria ramosa,* phylogeny.

As obligate parasites of plant-parasitic nematodes, species of the endospore-forming genus *Pasteuria* have arguably become the most promising agents for biocontrol of many nematodes that cause extensive damage to field crops, vegetables, turf grasses, and ornamentals. A relationship has been established between levels of soilborne endospores of *Pasteuria* spp. and suppression of nematode infection of plant roots (Chen and Dickson, 1998). The basis for their biocontrol potential is due in part to the fact that the propagation of the bacteria within the pseudocoel of the infected nematode host results in a loss of fecundity. The nematode host serves to amplify the infective endospores that are released into the soil to repeat a cycle of infection and amplification. This process has been studied most extensively in the infection of root-knot nematodes by *P. penetrans* as depicted in Figure 1.

The binding of a single endospore to a second-stage juvenile (J2) may be sufficient to allow infection and propagation of the bacteria. The infection process has not been differentiated from germination of the spore, and germination may in fact trigger infection. After germination the cells proliferate through a series of developmentally distinct vegetative stages prior to the onset of endospore formation. Discrete stages of spore formation comparable to those found in other endospore-forming bacteria occur (Chen et al., 1997a; Sayre and Starr, 1988). As many as 2×10^6 mature endospores are released as the female nematode ruptures. If each gall contains a *Pasteuria*-infected nematode, a single plant may release 10^7 to 10^8 endospores into the soil.

As the density of soilborne endospores of a particular species and biotype of *Pasteuria* increases, those soils may become more suppressive to a population of nematodes susceptible to infection by that particular species and biotype of *Pasteuria.* In addition to the loss of fecundity, the attachment of a significant number of endospores to the cuticle of a J2 may encumber the nematode to an extent that it is unable to reach and infect the root tip of a prospective host plant. While the number of bound endospores that effectively encumber a soilborne juvenile is known only approximately, a number greater than 40 likely will prevent root infection (Stirling, 1984). As shown in Figure 2, more than 100 endospores may bind to the cuticle of the nematode, predicting that a significant range in the density of soilborne endospores that will effectively suppress the infection of target plants by their respective parasitic nematodes.

The relationship between the levels of soilborne *Pasteuria* endospores and suppression of infection of plants by *Meloidogyne* spp. has been particularly well documented (Chen et al., 1997b; Weibelzahl-Fulton et al.,

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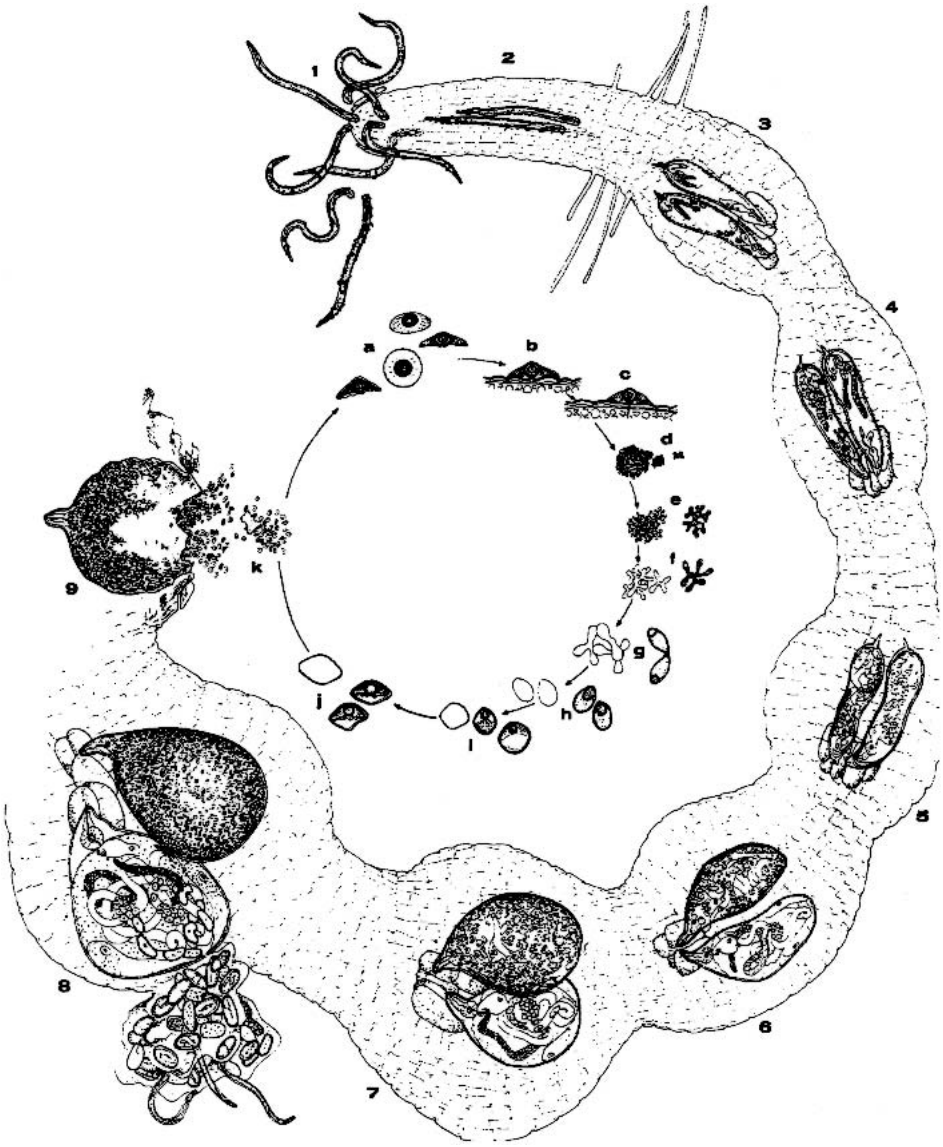

FIG. 1. The life cycle of a root-knot nematode, *Meloidogyne* sp., with (inner portion of outer circle) and without (outer portion of outer circle) infection by *Pasteuria penetrans*. The inner circle depicts the stages of *P. penetrans* following infection to release of endospores: (1) Entry of soilborne second-stage juveniles into root tip of plant host; (2) intracellular migration of J2 in cortex; (3) establishment of feeding site in vascular system (attached *P. penetrans* endospores germinate and infect); (4) molt to third-stage juvenile; (5) molt to fourth-stage juvenile; (6) development of young females; (7) egg formation in healthy female; little or no egg formation in female infected with *Pasteuria;* (8,9) healthy female releases egg masses; infected female releases mature endospores. The inner circle describes the life cycle of the *P. penetrans:* (a) mature endospores in the soil; (b) endospore attached to the cuticle of *Meloidogyne* sp.; (c) germination and formation of infection tube; (d) appearance of a microcolony of dichotomously branched septate mycelium; (e) release of colonies through lysis of intercalary cells, with rapid cell proliferation; (f) further differentiation with increase in numbers of colonies, containing larger cells; (g) formation of quartets and then diads that represent sporangia for onset of sporulation; (h) formation of forespore within mother cell; (i) engulfment and maturation with formation of parasporal fibers; (j) completion of maturation to form disk-shaped spores with polar orientation of parasporal fibers; (k) release of mature endospores into the environment (Weibelzahl-Fulton, 1998).

1996). In the case of *P. penetrans* P20, 10,000 endospores/g of soil have been demonstrated to effectively suppress the infection of peanut plants by *Meloidogyne arenaria* race 1 (Chen et al., 1996). There is evidence that most, if not all, plant-parasitic nematodes propagate a species of *Pasteuria* that restricts the fecundity of the nematode host. It is thus not surprising that these bacteria have received ever-increasing attention, with several research groups addressing the etiology and biochemistry of the basis for developing parasitic relationships with their respective hosts. Comprehensive reviews have been published on the genus *Pasteuria* and the species and isolates that have been implicated in the control of plant-parasitic nematodes (Chen and Dickson, 1998; Sayre, 1993; Sayre and Starr, 1988). The discussion presented here will extend these reviews to consider the phylogenic relationships of these bacteria as now discerned through DNA sequencing and immunochemical properties of spore adhesins.

Definition of genus Pasteuria: The type species of the

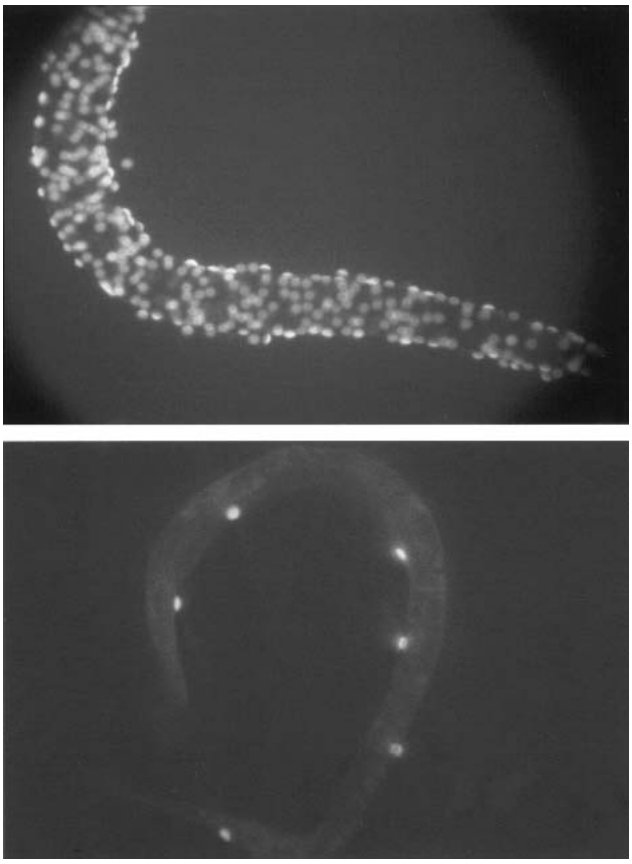

FIG. 2. Photomicrographs of second-stage juveniles of *Meloidogyne arenaria* race 1 with variable numbers of attached endospores of fluorescein-labeled endospores of *Pasteuria penetrans* P20 (magnification 1,000×). Endospores were reacted with fluorescein isothiocyanate (Charnecki et al., 1996) and incubated with freshly hatched second-stage juveniles (J2). Top panel: individual J2 with more than 350 endospores attached, where the attachment of more than 20 may encumber the nematode and suppress infection of a root. Bottom panel: individual J2 with six endospores attached where a single attached endospore may infect the nematode at an appropriate stage of development in the infected plant.

genus *Pasteuria* is *Pasteuria ramosa* (Metchnikoff, 1888). This was assigned by M. Elie Metchnikoff in 1888 to an endospore-forming bacterial parasite of water fleas (cladoceran crustaceans), *Daphnia magna* and *Daphnia pulex* (Metchnikoff, 1888). With his interest in other endospore-forming bacteria and collaboration with Louis Pasteur on the study of the anthrax bacillus, Metchnikoff identified the formation of a bacterial endospore as a late developmental stage following a unique vegetative growth phase. This phase was defined as starting with a mass that transformed into a "cauliflower"-like colony of cells that undergo successive longitudinal divisions and generate microcolonies with successively smaller numbers of cells. Thus Metchnikoff accepted the formation of the endospore rather than the mode of cell division as a basis for relating this pleiomorphic bacterium to endospore-forming bacteria that typically divide in the vegetative stage of growth by binary fission. The identity of this parasite as an endospore-forming bacterium was not generally accepted until nearly a century later, when the similarities to parasitic bacterial pathogens of plant-parasitic nematodes were considered (Sayre et al., 1983).

From the early 20th century, observations of microbes associated with plant-parasitic nematodes led to an interest in their potential role as biocontrol agents. Mankau (1975) applied electron microscopy to define some of these organisms at the structural level. He noted that the parasites found in the root-knot nematode *Meloidogyne javanica* divided with the formation of cross-walls typical of bacteria. Moreover, the spores that were formed resembled the endospores that were characteristic of *Bacillus* spp., and he designated these as belonging to a new species, *Bacillus penetrans* n. comb. Soon thereafter, Sayre and Wergin (1977), also using electron microscopy, determined the similarity of an endospore-forming bacterial parasite from *M. incognita* to *P. ramosa* originally described by Metchnikoff in 1888. A bacterial parasite of the cladoceran crustacean *Moina rectirostris* also was morphologically characterized and shown to be very similar to the *P. ramosa* described by Metchnikoff, and these new parasites of cladocerans now served as extant bacteria for comparison with parasites of plant-parasitic nematodes (Sayre et al., 1977; Sayre et al., 1979). The appearance of elongated and branching septate cells resembled the mycelial structures common to bacteria in the *Actinomycetales* compared to the members of *Bacillaceae* (Sayre et al., 1983). Based exclusively on morphological properties of forms during vegetative growth and endospore formation, the parasite of the root-knot nematodes was finally designated and accepted as *P. penetrans* (ex Thorne, 1940) (Sayre and Starr, 1985).

Diversity of Pasteuria spp. parasitic to plant-parasitic nematodes: The species *P. penetrans* was originally considered an endospore-forming bacterial parasite with a very broad host range, infecting a number of different genera of plant-parasitic nematodes (Sayre and Starr, 1985). Further examination of *Pasteuria* spp. growing and maturing in different genera of plant-parasitic nematodes has led to the description of species differentiated on the basis of morphometric properties and host specificity. Until recently, the genus *Pasteuria* was considered to include three species—*P. penetrans* (Sayre and Starr, 1985), *P. thornei* (Starr and Sayre, 1988), and *P. nishizawae* (Sayre and Starr, 1988; Sayre et al., 1991)—as obligate parasites of plant-parasitic nematodes with respective preferences for species of root-knot, lesion, and cyst nematodes. Using the same criteria for species assignment, *Pasteuria* sp. S1 obtained from the sting nematode *Belonolaimus longicaudatus* has been characterized to an extent that it may be considered another species (Giblin-Davis et al., 2001). The prevalence of structures resembling the defined endospores of *P. penetrans* on the free-living soilborne J2 forms of many species and genera suggests that plant-

pyrasitic nematodes serve as hosts for many different *Pasteuria* spp. (Chen and Dickson, 1998; Sayre and Starr, 1988). The inability to culture and archive any of the designated species outside of their respective hosts has led to the proposed designation of *Candidatus* status for each species, and the S1 isolate from *Belonolaimus longicaudatus* has been designated *Candidatus* Pasteuria usgae (Giblin-Davis et al., 2003).

Phylogenetic relationships based on 16S rDNA sequences: The inability to cultivate *Pasteuria* spp. outside of their hosts has made phylogenetic comparisons based on sequencing PCR-generated products the most productive approach to further define this important genus. The phylogenetic relationships of several *Pasteuria* isolates have now been established through sequence comparisons of genes encoding 16S rRNA. The first of these was obtained from *P. ramosa* by Ebert et al. (1996) and clearly established the relationship of the cladoceran parasite to other gram-positive endospore-forming bacteria, with greatest similarity to members of the family *Alicyclobacilliaceae.* This work also led to the isolation of *Daphnia* parasites from ponds in Russia and the United Kingdom, and thus provided an additional basis for the selection of *P. ramosa* for the type species of the genus. This was followed by the sequencing of 16S rRNA genes from two isolates of *P. penetrans,* P-20 and P-100, derived from root-knot nematodes, *Meloidogyne* spp. (Anderson et al., 1999), an isolate from soybean cyst nematode *Heterodera glycines,* designated as Hg *Pasteuria* (Atibalentja et al., 2000), and an isolate from the sting nematode *Belanolaimus longicaudatus,* designated as *Pasteuria* S1 (Bekal et al., 2001). These studies further confirmed the phylogenetic relationship of these to *P. ramosa* as a distinct clade within the gram-positive endospore-forming bacteria, most closely related to a clade including *Alicyclobacillus acidocaldarius, Alicyclobacillus cycloheptanicus,* and *Bacillus tusciae.* The phylogram in Figure 3 demonstrates the relatedness of 16S rDNA sequences that have been obtained for all *Pasteuria* spp. for which there is information on morphometrics and host preference. Included are relationships to other groups of endospore-forming bacteria that have been previously compared (Anderson et al., 1999; Atibalentja et al., 2000).

The 1,378 base pairs between sequence positions 31 and 1,409 in the 16S rRNA genes of *P. penetrans* P20 and P100 isolates were identical (Anderson et al., 1999). The *P. penetrans* P20 isolate showed a preference for

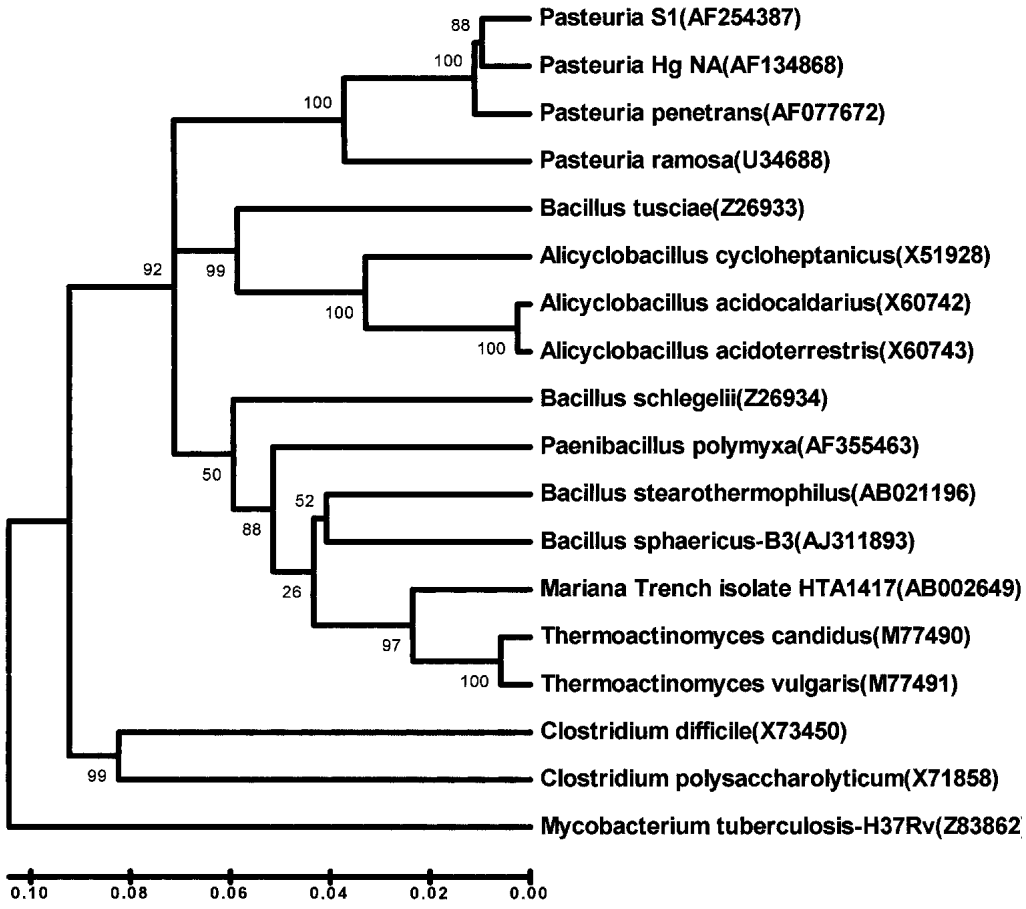

FIG. 3. Phylogenetic relationship of *Pasteuria* spp. to other endospore-forming bacteria based on nucleotide sequences of genes encoding 16S rRNA. The phylogenetic tree was constructed from rDNA sequences registered with GenBank, using MEGA2 (Kumar et al., 2001) with the Neighbor-Joining method (Saitou and Nei, 1987). The scale represents a relative evolutionary distance, and the whole numbers are bootstrap values for 1,000 analyses.

infection of *Meloidogyne arenaria* race 1, while the P100 isolate showed a preference for *M. arenaria* race 2, *M. javanica,* and *M. incognita.* Thus, preference for a particular host within the same genus would likely reflect a biotype rather than a species difference in the parasitic bacteria, and both the P20 and P100 isolates may be considered strains or biotypes of *P. penetrans.* The 16S rDNA sequences for *P. penetrans* P20, Hg *Pasteuria,* and *Pasteuria* S-1 isolates show 96% or less sequence similarity when compared to each other, and 93% similarity when compared to *P. ramosa* (Anderson et al., 1999; Giblin-Davis et al., 2003). A 16S rDNA sequence similarity of 97% or greater is currently accepted as a basis for delineating a bacterial species (Amann et al., 1992; Hagstrom et al., 2002), supporting the designation of *P. penetrans,* Hg *Pasteuria,* and *Pasteuria* S-1 as different species. The phylogram in Figure 3 supports a slightly closer evolutionary relationship between Hg *Pasteuria* and *Pasteuria* S-1 than between either of these and *P. penetrans.* The discovery of the Hg isolate from *Heterodera glycines* (Noel and Stanger, 1994), also referred to as the NA or North American strain, and the distinguishing sequence of its 16S rDNA (Atibalentja et al., 1998) supports its designation as the previously described species, *P. nishizawae* (Noel, pers. comm.). The *Pasteuria* sp. S1, with its host preference for *Belanolaimus longicaudatus,* as well as distinctive morphological properties of developing endospores, clearly supports this as a previously undescribed species (Bekal et al., 2001; Giblin-Davis et al., 2001). Additional 16S rRNA gene sequences from environmental samples from Florida have been deposited with GenBank and suggest the existence of additional species of *Pasteuria* (Duan et al., 2002).

Phylogenetic relationship of P. penetrans to other endospore forming bacteria based on deduced amino acid of sporulation genes: As endospore-forming bacteria, *Pasteuria* spp. are expected to have and express genes encoding proteins involved in the sporulation process. Typical of these are several transcription factors, identified and characterized from *Bacillus subtilis,* that direct the DNA-dependent RNA polymerase to transcribe genes within the forespore or the mother cell as spore maturation occurs (Haldenwang, 1995; Kroos et al., 1999; Min et al., 1993). A diagram summarizing the temporal relationships between the expression of genes encoding these factors and sporulation is presented in Figure 4. In *B. subtilis,* sigma factor E in the mother cell is required for the expression of the gene encoding sigma K that is required for transcription within the forespore. Genes required for the engulfment of the forespore by the mother cell are expressed under the combined control of both of these factors, and the subsequent formation of sigma factor G. Sigma G regulates expression of genes in the forespore required for DNA packaging and protection against environmental insult, and for later germination. Sigma factor K is the last of the transcription initiation factors to be formed and, along with sigma E, regulates the formation of components and assembly of the cortex and coat. Additional regulatory factors include SpoIIAB, the product of the *spoIIAB* gene that binds to sigma F and sigma G in the forespore. The release of the SpoIIAB protein is required for sigma F and sigma G to become active.

The genes encoding the sporulation factors are clustered in groups located in different regions of the *B. subtilis* genome (Kunst et al., 1997). We have chosen to first determine the sequences of *sigE* and *sigF* genes analogous to those that have been defined within the *B. subtilis* genome because they are found in syntenic clusters in the sequenced genomes of *B. subtilis* and *B. halo-*

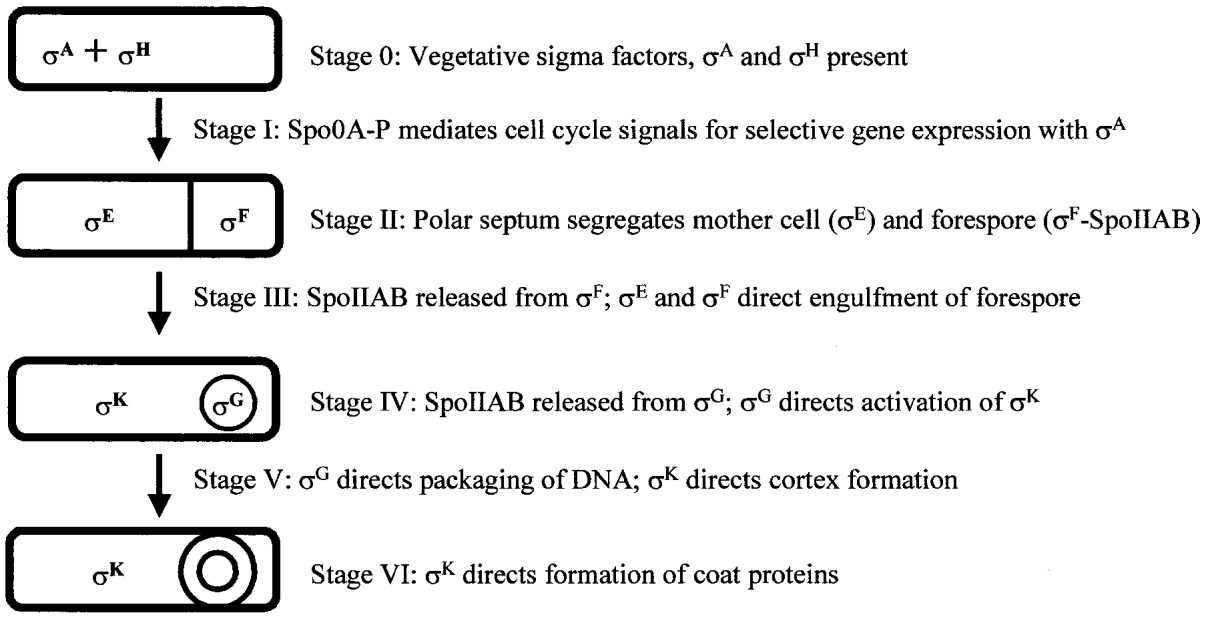

FIG. 4. Sequential expression of genes encoding transcription factors directing spore formation in *Bacillus subtilis.*

durans (Takami et al., 2000). As shown in Figure 5, the *sigE* and *sigG* genes are located in a cluster *spoIIGA, sigE, sigG* at a relative position of 135 degrees on the *B. subtilis* chromosome; the *sigF* and *spoIIAB* genes are located in a cluster *sigF, spoIIAB, spoIIAA* that is located at 150 degrees. Because these genes are essential for sporulation and are conserved in all gram-positive endospore-forming bacteria, the amino acid sequences can be used to establish phylogenetic relationships among *Pasteuria* spp. and other endospore-forming bacteria.

The approach to sequence and compare these genes used identified regions with conserved nucleotide sequences (identified in accessioned sequences from GenBank), synthesis of degenerate oligonucleotide primers, and amplification by PCR. Amplified products were resolved on agarose gels, cloned, and sequenced. The comparative phylogenetic relationships, based on the translated sequences of the *sigE* and *sigF* genes, are shown in Figures 6 and 7, respectively. The number of bacteria for which these genes have been sequenced is much less than that for which 16S rDNA sequences have been obtained. The relationship of *P. penetrans* to *Paenibacillus polymyxa,* as seen for the amino acid sequence of the sigma F transcription factor, is closer than that seen in the comparison of amino acid sequences for the sigma E transcription factor. While the genes encoding these proteins have not been sequenced for the *Alicyclobacillus* spp., which are the closest relatives based on 16S rDNA sequences, the different amino acid sequence relationships observed for sigma E and sigma F suggest a variability that may extend into the different species of *Pasteuria,* and should provide more informative phylogenetic comparisons than those provided by 16S rDNA comparisons. The sequence relationships of sporulation genes in *P. penetrans* must be considered preliminary pending publication along with sequences from other *Pasteuria* spp. Unique sequences for these and other *Pasteuria* genes

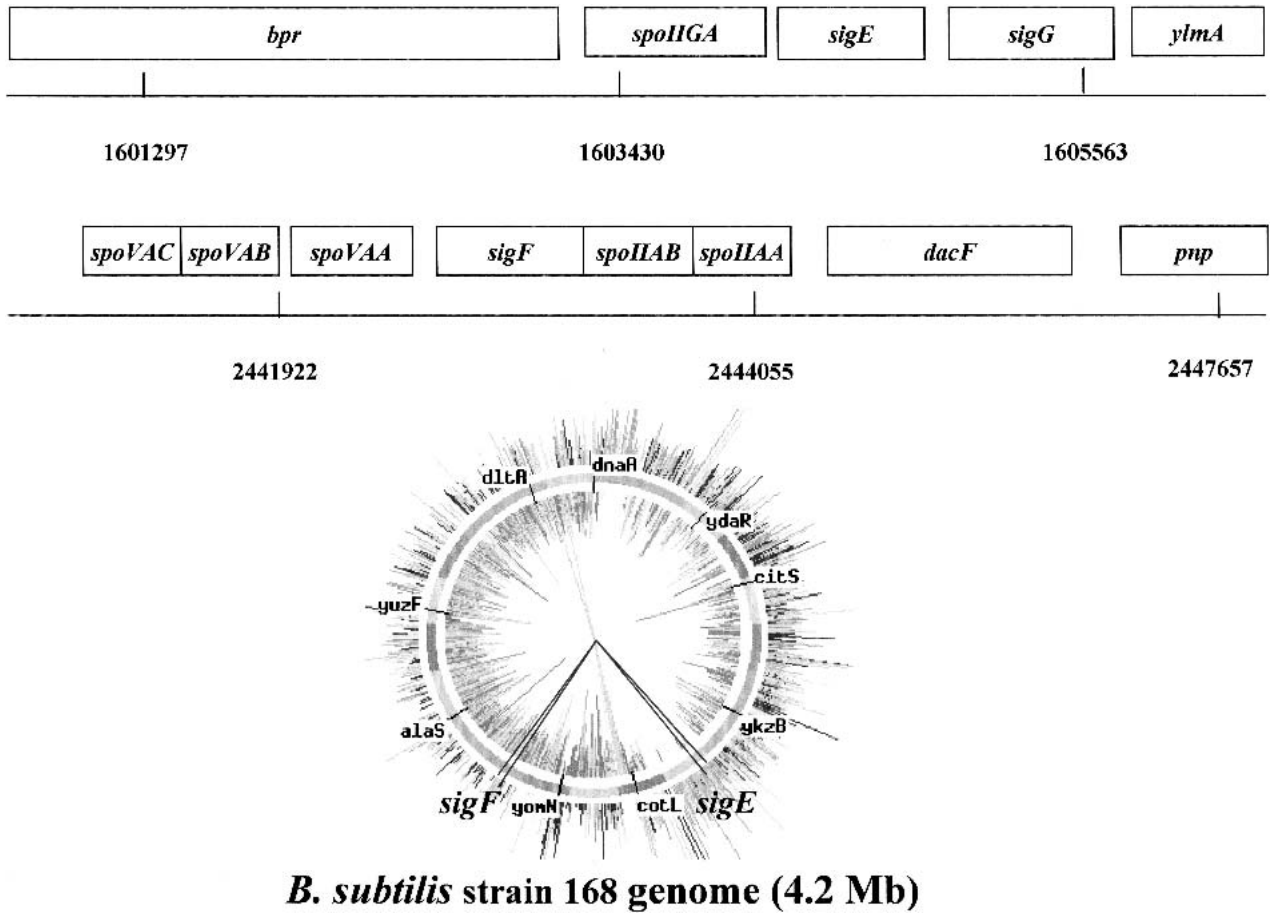

FIG. 5. The organization of gene clusters encoding *sigE* and *sigF* in the chromosome of *Bacillus subtilis.* The organization of genes is depicted as presented in the menus for the identification of genes within the microbial genome database archived by the National Center for Biotechnology Information. The enumeration of base pairs starts at the top of the circular depiction of the genome at *dnaA,* 410-1750 bp, the initiation site of chromosome replication, and proceeds clockwise through *rpmH,* 4214463-4214597 bp, encoding ribosomal protein L34 (not shown). The lines describing sectors define the regions of the *B. subtilis* genome encoding *sigE* and its neighboring genes from 1579165-1627695 bp, *sigF* and its neighboring genes from 2417657-2466187 bp. The synteny relationships of *spoIIGA, sigE, sigG* and *sigF, spoIIAB, spoIIAA* are identified on an expanded scale. For these two sets of sporulation genes, these relationships were the same in the genomes of *B. subtilis* and *B. halodurans,* and served as a basis for the selection of degenerate primers for PCR amplification, cloning, and sequencing of the *SigE* and *sigF* genes in *Pasteuria penetrans.*

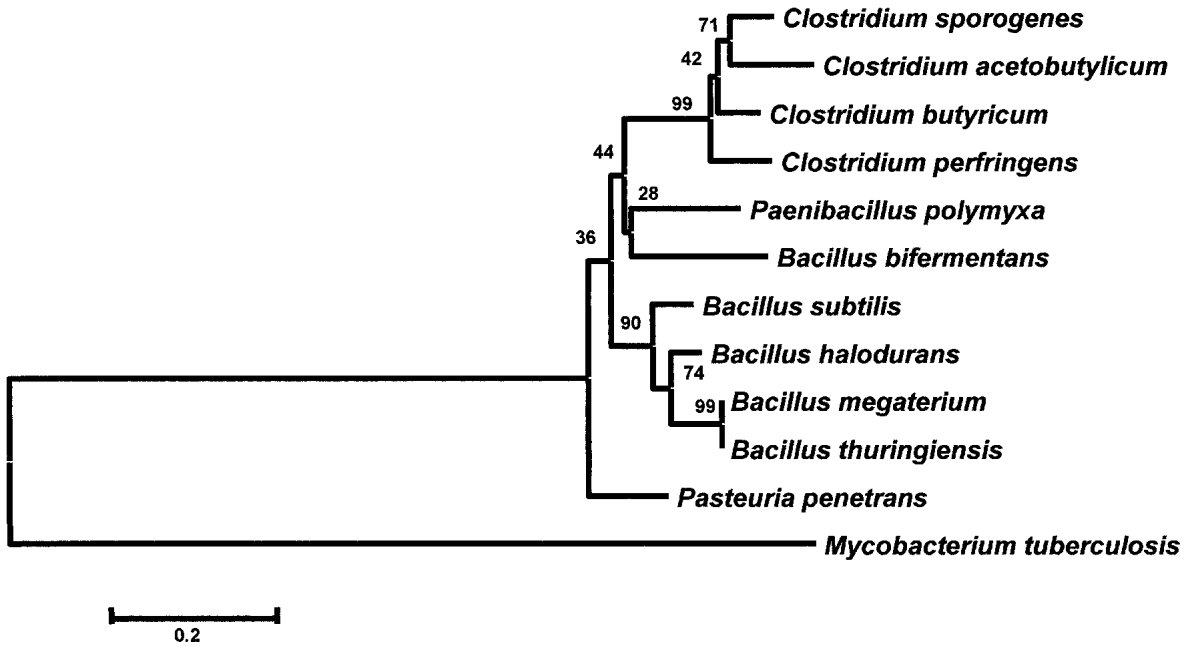

FIG. 6. The phylogenetic relationship of translated sequences of *sigE* gene from *Pasteuria penetrans* P20 and other bacteria. Partial nucleotide sequences for the *sigE* gene from *P. penetrans* P20 were obtained following cloning of PCR products using degenerate primers that were selected on the basis of conserved sequences within several different endospore-forming bacteria. Amino acid sequences were deduced from translated nucleotide sequences and compared with SigE sequences archived with SWISS-PROT or TrEMBL. The phylogenetic tree was constructed using MEGA2 (Kumar et al., 2001) with the Neighbor-Joining method (Saitou and Nei, 1987). The scale represents a relative evolutionary distance, and the whole numbers are bootstrap values for 1,000 analyses.

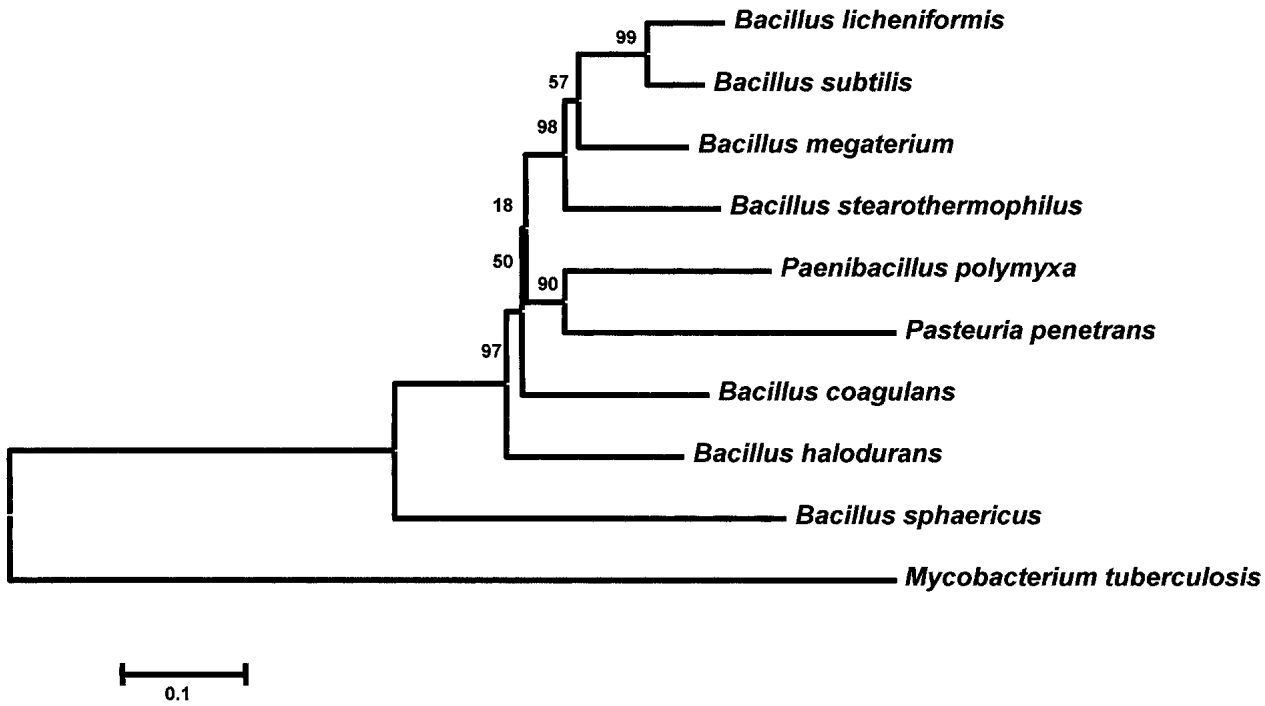

FIG. 7. The phylogenetic relationship of translated sequences of *sigF* gene from *Pasteuria penetrans* P20 and other bacteria. Partial nucleotide sequences for the *sigF* gene from *P. penetrans* P20 were obtained following cloning of PCR products using degenerate primers that were selected on the basis of conserved sequences within several different endospore-forming bacteria. Amino acid sequences were deduced from translated nucleotide sequences and compared with those from SigF sequences archived with SWISS-PROT or TrEMBL. The phylogenetic tree was constructed using MEGA2 (Kumar et al., 2001) with the Neighbor-Joining method (Saitou and Nei, 1987). The scale represents a relative evolutionary distance, and the whole numbers are bootstrap values for 1,000 analyses.

will be used to determine the levels of infection of nematodes *in planta*. Because these are genes unique to endospore-forming bacteria, selected oligonucleotide sequences for different sporulation genes may be synthesized to serve as probes or PCR primers to detect and differentiate *Pasteuria* spp. and possibly even biotypes in the field.

Comparison of Pasteuria species-biotypes based on immunodetection of epitopes on endospore envelope proteins: As the initial step in the cycle of infection, the endospore of a *Pasteuria* sp. must chemically recognize and then bind to the cuticle of its nematode host. Recognition and initial binding likely constitute reversible events that may be considered part of an irreversible attachment process (Fig. 1). The molecular processes associated with the initial binding of the endospores of *Pasteuria* spp. to their respective hosts have been explored in several laboratories (Charnecki et al., 1998; Davies and Danks, 1993; Davies et al., 1992; Persidis et al., 1991; Stirling et al., 1986). This has led to a model in which a carbohydrate ligand on the surface of the endospore binds to a lectin-like receptor on the cuticle of the nematode host. The sequential interaction of ligands distributed about the surface of the endospore with receptors distributed over the cuticle of the nematode host may drive a cooperative process that results in the conversion of a reversible-to-an-irreversible association of an endospore with its nematode host. Additional factors, including hydrophobic interactions (Esnard et al., 1997; Spiegel et al., 1996), also may contribute to the essentially irreversible association that allows a nematode to accumulate the endospores on its surface as it seeks the growing root tips of a host plant.

The carbohydrate ligands may be viewed as chemical determinants on polypeptides that comprise the parasporal fibers of the developing endospore. The polypeptides bearing these have been designated as adhesins, and represent the gene products on the spore envelope that participate in the attachment process. Monoclonal antibodies generated against *Pasteuria* endospores have been used to detect polypeptides on the surface of endospores that play a role in the attachment of an endospore to its respective nematode host (Brito et al., 1998; Charnecki et al., 1998; Davies and Redden, 1997; Schmidt, 2002). The ability of these antibodies to inhibit attachment of endospores to the cuticle of J2 supports a role for these peptides in the attachment of the spore to the host, and has further supported their designation as adhesins. A particular monoclonal antibody has been generated against endospores of *P. penetrans* P20 that binds to an epitope shared by several polypeptides extracted from these endospores and resolved by SDS-PAGE. The epitope includes a putative β-1,4-linked N-acetyl-D-glucosamine residue and has been detected in extracts from endospores from all of the several isolates of *Meloidogyne*-infecting *Pasteuria* that have been examined (Charnecki et al., 1998). The absence of this epitope in endospores of other spore-forming genera of bacteria allowed the development of an immunoassay for the quantification of *Pasteuria* spp. in soils and *in planta* (Schmidt et al., 2003).

Immunoblot patterns of polypeptides extracted from endospores of different isolates provide an approach to differentiate endospores in relation to their source. Figure 8 shows an immunoblot that compares the epitope-bearing polypeptides extracted from endospores of different *Pasteuria* spp. or biotypes that share the epitope recognized by the monoclonal antibody against *P. penetrans* P20 endospores. The polypeptide epitope patterns are different for the *P. penetrans* spores compared to those obtained for the spore extracts from either the Hg *Pasteuria,* the *Pasteuria* S1, or from a *Pasteuria* sp. isolated from a spiral nematode. The different patterns suggest different supramolecular structures that may be the basis for the attachment of endospores of different *Pasteuria* species or biotypes to their respective nematode hosts. These patterns should prove useful diagnostic probes with which to distinguish between spore isolates in the field, and may complement DNA sequence probes for the detection of *Pasteuria*-infected nematodes *in planta*. At present these results must be considered preliminary findings pending further definition of

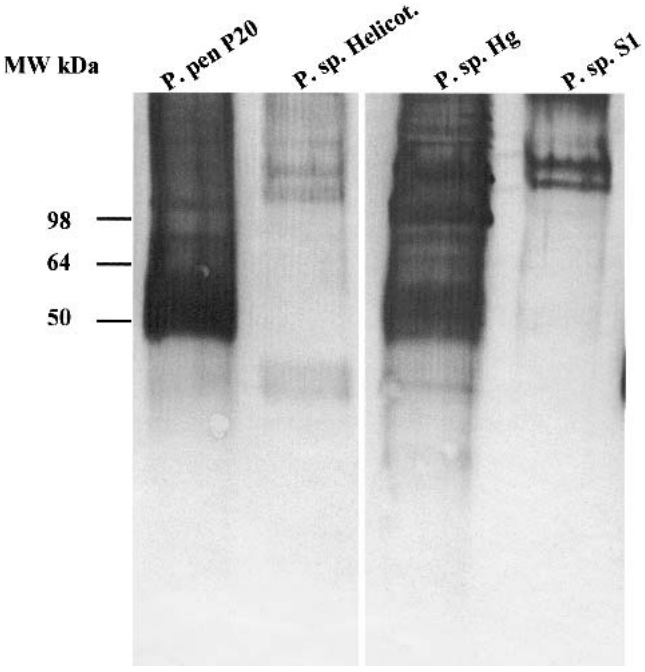

FIG. 8. Distribution of adhesin epitopes on endospores of *Pasteuria* spp. Proteins were extracted from mature endospores with 8 M urea, 0.04 M dithiothreitol, 0.00665 M CHES buffer, pH 10, subjected to SDS-PAGE, and detected on immunoblots with anti-P-20, IgM MAb in phosphate-buffered saline containing 0.2% Tween-20. Endospore extracts containing 600 ng protein in 20 µl were loaded into each well. Lane 1—*Pasteuria penetrans* P20 isolated from *Meloidogyne arenaria* race 1; Lane 2—*Pasteuria* sp. collected from *Helicotylenchus* sp.; Lane 3—*Pasteuria* sp. Hg. isolated from *Heterodera glycines;* and Lane 4—*Pasteuria* sp. S-1 from *Belonolaimus longicaudatus*. Molecular weight standards were run and stained in a separate lane to provide the indicated calibration values.

these patterns and their relationship to biotypes defined on the basis of host preference. Further details on the generation of the monoclonal antibody and the properties of the epitope it recognizes have been presented (Charnecki, 1997).

Application of genomics and bioinformatics in Pasteuria research: A goal of *Pasteuria* research of obvious practical benefit is to develop an effective biological control tactic as an alternative to the chemical control of plant-parasitic nematodes. The prospecting approaches that have been used to develop conditions for the propagation of *Pasteuria* spp. outside of the nematode host have not provided protocols that allow production of virulent endospores for field application. A logical approach to develop these is to sequence the genomes of one or more *Pasteuria* spp. with the expectation that bioinformatic methods will identify metabolic capabilities and limitations via comparison with the expanding genomic databases. A genomic-bioinformatic approach will be useful for the definition of the process of host recognition and attachment, as well as infection and germination. As the genomic approach is being pursued, the parsimonious approaches of selected gene and epitope typing of isolates of *Pasteuria* that infect different species of plant-parasitic nematodes will provide the information needed to decide which isolates will serve as species or biotypes for targeting particular species of nematodes.

The phylogenetic relationship of *P. ramosa* and the nematode-infecting *Pasteuria* spp. suggests common biochemical processes that have evolved in the parasitic relationships with the cladocerans and the plant-parasitic nematodes. Common components in the development of both are chitin polymers bearing the β-1,4-linked N-acetyl-D-glucosamine residues that are presumptive components of the epitope found on the surfaces of *Pasteuria* endospores. The role of the maturing female nematode in the synthesis and deposition of chitin in egg shells that encase the first-stage juveniles suggests a role for the maturing *Pasteuria*-infected female in the maturation of an adhesin that will program the endospore for its return to an accommodating host. Passage through the host may be necessary to provide a virulence determinant required for host recognition and attachment. It will thus be of interest to apply genomic-bioinformatic approaches to *Daphnia* spp. as well as *Meloidogyne* spp. infected with their respective *Pasteuria* spp. to further define the evolution of these parasitic relationships.

As naturally occurring parasitic bacterial pathogens with a narrowly selected host range, *Pasteuria* spp. lack the environmental concerns that limit development of other bacteria for suppressing nematode infestations. Even without the mass production and application of *Pasteuria* spp. as biocontrol agents, the knowledge we can gain with respect to host preference and virulence of different *Pasteuria* isolates, and their quantitative detection in the field, should allow the judicious application of chemical nematicides only where they are needed. The investment of time, talent, and resources may be all that is required to develop *Pasteuria* spp. as benign alternatives to chemical nematicides for the control of plant-parasitic nematodes.

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