Exploiting genomics to improve the biological control potential of *Pasteuria*spp.an organism with potential to control plant-parasitic nematodes

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Summary

The *Pasteuria* group of Gram positive bacteria are invertebrate parasites with the potential to be developed into biological control agents of plant-parasitic nematodes. A key step in the infection process is the attachment of endospores to the cuticle of plant-parasitic nematodes, possibly through a *Velcro*-like attachment system involving the collagen-like fibres of the exosporium (Davies, 2009). Phylogenetically these bacteria are members of the Firmicutes and closely related to the members of genus *Bacillus*. Some of the genes involved in the construction of the endospore and in particular the exosporium in *Bacillus* spp. have already been identified. The *Pasteuria* sequences in the public databases and the complete genomes of *Bacillus* spp. were investigated for the genes linked with the endospore and associated exosporium. On the basis of our *in silico* studies we report the presence of genes putatively similar to bclA, exsJ and vrrB in *Pasteuria*.

Key words: *Pasteuria*, *Bacillus*, plant-parasitic nematodes, endospore, exosporium, bclA, exsJ, vrrB

Introduction

Pasteuria group of Gram positive endospore-forming bacteria represent potentially ideal biocontrol agents for a wide range of economically important nematode pests (Starr & Sayre, 1988; Ciancio *et al*., 1994). The attachment of *Pasteuria* endospores to the cuticle plant-parasitic nematodes, the key step in the infection process, is highly specific and endospores from individual isolates of the bacterium do not adhere to or recognize all populations of nematodes (Sharma & Davies, 1996; Wishart *et al*., 2004). However, cross-generic attachment in some isolates has also been reported (Mohan *et al*., 2012). A suggested hypothetical reason for their host specificity is the heterogeneity of the collagen-like fibres present on the exosporium of the endospores (Davies & Curtis, 2011; Davies, 2009). The exact molecular mechanism is yet to be discovered in order to identify suitable populations of *Pasteuria* for deployment in the field. The skirt-like structure associated with the exosporium of *Pasteuria* spp. (Davies, 2009) seems to be covered with a hair-like nap in a similar manner to the exosporium of other closely related

Bacilli (Gerhardt & Ribi, 1964). It has been suggested that this hair-like nap, in *Bacillus* spp., is composed of a fibrous collagen-like glycoprotein coded for by the gene bclA (Sylvestre *et al*., 2002)which is amongst the 30 genes present in the Rhamnose Cluster Operon thought to be involved in exosporium synthesis in *B. anthracis*(Steichen *et al*., 2003). *Pasteuria* spp. are closely related to the *Bacilli* class (Charles *et al*., 2005) several of which have been fully sequenced and can serve as a basis for the comparative genomic studies of *Pasteuria.* In this study, we have investigated the presence of genes linked with its endospore and the associated exosporium in 46 different strains of *Bacillus cereus, Bacillus thuringiensis, Bacillus anthracis, Bacillus subtilis* and *Bacillus licheniformis*. We have studied the microsyntenies across these genes. We have also interrogated their putative presence in the unsequenced *Pasteuria* genome. The knowledge gained will serve as an aid in providing a comprehension of the molecular mechanisms governing the mode of *Pasteuria*-nematode interaction and the host specificity trait of *Pasteuria* endospores.

Materials and Methods

Amino acid sequences for 15 genes involved in endospore and exosporium formation and attachment, namely, bclA, rfbA, rfbB, rfbC, rfbD, yjcC, exsB, exsC, exsD, exsE, exsF, exsG, exsJ, clop, vrrB, (Todd *et al*., 2003; Schaff *et al*., 2011) were obtained from NCBI Protein database. The completely sequenced genomes of different strains of *Bacillus cereus, Bacillus thuringiensis, Bacillus anthracis, Bacillus subtilis* and *Bacillus licheniformis* were investigated for the presence of the genes above using a web-based software SyntTax. The microsynteny upstream and downstream of these genes wasstudied in all the strains. The sequences for the genes in question in different strains were retrieved using the GI number as derived from the SyntTax results. Using tBLASTn from the NCBI BLAST package (Altschul *et al*., 1990), the *Pasteuria* genome survey sequences present in the NCBI GSS database were compared to each gene sequence. The genes and the strains giving the most significant hits were selected and the sequences of their up– and downstream microsyntenic genes were retrieved and blasted, using tBLASTn, against *Pasteuria* GSS sequences.

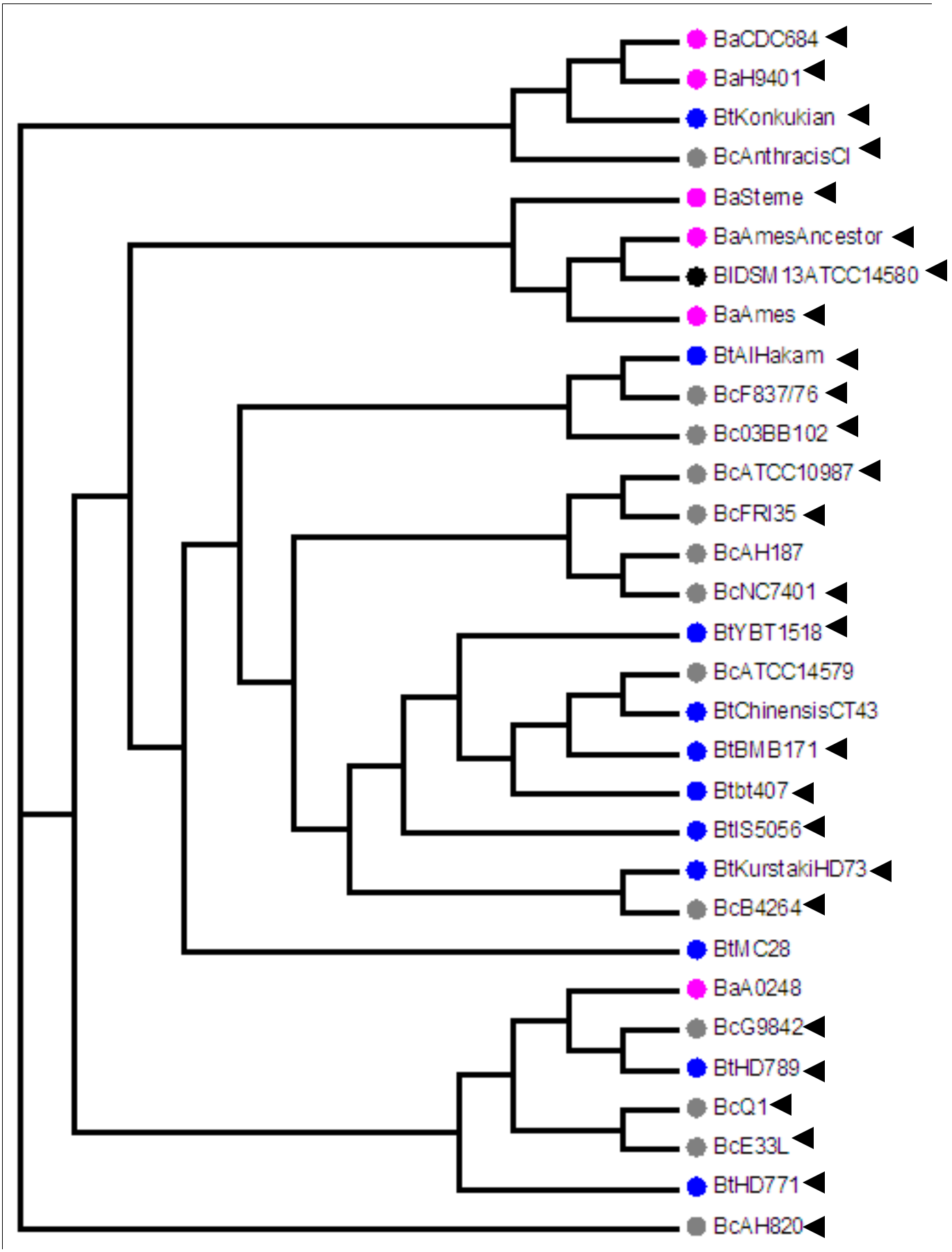
Results

Of the selected 46 strains, the non-animal parasitic strains, *B. subtilis and B. licheniformis,* showed the lowest percentage occurrence of all the genes in question while the animal parasitic strains showed the highest occurrence throughout. Figure 1 shows the percentage occurrence of the 15 selected genes across the different species. As suggested by the results of our *in silico* studies across the various *Bacillus* spp. there were considerable inter and intra specific variations in the microsyntenies. When the gene sequences retrieved from the genomes of *Bacillus* spp. were blasted against the GSS database using *Pasteuria* as search organism, out of all the 15 genes, three genes, namely, bclA, exsJ and vrrB gave the most significant hits. Cladograms were created on the basis of the pairwise sequence similarities of these three genes (Fig. 2). Interestingly, all the three cladograms placed the taxa very differently from a 16S rRNA-based cladogram. Two strains viz. *B. cereus* strain G9842 and *B. thuringiensis* strain HD771 were selected to study the micro-syntenies both upstream and downstream of the genes bclA, exsJ and

vrrB. Genes bclA, exsJ and vrrB from *B. cereus* strain G9842 gave significant hits with e-values $8e^{-14,}$ $1e^{-13}$ and $1e^{-09}$ respectively. While the genes bclA, exsJ and vrrB from *B. thuringiensis* gave hits with scores $3e^{-12}$, $6e^{-13}$ and $3e^{-07}$ respectively. Some neighboring genes also gave very significant hits against the *Pasteuria* GSS sequences (Fig. 3).

Gene	*B.cereus*	*B.thuringiensis*	*B.anthracis*	*B.subtilis*	*B.licheniformis*
rfbA	100%	100%	100%	100%	100%
rfbB	100%	100%	86%	100%	100%
yjcC	100%	100%	86%	100%	100%
bclA	100%	100%	86%	0%	50%
rfbD	100%	100%	86%	83%	0%
rfbC	100%	100%	86%	83%	0%
exsJ	100%	100%	86%	0%	0%
exsF	100%	100%	86%	0%	0%
exsE	100%	100%	86%	0%	0%
cloP	100%	100%	86%	0%	0%
exsD	69%	92%	86%	0%	0%
exsC	77%	92%	86%	0%	0%
vrrB	92%	92%	86%	0%	0%
exsB	92%	83%	86%	0%	0%
exsG	92%	75%	71%	8%	0%

Fig. 1. Percentage occurrence of 15 selected genes important in exosporium formation and potentially in attachment, across 46 strains from five species of *Bacillus*: *B.cereus* (n=13); *B. thuringiensis* (n=12); *B. anthracis*(n=7); *B. subtilis*(n=12) and *B. licheniformis* (n=2) where 'n' is the number of strains investigated.


BaCDC684
BaH9401
BtKonkukian
BcAnthracisCI
BaSteme
BaAmesAncestor
BIDSM13ATCC14580
BaAmes
BtAlHakam
BcF837/76
Bc03BB102
BcATCC10987
BcFRI35
BcAH187
BcNC7401
BtYBT1518
BcATCC14579
BtChinensisCT43
BtBMB171
Btbt407
BtIS5056
BtKurstakiHD73
BcB4264
BtMC28
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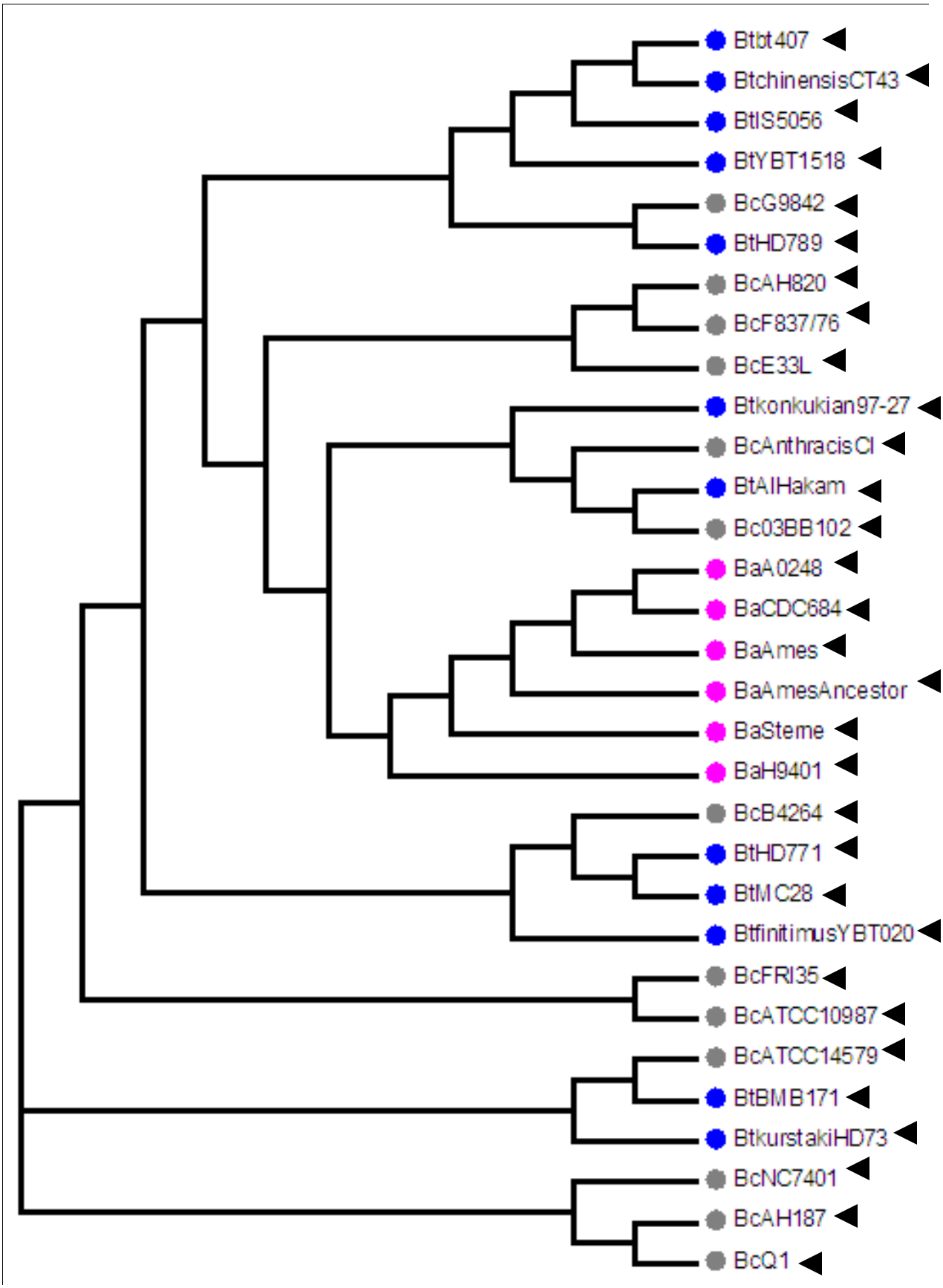
BcG9842
BtHD789
BcQ1
BcE33L
BtHD771
BcAH820

bclA


Btbt407
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BtIS5056
BtYBT1518
BcG9842
BtHD789
BcAH820
BcF837/76
BcE33L
Btkonkukian97-27
BcAnthracisCI
BtAlHakam
Bc03BB102
BaA0248
BaCDC684
BaAmes
BaAmesAncestor
BaSteme
BaH9401
BcB4264
BtHD771
BtMC28
BtfinitimusYBT020
BcFRI35
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BcATCC14579
BtBMB171
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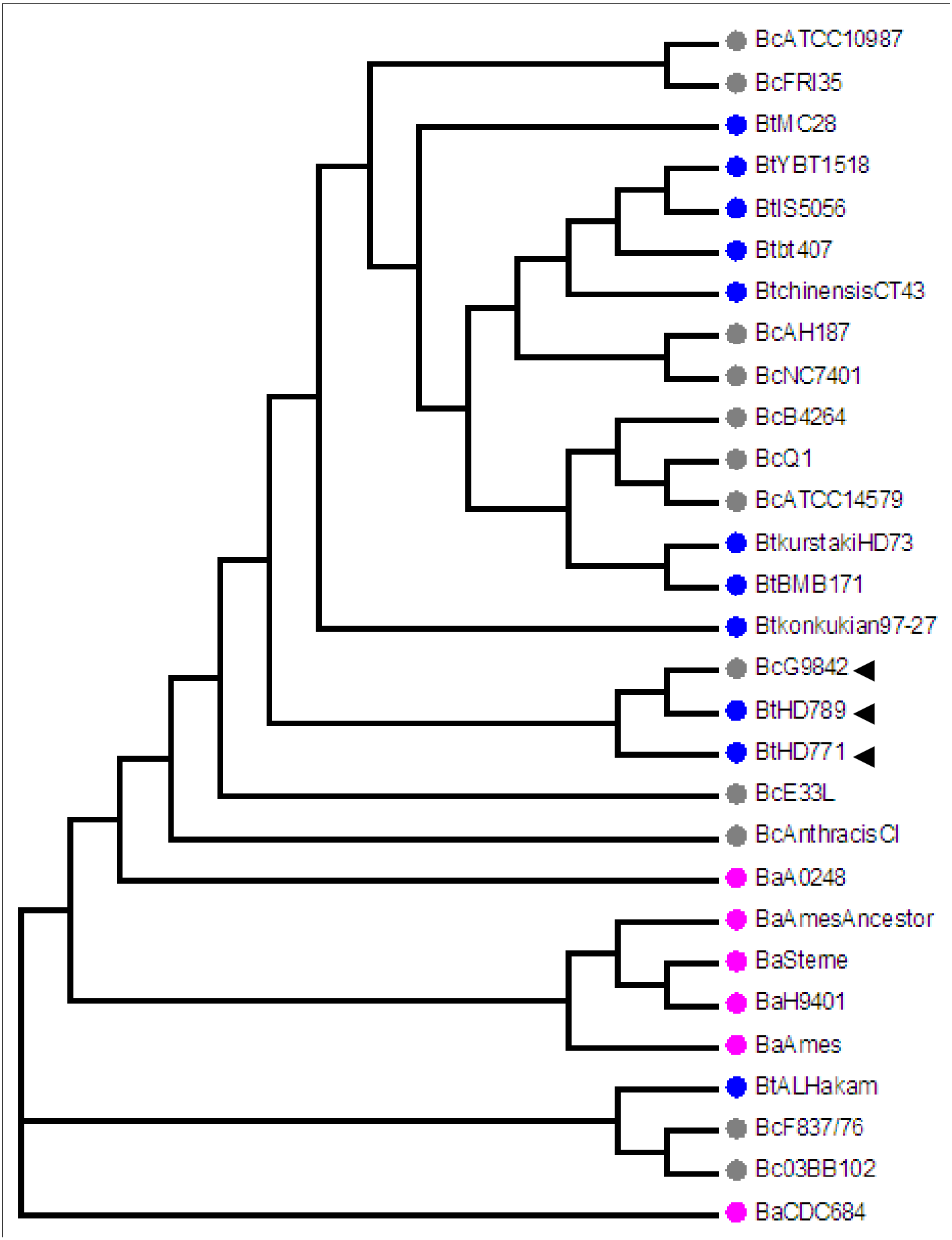
BcNC7401
BcAH187
BcQ1

exsJ


BcATCC10987
BcFRI35
BtMC28
BtYBT1518
BtIS5056
Btbt407
BtchinensisCT43
BcAH187
BcNC7401
BcB4264
BcQ1
BcATCC14579
BtkurstakiHD73
BtBMB171
Btkonkukian97-27
BcG9842
BtHD789
BtHD771
BcE33L
BcAnthracisCI
BaA0248
BaAmesAncestor
BaSteme
BaH9401
BaAmes
BtALHakam
BcF837/76
Bc03BB102
BaCDC684

vrrB

Fig. 2.Cladograms constructed using Clustal omega based on genes bclA, exsJ and vrrB in different *Bacillus* spp. (*B.anthracis; B. thuringiensis; B. cereus*; *B. licheniformis*). The arrows along some of the taxa represent the *Pasteuria*(tBLASTn) hits.

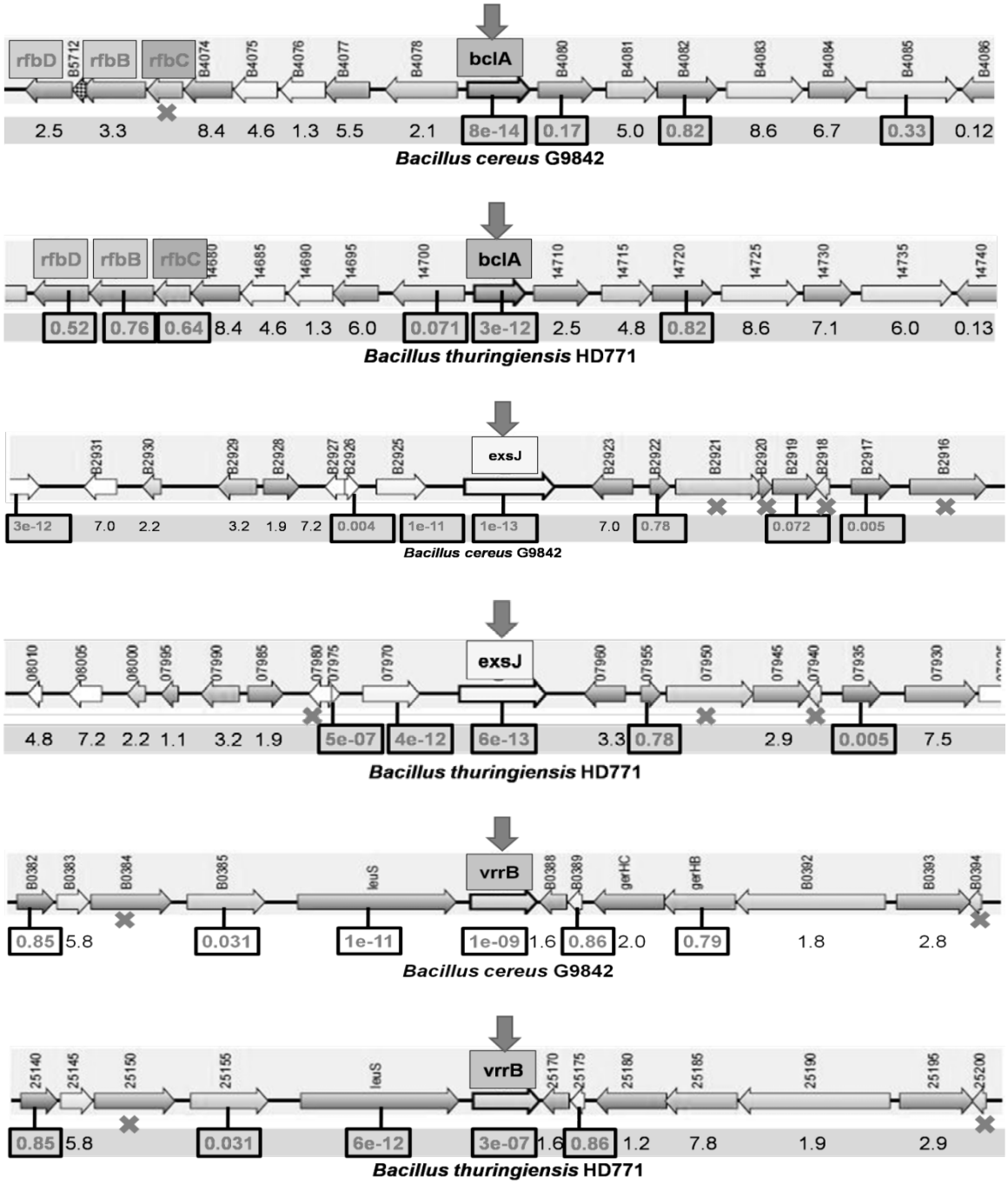


Fig. 3. The micro-syntenies across genes bclA, exsB and vrrB in *Bacillus cereus* strain G9842 and *Bacillus thuringiensis* strain HD771 (as depicted by the program SyntTax). The arrows represent the genes in *Bacillus* spp. The numbers below the genes represent the tBLASTn hits against *Pasteuria* GSS sequences. The numbers in boxes represent the hits with an E-value of <0.

The crosses below the arrows show an absence of similar gene sequence in *Pasteuria.* The diagram has been reconstructed from the SyntTax results.

Discussion

The highly variable attachment profiles of *Pasteuria* spp. pose an intriguing question on the molecular mechanisms governing the *Pasteuria*-nematode interaction. It has been shown amongst the root-knot nematodes that *Pasteuria* endospore attachment was not linked to the phylogeny of the nematode species and that attachment profiles of a particular *Pasteuria* isolate was highly specific(Davies et al., 2001). However, more recent research, of an isolate of *Pasteuria*from *Heterodera cajani* was much less specific, and indeed adhered to and infected *Globodera* spp. (Mohan *et al*., 2012).This study is a step towards identifying the genes involved in the molecular attachment mechanisms and the biochemical nature of endospore adhesion that regulates host range amongst *Pasteuria* isolates.

The genes in the Rhamnose cluster operon, including the gene bclA, are considered important in endospore and exosporium formation and their interaction and attachment with the host surface. Several other genes thought to play significant roles in endospore formation are scattered all over the genome of *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus subtilis* (Todd *et al*., 2003). We looked for 15 such genes in 46 strains across five species of *Bacillus.*The lowest occurrence of these 15 genes in *B. cereus*, *B. anthracis* & *B. thuringiensis*, (i.e. the animal parasitic strains) was 69%, compared to the fact that in non-parasitic strains (i.e. *B. subtilis* and *B. licheniformis*) only rfbA, rfbB and yjcC occurred to a high degree whereas the majority of genes were absent and not detected. These genes were present in most of the strains of *B. anthracis*, *B. cereus* and *B. thuringiensis* and their absence or low occurrence in *B. subtilis and B. licheniformis.* The most likely explanation for this is that the genes rfbA, rfbB and yjcC present in *B. subtilis and B. licheniformis* are not involved with attachment and subsequent infection but are likely to be involved structurally in endospore development and formation. In contrast the other genes present in parasitic species are likely to be either directly or indirectly involved with endospore attachment and the infection of hosts.

The use of Genomics can expand the possibilities of deciphering the intricate mechanisms behind host range and specificity of *Pasteuria* spp. The complete genome of *Pasteuria* is yet to be sequenced. However some 4000+ nucleotide sequences are available in public databases (Bird *et al*., 2003).We interrogated these sequences for the presence of genes and syntenic regions of key importance in the endospore-cuticle interactions. Our results show there is a high degree of genetic conservation between genes associated with the exosporium of the animal parasitic strains of *Bacillus* and *Pasteuria.* The preliminary analysis of the incompletely sequenced *Pasteuria* genome yielded many nucleotide stretches with significant similarities (e-values $\leq 1.0 \text{ X } 10^{-8}$) to some genes associated with the endospores in *Bacillus* spp. Earlier Schaff *et al.* (2011) reported genes analogous to some 12 genes from the Rhamnose Cluster Operon to be present in *P. penetrans.*

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References

Altschul SF, Gish W, Miller W, Myers E W, Lipman D J. 1990. Basic local alignment search tool. *Journal of Molecular Biology* **215**(3):403–410.

Bird D M, Opperman C H, Davies, K G. 2003. Interactions between bacteria and plant-parasitic nematodes: now and then. *International Journal for Parasitology* **33**(11):1269–1276.

Charles L, Carbone I, Davies K G, Bird D, Burke M, Kerry B R, Opperman C H. 2005. Phylogenetic Analysis of Pasteuria penetrans by Use of Multiple Genetic Loci Phylogenetic Analysis of *Pasteuria penetrans* by Use of Multiple Genetic Loci. *Journal of Bacteriology* **187**(16):5700–5708.

Ciancio A, Bonsignore R, Vovlas N,Lamberti F. 1994. Host Records and Spore Morphometrics of *Pasteuria penetrans* Group Parasites of Nematodes. *Journal of Invertebrate Pathology* **63**(3):260–267.

Davies KG. 2009. Understanding the interaction between an obligate hyperparasitic bacterium, Pasteuria penetrans and its obligate plant-parasitic nematode host, *Meloidogyne* spp. *Advances in Parasitology* **68**:211–245.

Davies K G, Curtis R H C. 2011. Cuticle surface coat of plant-parasitic nematodes. *Annual Review of Phytopathology* **49**:135–156.

Davies KG, Fargette M, Balla G, Daud A I, Duponnois R, Gowen S R,Mateille T, Phillips MS, Sawadogo A, Trivino C, Vouyoukalou E, Trudgill DL. 2001. Cuticle heterogeneity as exhibited by *Pasteuria* spore attachment is not linked to the phylogeny of parthenogenetic root-knot nematodes (*Meloidogyne* spp.). *Parasitology* **122**(Pt 1):111–120.

Gerhardt P, Ribi E. 1964. Ultrastructure of the exosporium enveloping spores of *Bacillus cereus*. *Journal of Bacteriology* **88**:1774–1789.

Mohan S, Mauchline T H, Rowe J, Hirsch P R, Davies K G. 2012. *Pasteuria* endospores from *Heterodera cajani* (Nematoda: Heteroderidae) exhibit inverted attachment and altered germination in cross-infection studies with *Globodera pallida* (Nematoda: Heteroderidae). *FEMS Microbiology Ecology* **79**(3):675–684.

Schaff J E, Mauchline T H, Opperman C H and Davies K G. 2011. Exploiting Genomics to Understand the Interactions Between Root-knot Nematodes and *Pasteuria penetrans.* In K Davies Y Spiegel (Ed.). *Biological Control of Plant-Parasitic Nematodes: Building Coherence between Microbial Ecology and Molecular Mechanisms.* Springer. pp. 91-113.

Sharma SB, Davies K G. 1996. Characterisation of *Pasteuria* Isolated from *Heterodera cajani* Using Morphology, Pathology and Serology of Endospores. *Systematic and Applied Microbiology* **19**(1):106–112.

Starr MP, Sayre R M. 1988. *Pasteuria thornei* sp. nov. and *Pasteuria penetrans sensu stricto* emend., mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera Pratylenchus and Meloidogyne. *Annales de l'Institut Pasteur / Microbiologie* **139**(1):11–31.

Steichen C,Chen P, Kearney J F, Turnbough C L Jr. 2003. Identification of the Immunodominant Protein and Other Proteins of the *Bacillus anthracis Exosporium*. *Journal of Bacteriology* **185**(6):1903–1910.
Sylvestre P, Couture-Tosi E, Mock M. 2002. A collagen-like surface glycoprotein is a structural component of the *Bacillus anthracis exosporium*. *Molecular Microbiology* **45**(1):169–178.
Todd S J, Moir A J G, Johnson M J, Moir M. 2003. Genes of *Bacillus cereus* and Bacillus anthracis Encoding Proteins of the Exosporium. *Journal of Bacteriology* **185**(11):3373–3378.
Wishart J, Blok V C, Phillips M, Davies K S G. 2004. *Pasteuria penetrans* and *P. nishizawae* attachment to *Meloidogyne chitwoodi*, *M . fallax* and *M . hapla*. *Nematology* **6**(4):507–510.