

Exploiting genomics to improve the biological control potential of *Pasteuriaspp.* 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 & Ribí, 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 Rhamnase 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*, *vrkB*, (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 was studied 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.

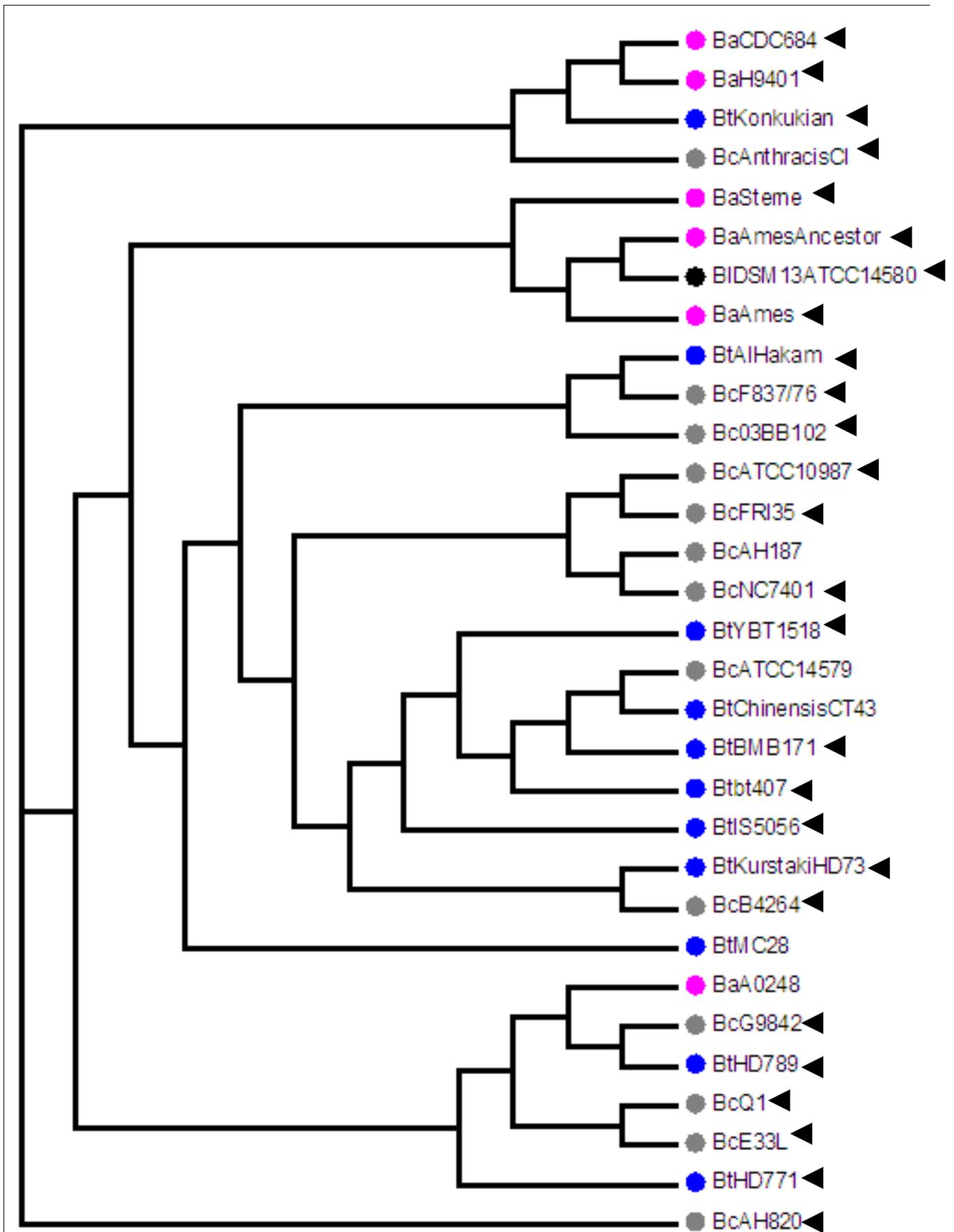
Results

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 *vrkB* 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

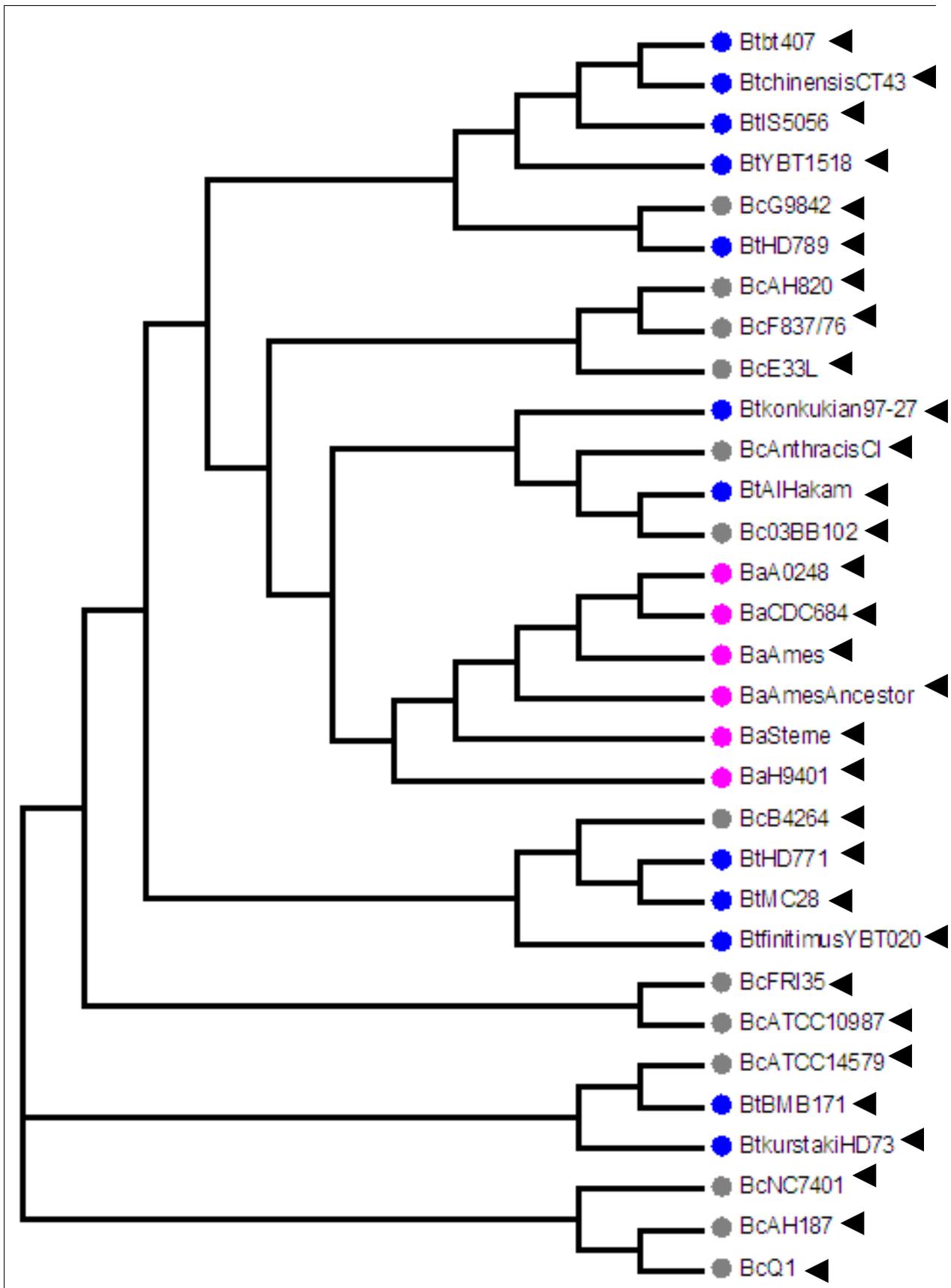
vrrB. Genes bclA, exsJ and vrrB from *B. cereus* strain G9842 gave significant hits with e-values $8e^{-14}$, $1e^{-13}$ and $1e^{-09}$ respectively. While the genes bclA, exsJ and vrrB from *B. thuringiensis* gave hits with scores $3e^{-12}$, $6e^{-13}$ and $3e^{-07}$ respectively. Some neighboring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

| Gene | <i>B.cereus</i> | <i>B.thuringiensis</i> | <i>B.anthraxis</i> | <i>B.subtilis</i> | <i>B.licheniformis</i> |
|------|-----------------|------------------------|--------------------|-------------------|------------------------|
| 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% |

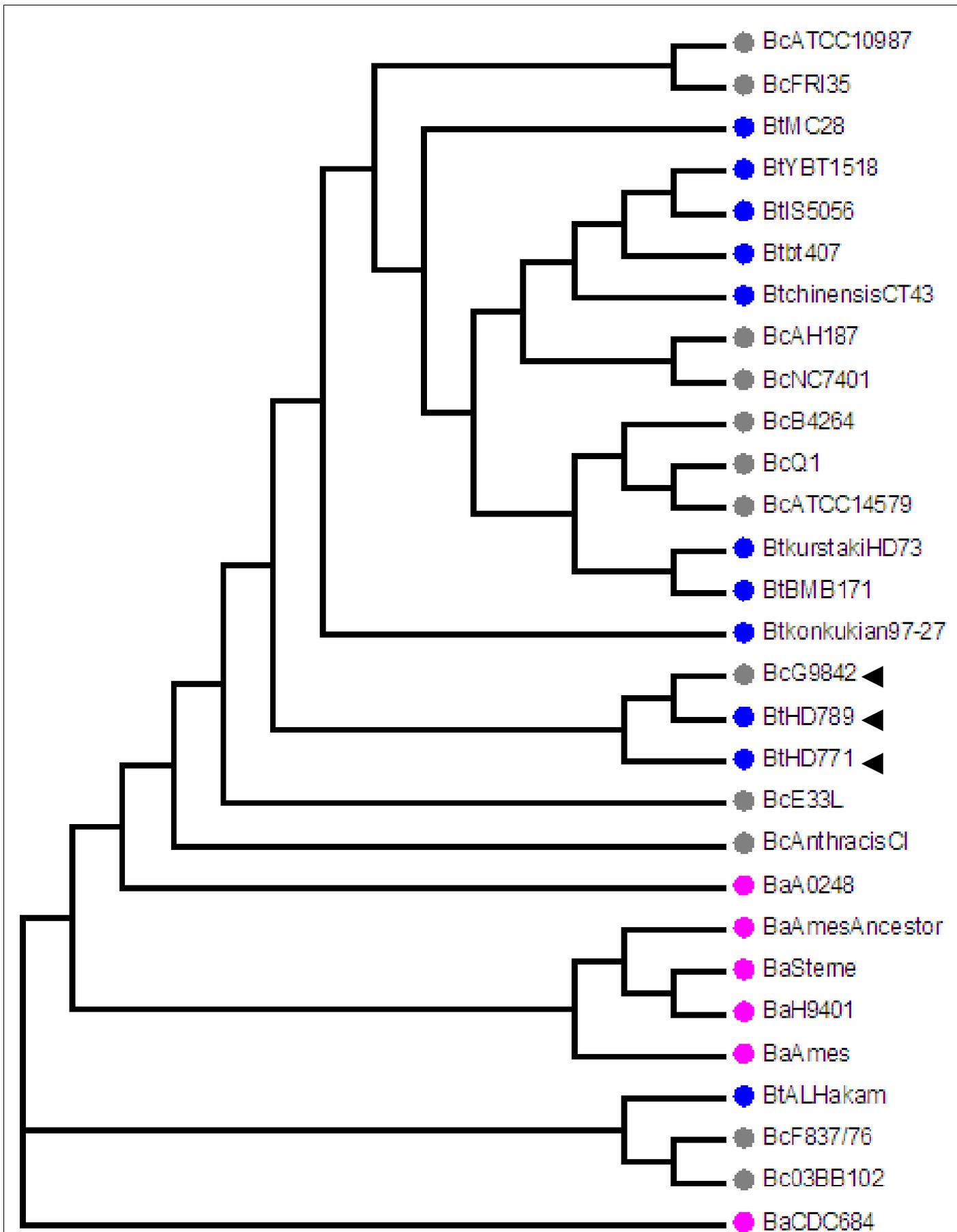
Fig. 1. Percentage occurrence of 15 selected genes important in exosporium formation and potentially in attachment, across 46 strains from five species of *Bacillus*: *B.cereus* (n=13); *B. thuringiensis* (n=12); *B. anthracis*(n=7); *B. subtilis*(n=12) and *B. licheniformis* (n=2) where ‘n’ is the number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



bclA



exsJ



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.

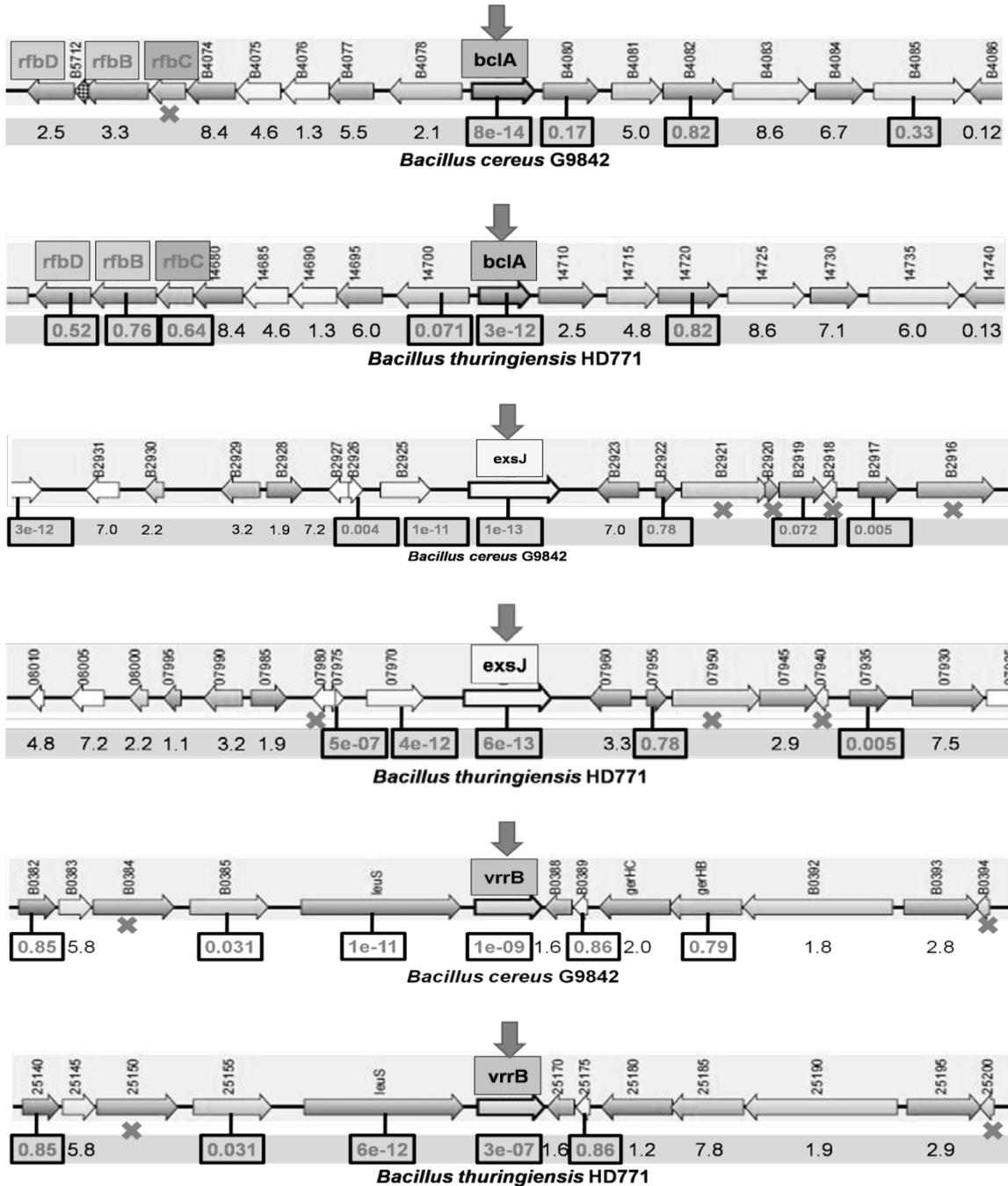


Fig. 3. The micro-synteny across genes *bclA*, *exsB* and *vrrB* in *Bacillus cereus* strain G9842 and *Bacillus thuringiensis* strain HD771 (as depicted by the program SyntTax). The arrows represent the genes in *Bacillus* spp. The numbers below the genes represent the tBLASTn hits against *Pasteuria* 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 $\leq 1.0 \times 10^{-8}$) 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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