Draft genome sequence of *Bacillus amyloliquefaciens* HB-26

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Keywords: *Bacillus amyloliquefaciens* HB-26, next-generation sequencing, *Plasmodiophora brassicae*

*Bacillus amyloliquefaciens* HB-26, a Gram-positive bacterium was isolated from soil in China. SDS-PAGE analysis showed this strain secreted six major protein bands of 65, 60, 55, 34, 25 and 20 kDa. A bioassay of this strain reveals that it shows specific activity against *P. brassicae* and nematode. Here we describe the features of this organism, together with the draft genome sequence and annotation. The 3,989,358 bp long genome (39 contigs) contains 4,001 protein-coding genes and 80 RNA genes.

**Introduction**

*Bacillus amyloliquefaciens* is a species of bacterium in the genus *Bacillus* with high affinity of *B. subtilis*. In the growth process, *B. amyloliquefaciens* can produce numerous antimicrobial or, more generally, bioactive metabolites with well-established activity in vitro such as surfactin, iturin and fengycin [1,2]. The production of all of these antibiotic compounds highlights *B. amyloliquefaciens* as a good candidate for the development of biocontrol agents [3,4].

Strain HB-26 belongs to the species *B. amyloliquefaciens*. The type strain of the species produces much bioactive metabolites showing specific activity against *Plasmodiophora brassicae* which could cause Clubroot, one of the most serious diseases of brassica crops worldwide [5-7]. Heavy infection by this pathogen of Chinese cabbage, cabbage, broccoli, turnip, oilseed rape, and other crucifers can lead to severe economic losses [8-11]. The root systems of infected plants show gall formation, which inhibits nutrient and water transport, stunts plant growth, and increases susceptibility to wilting [12,13]. Otherwise, bioassay results showed strain HB-26 also had some root-knot nematicidal activity.

Here, we present a summary classification and a set of features for *B. amyloliquefaciens* HB-26, together with the description of the genomic sequencing and annotation in order to improve the understanding of the molecular basis for its ability to inhibit *Plasmodiophora brassicae* and nematode.

**Classification and features**

Strain HB-26 colonies were milky white and matte with a wrinkled surface. Microscopy observations indicated that it was a *Bacillus* species (Figure 1A, Figure 1B and Table 1). SDS-PAGE analysis showed this strain secreted six major protein bands of 65, 60, 55, 34, 25 and 20 kDa (Figure 1C). A representative genomic 16S rDNA sequence of strain HB-26 was searched against GenBank database using BLAST [29]. Sequences showing more than 99% sequence identity to 16S rDNA of HB-26 were selected for phylogenetic analysis, and 15 sequences were aligned with ClustalW algorithm. The tree was reconstructed by neighbor-Joining by using Kimura 2-parameter for distance calculation. The phylogenetic tree was assessed by bootstrapped for 1,000 times, and the consensus tree was shown in Figure 2.
Bacillus amyloliquefaciens HB-26

Figure 1. General characteristics of B. amyloliquefaciens HB-26. (A) The colonial morphology pictures of strain HB-26. (B) Phase contrast micrograph of HB-26. (C) SDS-PAGE analysis of proteins of HB-26. Lane M, protein molecular weight marker; Lane 1, proteins of strain HB-26.

Table 1. Classification and general features of B. amyloliquefaciens HB-26

| MIGS ID | Property                  | Term                                 | Evidence code |
|---------|---------------------------|--------------------------------------|---------------|
|         | Domain                    | Bacteria                             | TAS [14]      |
|         | Phylum                    | Firmicutes                           | TAS [15-17]   |
|         | Class                     | Bacilli                              | TAS [18,19]   |
|         | Current classification    |                                      |               |
|         | Order                     | Bacillales                           | TAS [20,21]   |
|         | Family                    | Bacillaceae                          | TAS [20,22]   |
|         | Genus                     | Bacillus                             | TAS [20,23,24]|
|         | Species                   | Bacillus amyloliquefaciens           | TAS [25-27]   |
|         | Gram stain                | Gram-positive                        | NAS           |
|         | Cell shape                | rod-shaped                            | IDA           |
|         | Motility                  | motile                               | NAS           |
|         | Sporulation               | spore-forming                        | IDA           |
|         | Temperature range          | room temperature                     | NAS           |
|         | Optimum temperature       | pH 7.0                               | IDS           |
|         | Carbon source             | organic carbon source                | NAS           |
|         | Energy source             | organic carbon source                | NAS           |
|         | MIGS-6                    | Habitat                              | soil          | IDA |
|         | MIGS-6.3                  | Salinity                             | salt tolerant | NAS |
|         | MIGS-22                   | Oxygen                               | aerobic       | NAS |
|         | MIGS-14                   | Pathogenicity                        | avirulent     | NAS |
|         | MIGS-4                    | Geographic location                  | Hubei, China  | IDA |
|         | MIGS-4.1                  | Latitude                             | 30.07N        |     |
|         | MIGS-4.2                  | Longitude                            | 112.23E       |     |
|         | MIGS-4.3                  | Depth                                | 5-10cm        |     |
|         | MIGS-4.4                  | Altitude                             | about 35m     |     |
|         | MIGS-5                    | Sample collection time              | 2009          | IDA |

*Evidence codes - IDA: Inferred from Direct Assay; TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [28]
Figure 2. Neighbor-Joining Phylogenetic tree was generated using MEGA 4 based on 16S rRNA sequences. The strains and their corresponding GenBank accession numbers for 16S rDNA sequences are: A: B. amyloliquefaciens ML581 (KC692179.1); B: B. amyloliquefaciens JM-21 (KC752450.1); C: Bacillus strain HB-26 (HM138476); D: B. vallismortis WA3-7 (JF496475.1); E: B. sp.BYK1448 (HF549161.1); F: B. subtilis 2B (KF112078.1); G: B.methylotrophicus GZGL8 (JN999861.1); H: B.vallismortis D20 (KC441761.1); I: B.tequilensis L10 (JN700126.1); J: B. sp. C4(2013) (KC310834.1); K: B. subtilis WBZ (KC460988.1); L: B. Amyloliquefaciens CA81 (KF040978.1) ; M: B. sp. SWB30 (JX861886.1); N: B.methylotrophicus Ns7-22 (HQ831412.1); O: B. subtilis 26A (KC295415.1). The phylogenetic tree was constructed by using the neighbor-joining method within the MEGA software [30].

Genome sequencing information

Genome project history
This Bacillus strain was selected for sequencing due to its specific activity against Plasmodiophora brassicae and nematode. The complete high quality draft genome sequence is deposited in GenBank. The Beijing Genomics Institute (BGI) performed the sequencing and the NCBI staffs used the Prokaryotic Genome Annotation Pipeline (PGAAP) to complete the annotation. A summary of the project is given in Table 2.

Table 2. Project information

| MIGS ID  | Property             | Term                                                                 |
|----------|----------------------|----------------------------------------------------------------------|
| MIGS-31  | Finishing quality    | Draft                                                                |
| MIGD-28  | Libraries used       | One genomic libraries, one Illumina paired-end library (700 bp inserted size) |
| MIGS-29  | Sequencing platform  | Illumina Hiseq 2000                                                 |
| MIGS-31.2| Sequencing coverage  | 192 ×                                                               |
| MIGS-30  | Assemblers           | SOAPdenovo 1.05 version                                              |
| MIGS-32  | Gene calling method  | Glimmer and GeneMark                                                |
|          | GenBank Data of Release | August 31, 2016                                                 |
|          | NCBI project ID      | AUWK000000000                                                      |
|          | Project relevance    | Agricultural                                                        |

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Growth conditions and DNA isolation

*B. amyloliquefaciens* HB-26 was grown in 50 mL Luria-Broth for 6 h at 28°C. DNA was isolated by incubating the cells with lysozyme (10 mg/mL) in 2 mL TE (50 mM Tris base, 10 mM EDTA, 20% sucrose, pH 8.0) at 4°C for 6 h. 4 mL of 2% SDS were added and the mixture was incubated at 55°C for 30 min; 2 mL 5M NaCl were added, and the mixture was incubated at 4°C for 10 min. DNA was purified by organic extraction and ethanol precipitation.

Genome sequencing and assembly

The genome of *B. amyloliquefaciens* HB-26 was sequenced using Illumina Hiseq 2000 platform (with a combination of a 251-bp paired-end reads sequencing from a 700-bp genomic library). Reads with average quality scores below Q30 or more than 3 unidentified nucleotides were eliminated. 2,605,589 paired-end reads (achieving ~192 fold coverage [0.94 Gb]) was *de novo* assembled using SOAPdenovo 1.05 version [9]. The assembly consists of 39 contigs arranged in 39 scaffolds with a total size of 3,989,358 bp (including chromosome and plasmids).

Genome annotation

Genome annotation was completed using the Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP). Briefly, Protein-coding genes were predicted using a combination of GeneMark and Glimmer [31-33]. Ribosomal RNAs were predicted by sequence similarity searching using BLAST against an RNA sequence database and/or using Infernal and Rfam models [34,35]. Transfer RNAs were predicted using tRNAscan-SE [36]. In order to detect missing genes, a complete six-frame translation of the nucleotide sequence was done and predicted proteins (generated above) were masked. All predictions were then searched using BLAST against all proteins from complete microbial genomes. Annotation was based on comparison to protein clusters and on the BLAST results. Conserved domain Database and Cluster of Orthologous Group information is then added to the annotation.

### Table 3. Nucleotide content and gene count levels of the genome

| Attribute                          | Value       | % of total<sup>a</sup> |
|------------------------------------|-------------|------------------------|
| Genome size (bp)                   | 3,989,358   | 100.00                 |
| DNA coding region (bp)             | 3,486,615   | 87.39                  |
| DNA G+C content (bp)              | 1,889,758   | 47.37                  |
| Number of scaffolds                | 39          | -                      |
| Extrachromosomal elements          | unknown     | -                      |
| Total genes                        | 4,114       | 100.00                 |
| tRNA genes                         | 76          | 1.85                   |
| rRNA genes                         | 4           | 0.1                    |
| rRNA operons                       | 0<sup>b</sup> | -                      |
| Protein-coding genes               | 4,001       | 97.25                  |
| Pseudo gene (Partial genes)        | 0 (36)      | 0 (0.87%)              |
| Genes with function prediction (proteins) | 2224 | 54.06% |
| Genes assigned to COGs             | 2,336       | 56.78%                 |
| Genes with signal peptides         | 328         | 7.97                   |
| CRISPR repeats                     | 0           | 0                      |

<sup>a</sup>The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

<sup>b</sup>None of the rRNA operons appears to be complete due to unresolved assembly problems.
Genome properties

The draft assembly of the genome consists of 39 contigs in 39 scaffolds, with an overall 47.37% G+C content. Of the 4,114 genes predicted, 4,001 were protein-coding genes, and 80 RNAs were also identified. The majority of the protein-coding genes (54.06%) were assigned a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 3, Table 4 and Figure 3.

Table 4. Number of genes associated with the 25 general COG functional categories

| Code | Value | %agea | Description                                             |
|------|-------|-------|---------------------------------------------------------|
| J    | 130   | 3.160 | Translation, ribosomal structure and biogenesis         |
| A    | 0     | 0.0   | RNA processing and modification                         |
| K    | 262   | 6.368 | Transcription                                           |
| L    | 122   | 2.965 | Replication, recombination and repair                   |
| B    | 1     | 0.024 | Chromatin structure and dynamics                        |
| D    | 34    | 0.826 | Cell cycle control, cell division, chromosome partitioning |
| Y    | 0     | 0     | Nuclear structure                                       |
| V    | 52    | 1.264 | Defense mechanisms                                      |
| T    | 153   | 3.719 | Signal transduction mechanisms                          |
| M    | 182   | 4.424 | Cell wall/membrane/envelope biogenesis                  |
| N    | 53    | 1.288 | Cell motility                                           |
| Z    | 0     | 0.000 | Cytoskeleton                                            |
| W    | 1     | 0.024 | Extracellular structures                                |
| U    | 43    | 1.045 | Intracellular trafficking, secretion, and vesicular transport |
| O    | 97    | 2.358 | Posttranslational modification, protein turnover, chaperones |
| C    | 177   | 4.302 | Energy production and conversion                        |
| G    | 249   | 6.053 | Carbohydrate transport and metabolism                   |
| E    | 340   | 8.264 | Amino acid transport and metabolism                     |
| F    | 79    | 1.920 | Nucleotide transport and metabolism                     |
| H    | 123   | 2.990 | Coenzyme transport and metabolism                       |
| I    | 117   | 2.844 | Lipid transport and metabolism                          |
| P    | 205   | 4.983 | Inorganic ion transport and metabolism                  |
| Q    | 116   | 2.820 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 435   | 10.574| General function prediction only                        |
| S    | 287   | 6.976 | Function unknown                                        |
| 856  | 20.81 |       | Not in COGs                                             |

aThe total is based on the total number of protein coding genes in the annotated genome.
**Figure 3.** Graphical circular map of the *Bacillus amyloliquefaciens* HB-26 genome. From the outside to the center: genes on forward strand (color by COG categories), genes on reverse strand (color by COG categories), GC content, GC skew. The map was generated with the CGviewer server (Stothard Research Group: http://stothard.afns.ualberta.ca/cgview_server/).

**Acknowledgments**

This work was financially supported by the National Science and Technology Support Program (2008BADA5B03), the National 863 High Technology Research Program of China (2011AA10A201, 2011AA10A203), China 948 Program of Ministry of Agriculture (2011-G25), the National Science and Technology Support Program (2011BAB06B004-02), Hubei Province Development Plan (YJN0077) and the Science and Technology Support Program of Academy of Agricultural Sciences of Hubei Province (2012NKYJJ21).

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