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Author(s)	Watanabe, Miho; Kojima, Hisaya; Fukui, Manabu
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**Complete genome sequence and cell structure of *Limnochorda pilosa*, a Gram-negative spore
former within the phylum *Firmicutes***

Miho Watanabe^{1,2*}, Hisaya Kojima¹ and Manabu Fukui¹.

¹ The Institute of Low Temperature Science, Hokkaido University, Sapporo, Japan

² Graduate School of Environmental Science, Hokkaido University, Sapporo, Japan

* Corresponding author. Tel/fax number: +81 11 706 5460.

The Institute of Low Temperature Science, Hokkaido University, Nishi 8, Kita 19, Kita-ku Sapporo, Hokkaido
060-0819, Japan.

E-mail: m.watanabe@pop.lowtem.hokudai.ac.jp

Running head: Complete genome sequence and cell structure of *Limnochorda pilosa*

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The GenBank/EMBL/DDBJ accession numbers for the complete genome sequence of HC45^T is AP014924.

24 **Abstract**

25 *Limnochorda pilosa* is a pleomorphic facultative anaerobe and the sole species in the class *Limnochordia*, which
26 has tentatively been placed in the phylum *Firmicutes*. In the present study, the complete genome sequence of *L.*
27 *pilosa* HC45^T was obtained and analyzed. The genome size was 3.82 Mbp and the G+C content was 69.73%.
28 Phylogenetic analyses based on the 30S-50S ribosomal proteins and 23S rRNA gene consistently indicated that *L.*
29 *pilosa* is phylogenetically isolated from the other members of the phylum *Firmicutes*. Ultrastructural observation
30 revealed that *L. pilosa* possesses a Gram-negative-type cell wall and the capacity to form endospores. Accordingly,
31 the *L. pilosa* genome has characteristics that are specific to Gram-negative bacteria and contains many genes that
32 are involved in sporulation. On the other hand, several sporulation genes were absent in *L. pilosa* genome although
33 they have been regarded as essential for endospore-forming system of the phylum *Firmicutes*. The *gyrB* gene of *L.*
34 *pilosa* possesses an intein sequence. The genome has a high percentage of GTG start codons and lacks several
35 conserved genes related to cell division.

36

37 **Introduction**

38 The phylum *Firmicutes* has been traditionally regarded as a taxon encompassing Gram-positive
39 endospore-forming bacteria characterized by genomes with low G+C content (Ludwig et al., 1999; Schleifer et al.,
40 2009). *Firmicutes* is also thought to be a possible ancestral lineage of *Bacteria* (Ciccarelli et al., 2013; Koch, 2003).
41 *Firmicutes* consists of bacteria with an enormous genetic range and contains six established classes (*Bacilli*,
42 *Clostridia*, *Negativicutes*, *Thermolithobacteria*, *Erysipelotrichia*, *Tissierellia* and *Limnochordia*) (Garrity et al.,
43 2005; Ludwig et al., 2009; Marchandin et al., 2010; Sokolova et al., 2007; Alauzet et al., 2014, Watanabe et al.,
44 2015). The class *Negativicutes* was defined as a class of Gram-negative spore-forming organisms in this phylum
45 (Marchandin et al., 2010), but phylogenetic analyses indicated that the class could be incorporated into the class
46 *Clostridia* (Galperin, 2013; Yutin et al., 2013). The class *Limnochordia* was recently proposed to accommodate the
47 *Limnochorda pilosa* HC45^T, which was isolated from the sediment of a meromictic lake in Japan (Watanabe et al.,
48 2015). Analysis of the 16S rRNA gene sequence revealed that *L. pilosa* is distantly related to the genera

49 *Symbiobacterium*, *Sulfobacillus* and *Thermaerobacter*, which are facultative anaerobic Gram-positive clostridial
50 genera (Beppu et al., 2009; da Costa et al., 2009; Spanevello et al., 2009). These 3 genera are characterized by
51 high-G+C content (except for sulfobacilli [46-62 mol%]), and their taxonomic affiliations of still controversial. *L.*
52 *pilosa* HC45^T has some characteristics in common with these genera; the capability of facultative anaerobic growth
53 and high G+C content (71 mol%, as estimated by high-performance liquid chromatography analysis), but it is
54 Gram-stain-negative (Watanabe et al., 2015).

55 In the present study, the complete genome sequence of *L. pilosa* HC45^T was obtained as a representative of a
56 distinct phylogenetic lineage. Using the genome data, detailed phylogenetic analyses were performed. In addition,
57 an ultrastructural analysis was conducted to elucidate the cell wall structure and other morphological characteristics
58 of *L. pilosa*.

59

60 **Materials and methods**

61 DNA preparation, genome sequencing and annotation

62 *L. pilosa* HC45^T was grown at 45°C for 10 days in R2A liquid medium supplemented with 2% NaCl under
63 aerobic conditions. Genomic DNA was purified from collected cells using a Wizard® Genomic DNA Purification
64 Kit (Promega; Madison, WI, USA). The extracted genomic DNA was sequenced at Takara Bio, Inc. (Otsu, Shiga,
65 Japan). Library preparation (approximately 10 kb) was performed using the SMRTBell™ Template Prep Kit 1.0.
66 Sequence reactions with four single-molecule real-time (SMRT)® cells were performed on the PacBio RS II
67 sequencer (Pacific Biosciences; Menlo Park, CA, USA). SMRT Analysis portal version 2.2 was utilized for de novo
68 assembly using the Hierarchical Genome Assembly Process (HGAP) (Chin et al., 2013) (PacBio DevNet; Pacific
69 Biosciences), which is the execution program of SMRT Analysis packages, yielding a finished genome sequence.

70 The genome was automatically annotated using the Microbial Genome Annotation Pipeline (MiGAP) (Sugawara
71 et al., 2009). In the pipeline, RNAmmer (Lagesen et al., 2007) and tRNAscan-SE (Lowe et al., 1997) were used to
72 identify rRNA and tRNA genes, respectively. MetaGene Annotator (Noguchi et al., 2008) was used to predict open
73 reading frames likely to encode proteins (coding sequences [CDSs]), and functional annotation was performed

74 based on reference databases, including Reference Sequence (RefSeq), TrEMBL, and Clusters of Orthologous
75 Groups (COG). Manual annotation was performed using IMC-GE software (In Silico Biology; Yokohama, Japan).
76 Putative CDSs were confirmed again by a sequence similarity search against the GenBank protein database using
77 the BLASTP tool. Putative CDSs possessing BLASTP matches with more than 70% coverage and 35% identity and
78 E-values less than $1 \times e^{-5}$ were considered potentially functional genes. When these standards were not satisfied,
79 the CDSs were annotated as hypothetical proteins. Transcription start sites of predicted proteins were corrected
80 based on multiple sequence alignments. Short putative CDSs (approximately <500 bp) overlapping with longer
81 CDSs or rRNAs were removed. Clustered regularly interspaced short palindromic repeat (CRISPR) loci were
82 distinguished using the CRISPR Recognition Tool (Bland et al., 2007). The complete genome sequence of *L. pilosa*
83 has been deposited into the DDBJ/EMBL/GenBank database and assigned the accession number AP014924.

84

85 Phylogenetic analyses

86 The ribosomal protein amino acid sequences and the 23S rRNA gene sequence were obtained from the *L. pilosa*
87 genome. Data of 79 bacterial genomes retrieved from the NCBI genome database were used as the source of
88 ribosomal proteins and 23S rRNA gene sequences. The reference sequences were included those of 5 classes
89 (*Clostridia*, *Bacilli*, *Negativicutes*, *Tissierellia* and *Erysipelotrichia*) of the phylum *Firmicutes*, as well as all orders
90 in the classes *Clostridia* (*Clostridiales*, *Thermoanaerobacterales*, *Natranaerobiales* and *Halanaerobiales*) and
91 *Bacilli* (*Bacillales*, *Lactobacillales*). Universal 32 ribosomal proteins in the three domains of life (S2, S3, S4, S5,
92 S7, S8, S9, S10, S11, S12, S13, S14, S15, S17, S19, L1, L2, L3, L4, L5, L6, L10, L11, L12, L13, L14, L15, L18,
93 L22, L23, L24, and L29) were selected for phylogenetic analysis (Yutin et al., 2012). Each ribosomal protein was
94 separately aligned, and the 32 alignments of the proteins were then concatenated to perform a multilocus sequence
95 analysis (MLSA). All sequences were aligned with reference sequences using the ClustalX version 2.1 program
96 (Larkin et al., 2007). Phylogenetic trees were constructed using the maximum-likelihood method, by using the
97 program *MEGA* version 5.1 (Tamura et al., 2011). Bootstrap analyses were performed with 1,000 replicates.

98

99 Ultrastructural analysis

100 Cell morphology and intracellular structures were observed by transmission electron microscopy (TEM).
101 Cultivation and fixation of *L. pilosa* cells were performed as described previously (Watanabe et al., 2015). TEM
102 was performed by post-fixing cell samples with osmium tetroxide, followed by dehydration via a graded ethanol
103 series (50-100%). Samples were transferred to propylene oxide and embedded in epoxy resin. Ultrathin sections
104 were cut with an ultramicrotome and double-stained with uranyl acetate and lead citrate. For negative-stained
105 electron microscopy, cells were adsorbed onto a grid coated with a carbon support film and stained with uranyl
106 acetate. Sample observation was performed using a JEM-1400 Plus transmission electron microscope (JEOL;
107 Akishima, Tokyo, Japan).

108

109 **Results**

110 General features of the *L. pilosa* genome

111 Genome sequencing of *L. pilosa* HC45^T revealed a single circular chromosome of 3,817,036 bp with a mol%
112 G+C content of 69.73. The genome contained 2 rRNA operons, 47 tRNAs, and 2 CRISPR loci. In the genome,
113 3,603 CDSs were predicted, 47% of which had ATG start codons. The second most frequent start codon was GTG
114 (43%), and the TTG start codon was found in 10% of CDSs. The total length of CDSs was 3,387,686 bp, and the
115 CDS density was 88%.

116

117 Phylogeny

118 Phylogenetic trees based on ribosomal proteins and the 23S rRNA gene sequence are shown in Fig. 1 and 2. The
119 taxonomic clusters of the class- or order-level were supported in ribosomal protein and the 23S rRNA gene
120 phylogeny, but the general topology was not consistent with each result of phylogenetic analysis. The phylogenetic
121 analyses showed that *L. pilosa* is coherently related to the 3 genera *Symbiobacterium*, *Sulfobacillus* and
122 *Thermaerobacter*.

123

124 Genomic insights into the cell wall structure

125 The cell wall structure has been regarded as an important criterion for classification of bacteria at higher
126 taxonomic levels. It has been suggested that *L. pilosa* HC45^T has Gram-negative type cell wall, on the basis of a
127 Gram stain test and a trial to analyze amino acid component of the cell wall (Watanabe et al., 2015). Accordingly,
128 the genome of *L. pilosa* HC45^T had characteristics that are specific to Gram-negative bacteria. Lipopolysaccharide
129 (LPS) is a structural component of the outer membrane of Gram-negative bacteria, and Kdo₂-lipid A composes an
130 essential part of LPS. Kdo₂-lipid A is synthesized in 9 enzymatic steps (Opiyo et al., 2010), and *L. pilosa* HC45^T
131 was found to encode genes for 4 of the enzymes (*lpxA*: LIP_3063, *lpxC*: LIP_3065, *lpxD*: LIP_3069 and *lpxB*:
132 LIP_3061) involved in these steps. The pathway that consists of these 4 enzymes was also identified in the
133 genomes of the phyla *Cyanobacteria* and *Dictyoglomi* (Opiyo et al., 2010). The LPS-glycosyl transferase was also
134 conserved in the *L. pilosa* HC45^T genome, although almost none of the genes for LPS transport machinery (*lptA*,
135 *lptC*, *lptD*, and *lptE*) were encoded, with the exception of the *lptB* (LIP_3055) gene. S-layer-associated proteins
136 were not identified. Genes for flagella biosynthesis were also identified in the *L. pilosa* HC45^T genome, and their
137 composition suggests that *L. pilosa* HC45^T is Gram-negative bacterium. L-ring and P-ring proteins, components of
138 flagella specifically observed in Gram-negative bacteria (Aizawa, 2014), were conserved in the genome of *L. pilosa*
139 (LIP_3076–3077). The flagella motor (switch complex [C-ring]) of Gram-negative bacteria consists of three
140 components (FliG, FliM and FliN), and *L. pilosa* HC45^T encoded genes for all of them (LIP_1652, LIP_2960 and
141 LIP_2959, respectively).

142

143 Genes involved in sporulation and cell division

144 In a previous study, endospore-like structures were observed in *L. pilosa* HC45^T by phase-contrast microscopy
145 (Watanabe et al., 2015). In another study, essential sporulation genes that are conserved in the *Bacilli* and *Clostridia*
146 classes were identified (Galperin et al., 2012). The *L. pilosa* HC45^T genome harbored many of these genes, but
147 some important genes were not detected (Table 1). *L. pilosa* HC45^T lacked half of the genes for stage II of spore
148 formation (post-septation) and several genes for stages III-IV (post-engulfment) that are conserved in the

149 spore-forming bacteria of *Bacilli* and *Clostridia*. In addition to the genes listed Table 1, *L. pilosa* HC45^T lacked the
150 *divIVA* gene. The *divIVA* gene is widely conserved among high-G+C and low-G+C spore-forming bacteria and is
151 involved in sporulation, cell growth and cell division.

152 As in the case of *divIVA*, many sporulation genes are also relevant to cell division. Upon inspection of such genes,
153 it was found that *L. pilosa* HC45^T lacked several *fts* genes, which are widely conserved genes involved in cell
154 division. The *ftsA*, *B*, *L*, *N* and *Q* genes were not found in the *L. pilosa* HC45^T genome, although genes for FtsZ,
155 FtsI (SpoVD), FtsW (SpoVE) and FtsK/SpoIIIE family protein were identified (LIP_1859, LIP_1869, LIP_1865,
156 LIP_1851, respectively). The FtsA protein might be substituted by a protein coded by the *mreB* gene (LIP_2790),
157 which included a conserved domain of an FtsA-like protein that is essential for the membrane attachment of FtsZ.
158 The genes *ftsQ*, *ftsB* and *ftsL* encoding divisome proteins were absent in *L. pilosa* HC45^T, and their functions are
159 known to be substituted by the *divIB*, *divIC*, and *yIIID* genes in Gram-positive bacteria, respectively [12, 48].
160 However, these alternative genes were also absent from the *L. pilosa* HC45^T genome.

161 Regarding other genes related to cell division, *L. pilosa* was found to possess the *sepF* gene (LIP_2545), which is
162 specifically found in Gram-positive bacteria. However, *L. pilosa* HC45^T was also found to possess the genes
163 encoding the MinCDE system (*minCDE*: LIP_2784–2786), which is thought to be specific to Gram-negative
164 bacteria, as most spore-forming Gram-positive bacteria encode the MinCDJ/DivIVA system instead of the MinCDE
165 system (Barak, 2013). As mentioned above, *L. pilosa* HC45^T lacked the *divIVA* gene, and the *minJ* gene was also
166 absent from the *L. pilosa* HC45^T genome.

167

168 Intein in DNA gyrase subunit B

169 As a candidate for a phylogenetic marker, the sequence of the *L. pilosa* HC45^T gene encoding DNA gyrase
170 subunit B, *gyrB* (LIP_0006), was investigated. The *gyrB* gene was found to possess an intein sequence. The intein
171 included the sequence of the LAGLIDADG3 homing-endonuclease, and the total length of the intein sequence was
172 506 amino acids. GyrB inteins have been identified in bacterial phyla including *Proteobacteria* (MacGregor et al.,
173 2013; Soucy et al., 2014), *Chloroflexi* (Kiss et al., 2011), *Actinobacteria* (Soucy et al., 2014), *Firmicutes* (Soucy et

174 al., 2014) and *Cyanobacteria* (Nakamura et al., 2003) and in the archaeal phylum *Euryarchaeota* (Soucy et al.,
175 2014).

176

177 Ultrastructure of *L. pilosa* HC45^T cells

178 As direct evidence for a Gram-negative-type cell wall in *L. pilosa* HC45^T, TEM analysis clearly showed a
179 three-layer structure consisting of an outer membrane, a peptidoglycan layer and a plasma membrane (Fig. 3A).
180 The cell surface of *L. pilosa* HC45^T was shaggy in appearance (Watanabe et al., 2015), which was also observed in
181 the TEM images (Fig. 3). Different internal structures in round and filamentous cells were observed (Fig. 3B, 3C).
182 Cell surfaces were often irregularly hollowed (Fig. 3D). TEM analysis revealed the presence of projecting cylinders
183 (Fig. 4A-C). A complete septum was not observed at the division site of *L. pilosa* HC45^T cells, thus suggesting a
184 constriction mode of cell division (Fig. 5). The presence of both forespores and endospores was also confirmed (Fig.
185 6A and 6B). TEM analysis of the negatively stained *L. pilosa* HC45^T revealed the presence of small spherical
186 structures (ca. 200 nm in diameter) adhering to cells (Fig. 7A) that could not be detected using other methods.

187

188 Discussion

189 It had been already shown that *L. pilosa* is characterized by a high G+C content and is phylogenetically
190 separated from low-G+C Gram-positive bacteria in the phylum *Firmicutes* (Watanabe et al., 2015). In this study,
191 ultrastructural and genetic analyses confirmed that *L. pilosa* HC45^T is a Gram-negative bacterium representing a
192 novel independent lineage. In the phylogenetic analyses based on ribosomal proteins and the 23S rRNA gene,
193 phylogenetic uniqueness of *L. pilosa* within the phylum *Firmicutes* was consistently indicated (Fig. 1, Fig. 2).
194 Because of the longer informative sequence, the 23S rRNA gene is considered a better phylogenetic tool than the
195 16S rRNA gene (Ludwig et al., 2001; Ludwig et al., 1999; Ludwig et al., 1998; Yarza et al., 2010). The sequences
196 of ribosomal proteins have also been utilized as useful markers in phylogenetic analyses at levels of taxa ranging
197 from domain to species (Ciccarelli et al., 2005; Jolley et al., 2012; Yutin et al., 2013).

198 As a representative of a novel lineage without close relatives, *L. pilosa* HC45^T possessed several unique features.

199 The *L. pilosa* HC45^T genome was characterized by a high percentage of GTG start codons (43%) in the coded
200 proteins. A majority of known bacteria primarily use the ATG start codon, and the highest prevalence of the GTG
201 start codon usage that has been reported so far is 30-35% in mycobacterial genomes, which have high G+C content
202 (66-67%) (Magee et al., 2011; Newton-Foot et al., 2013). The high incidence of the GTG start codon in the *L.*
203 *pilosa* HC45^T genome may be related to its high G+C content (70%). Another feature of the *L. pilosa* HC45^T
204 genome was the lack of several conserved genes involved in cell division. The ultrastructural analysis suggested
205 that the cell division system in *L. pilosa* HC45^T resembles the Gram-negative type without a complete septum, or
206 the so-called constriction mode (Egan et al., 2013). *L. pilosa* HC45^T may have a unique, previously unidentified
207 cell division system, which may be related to its pleomorphy.

208 An enormous number of small spherical structures were observed at the negatively stained cell surface of *L.*
209 *pilosa* HC45^T (Fig. 7A). Their appearances were very similar to those of external membrane vesicles (MVs),
210 produced by organisms in all 3 domains of life (Deatherage et al., 2012). The *L. pilosa* HC45^T MVs fell within the
211 range of Gram-negative bacterial MVs (10-300 nm in diameter) and were larger than those of Gram-positive
212 bacteria (50-150 nm) (Deatherage et al., 2012). In Gram-negative bacteria, MVs are often produced by the faster
213 growth of the outer membrane than the peptidoglycan layer (Bernadac et al., 1998). Accordingly, MVs derived
214 from overproduced outer membranes were clearly observed at the cell surface of *L. pilosa* HC45^T (Fig. 7B).

215 *L. pilosa* HC45^T endospore formation was confirmed by TEM analysis. The phylogenetic expanse of
216 endospore-forming organisms was once thought to be restricted to the classes *Bacilli* and *Clostridia*, but
217 endospore-formers were reported in *Mycobacterium* spp. (Ghosh., 2009; Lamont et al., 2012; Singh et al., 2010)
218 and *Streptomyces* sp. (Filippova et al., 2005) within the phylum *Actinobacteria* although disputed on the basis of
219 genome comparisons (Traag et al, 2010; Galperin et al., 2012; Abecasis et al., 2013). Moreover, members of the
220 phylum *Proteobacteria* were also reported to generate endospores although they are typical Gram-negatives.
221 (Ajithkumar et al., 2003; Girija et al., 2010). As shown in Table 1, *L. pilosa* HC45^T was found to encode many
222 genes involved in sporulation. The genes listed in Table 1 were identified by genome analysis of spore formers in
223 the classes *Bacilli* and *Clostridia*, including the uncultivated *Candidatus* Arthromitus spp., which have a greatly

224 reduced gene set for regulating sporulation (Chase et al., 1976, Kuwahara et al., 2011). The list was used for the
225 rapid screening of sporulation genes, and the absence of some genes in this list does not necessarily indicate a lack
226 of sporulation capacity. For instance, *L. pilosa* HC45^T was found to lack the genes *spoIIE*, *spoIIGA*, *spoIIR*, *spoIIM*
227 and *yqfD*, but the absence of these genes is a common property among actinobacterial spore-formers (Abecasis et
228 al., 2013). However, the lack of the *divIVA* gene may be a unique property of *L. pilosa* HC45^T among
229 spore-forming bacteria, as the gene is widely conserved in spore-forming *Firmicutes*, sporogenous *Actinobacteria*
230 and exosporulating *Deltaproteobacteria* (Abecasis et al., 2013). *L. pilosa* HC45^T is phylogenetically far-distant
231 from other known sporogenous bacteria, and other unidentified proteins may have roles in its endospore expression.

232

233 **Conclusion**

234 Polyphasic phylogenetic analyses showed that *L. pilosa* is assigned to an independent lineage within the phylum
235 *Firmicutes*. These results were not in conflict with the 16S rRNA gene-based phylogeny results. Ultrastructural
236 observation revealed that *L. pilosa* HC45^T has a Gram-negative-type cell wall and a faculty for endospore
237 formation. The genomic information generally supported these results, but *L. pilosa* HC45^T is proposed to possess
238 sporulation machinery that is somewhat different from that of other bacteria in the phylum *Firmicutes*.

239

240

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244

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437 **Figure legends**

438 Fig. 1 Phylogenetic tree based on amino acid sequences of 30S-50S ribosomal proteins. The tree was generated by
439 the Maximum-Likelihood method. Bootstrap values (percentages of 1000 replications) only 50% or more are
440 shown at nodes.

441

442 Fig. 2 Maximum-Likelihood tree showing the phylogenetic position of *L. pilosa* based on 23S rRNA gene sequence.
443 Bootstrap values (percentages of 1000 replications) only 50% or more are shown at nodes.

444

445 Fig. 3 Transmission electron microscope images of A: Gram-negative type cell wall; B: round vacuolated cell; low
446 electron-dense granules and D: cell surface hollows of *L. pilosa* HC45^T. Arrows indicate outer membrane (OM),
447 peptidoglycan layer (PG) and plasma membrane (PM). Solid triangle indicates a low electron-dense granule. Open
448 triangles indicate asymmetric invaginations.

449

450 Fig. 4 Transmission electron microscope image of A: bipolarly; B: sub-polarly and C: laterally projecting cylinders
451 of *L. pilosa* HC45^T. Cells were negatively-stained in image B and C.

452

453 Fig. 5 Transmission electron microscope image of division site of *L. pilosa* HC45^T cell. An arrow indicate the
454 division site with constriction of envelope.

455

456 Fig. 6 Transmission electron microscope image of A: forespore-like structures and B: endospore-like structure of *L.*
457 *pilosa* HC45^T.

458

459 Fig. 7 Transmission electron microscope images of negatively-stained cells of *L. pilosa* HC45^T. Image A shows
460 MVs attached to cell surface, and image B shows MVs generated from redundant outer-membrane.

461 Table 1 List of conserved or absent essential sporulation genes in *L. pilosa* genome. For details see the text.

Sporulation stage	Identified sporulation genes in <i>L. pilosa</i> genome	Absent sporulation genes in <i>L. pilosa</i> genome
Stage 0	<i>spo0A</i> (LIP_2584), <i>sigH</i> (LIP_3293), <i>obgE</i> (LIP_2777), <i>spo0J</i> (LIP_3647)	
Stage II	<i>spoIIAA</i> (LIP_1706), <i>spoIIAB</i> (LIP_1707), <i>sigE</i> (LIP_1858), <i>spoIID</i> (LIP_2799), <i>spoIIP</i> (LIP_1847) <i>cwlD</i> (LIP_1224), <i>dapA</i> (LIP_2564), <i>dapB</i> (LIP_2569), <i>spmA</i> (LIP_0753), <i>spmB</i> (LIP_0754), <i>spoIIIAA</i> (LIP_2607), <i>spoIIIAB</i> (LIP_2606), <i>spoIIIAC</i> (LIP_2605), <i>spoIIIAD</i> (LIP_2604), <i>spoIIIAE</i> (LIP_2603), <i>spoIIID</i> (LIP_3082), <i>spoIIIE</i> (LIP_1851), <i>spoIIIAG</i> (LIP_2601), <i>spoIIIJ</i> (LIP_3652), <i>jag</i> (LIP_3651), <i>spoIVA</i> (LIP_1775), <i>spoIVB</i> (LIP_2585), <i>sigG</i> (LIP_1856), <i>sigK</i> (LIP_2614), <i>spoVAC</i> (LIP_1799), <i>spoVAD</i> (LIP_1798), <i>spoVAEB</i> (LIP_1797), <i>spoVC</i> (LIP_3596), <i>spoVD</i> (LIP_1869), <i>spoVT</i> (LIP_3594), <i>stoA</i> (LIP_0685), <i>yabP</i> (LIP_3590), <i>yabQ</i> (LIP_3589), <i>yblJ</i> (LIP_1610), <i>ylmC</i> (LIP_1854), <i>yqfC</i> (LIP_2648), <i>ytlv</i> (LIP_2524), <i>yycA</i> (LIP_3643)	<i>sigF</i> , <i>spoIIE</i> , <i>spoIIGA</i> , <i>spoIIM</i> , <i>spoIIB</i>
Stage III-VI		<i>dacB</i> , <i>spoIIIAF</i> , <i>spoIIIAH</i> , <i>spoVB</i> , <i>spoVG</i> , <i>yqfD</i>
Spore coat	<i>spoIVA</i> (LIP_1775), <i>yncD</i> (LIP_1264)	
Germination	<i>gpr</i> (LIP_2672), <i>lgt</i> (LIP_1407)	

462