



# HOKKAIDO UNIVERSITY

|                  |                                                                                                                                                                                                                                                                       |
|------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Title            | <i>Limnochorda pilosa</i> gen. nov., sp nov., a moderately thermophilic, facultatively anaerobic, pleomorphic bacterium and proposal of <i>Limnochordaceae</i> fam. nov., <i>Limnochordales</i> ord. nov and <i>Limnochordia</i> classis nov in the phylum Firmicutes |
| Author(s)        | Watanabe, Miho; Kojima, Hisaya; Fukui, Manabu                                                                                                                                                                                                                         |
| Citation         | International journal of systematic and evolutionary microbiology, 65, 2378-2384<br><a href="https://doi.org/10.1099/ijs.0.000267">https://doi.org/10.1099/ijs.0.000267</a>                                                                                           |
| Issue Date       | 2015-08                                                                                                                                                                                                                                                               |
| Doc URL          | <a href="https://hdl.handle.net/2115/62587">https://hdl.handle.net/2115/62587</a>                                                                                                                                                                                     |
| Type             | journal article                                                                                                                                                                                                                                                       |
| File Information | Supplementary files150414 .pdf                                                                                                                                                                                                                                        |



International Journal of Systematic and Evolutionary Microbiology  
Supplementary material

***Limnochorda pilosa* gen. nov., sp. nov., a moderately thermophilic, facultative anaerobic pleomorphic bacterium and proposal of *Limnochordaceae* fam. nov., *Limnochordales* ord. nov. and *Limnochordia* classis nov. in the phylum *Firmicutes***

Miho Watanabe, Hisaya Kojima, Manabu Fukui

Corresponding author: Miho Watanabe  
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

**Supplementary Figure S1:** The culturing procedure of strain HC45<sup>T</sup>.

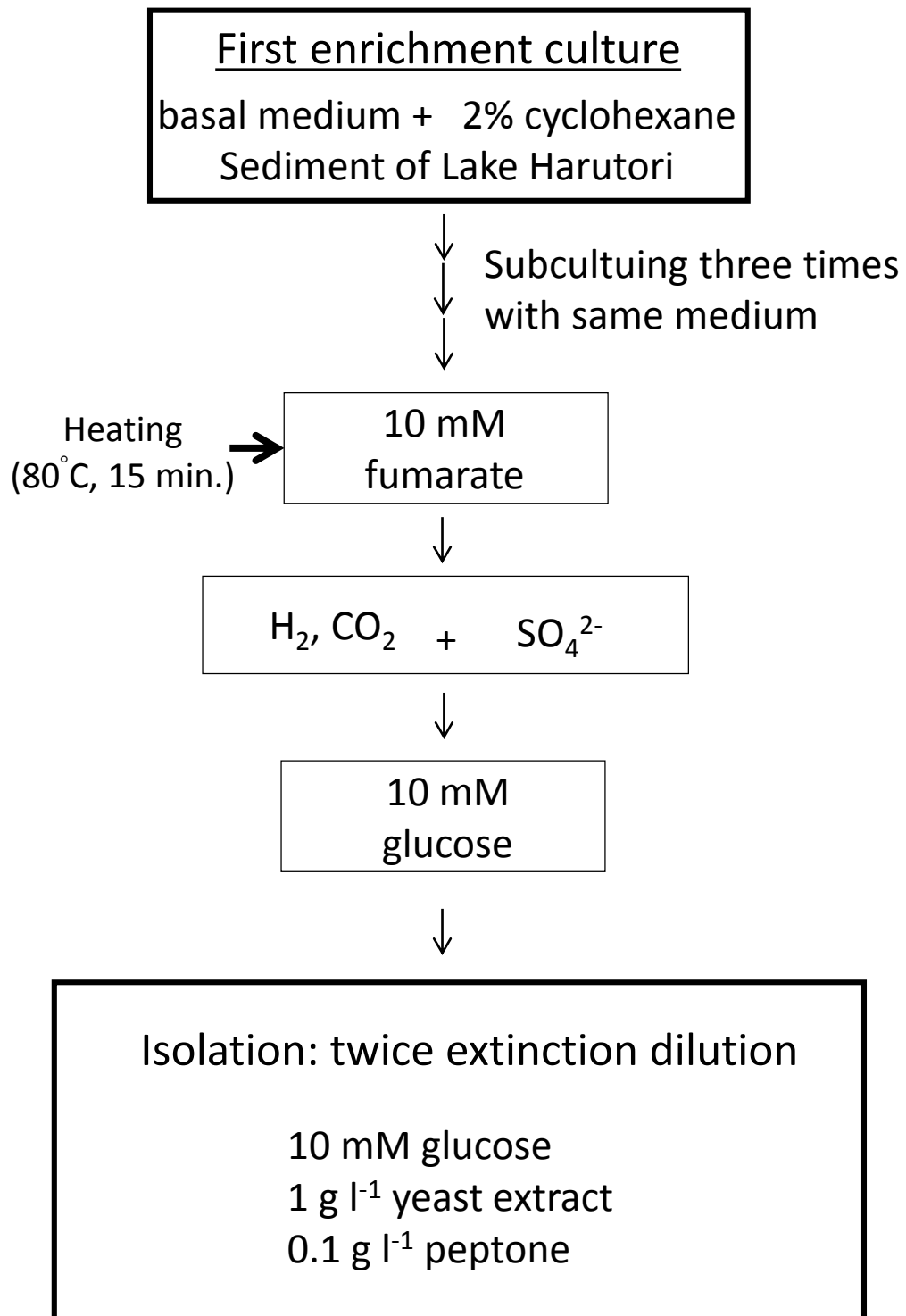
**Supplementary Figure S2:** Phase-contrast micrograph showing endospore-like structure of the strain HC45<sup>T</sup> (arrow) grown on NaCl-R2A liquid medium for a week.

**Supplementary Figure S3:** Maximum-likelihood tree based on 16S rRNA gene sequences of the strain HC45<sup>T</sup>, related environmental sequences and representatives from all classes in the phylum *Firmicutes*. This phylogenetic tree is based on a comparison of 1369-1449 nucleotides. Bootstrap values (percentages of 1000 replications) only 50% or more are shown at nodes.

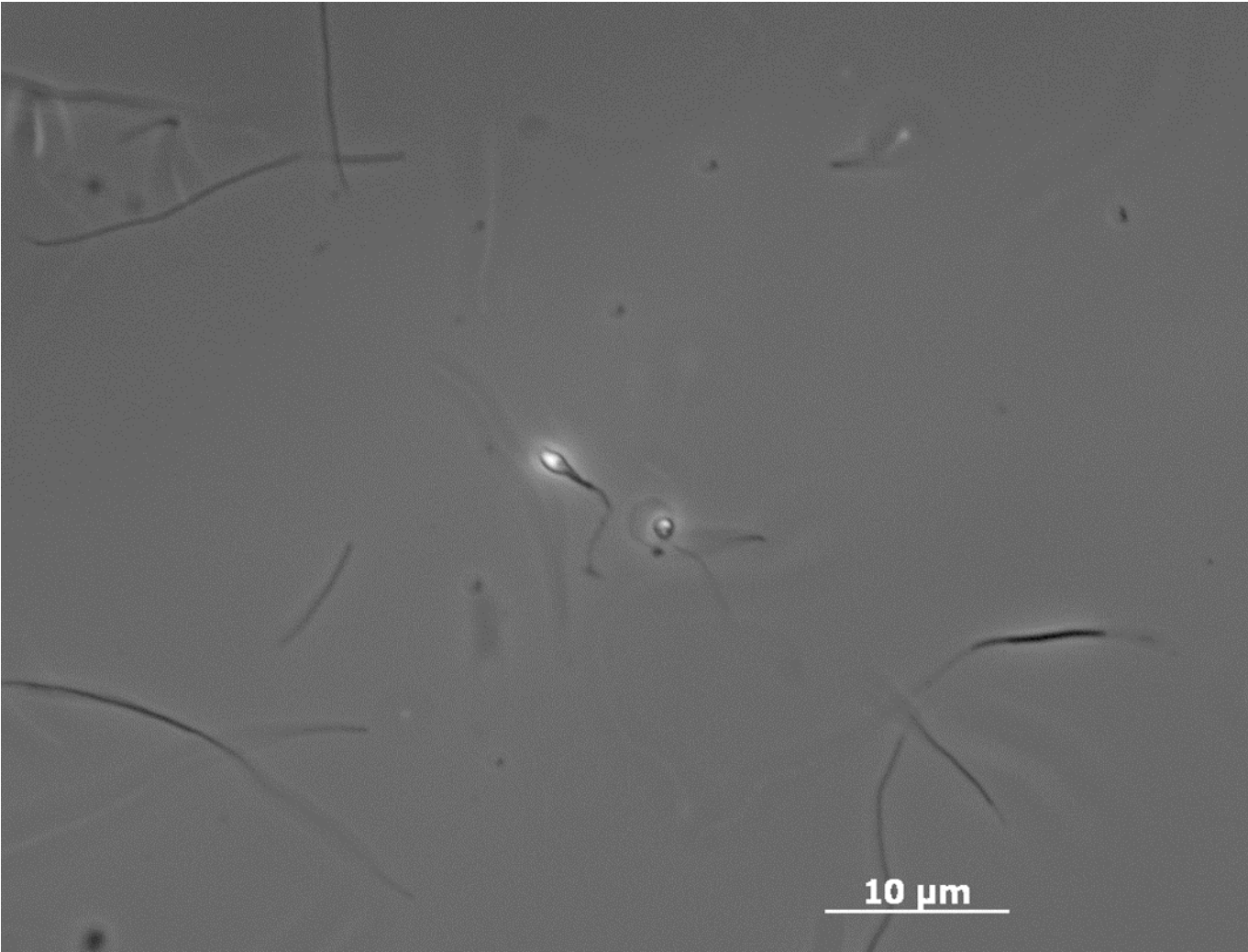
**Supplementary Figure S4:** Neighbour-joining tree based on 16S rRNA gene sequences of the strain HC45<sup>T</sup>, related environmental sequences and representatives from all classes in the phylum *Firmicutes*. This phylogenetic tree is based on a comparison of 1369-1449 nucleotides. Bootstrap values (percentages of 1000 replications) only 50% or more are shown at nodes.

**Supplementary Figure S5:** Minimum-evolution tree based on 16S rRNA gene sequences of the strain HC45<sup>T</sup>, related environmental sequences and representatives from all classes in the phylum *Firmicutes*. This phylogenetic tree is based on a comparison of 1369-1449 nucleotides. Bootstrap values (percentages of 1000 replications) only 50% or more are shown at nodes.

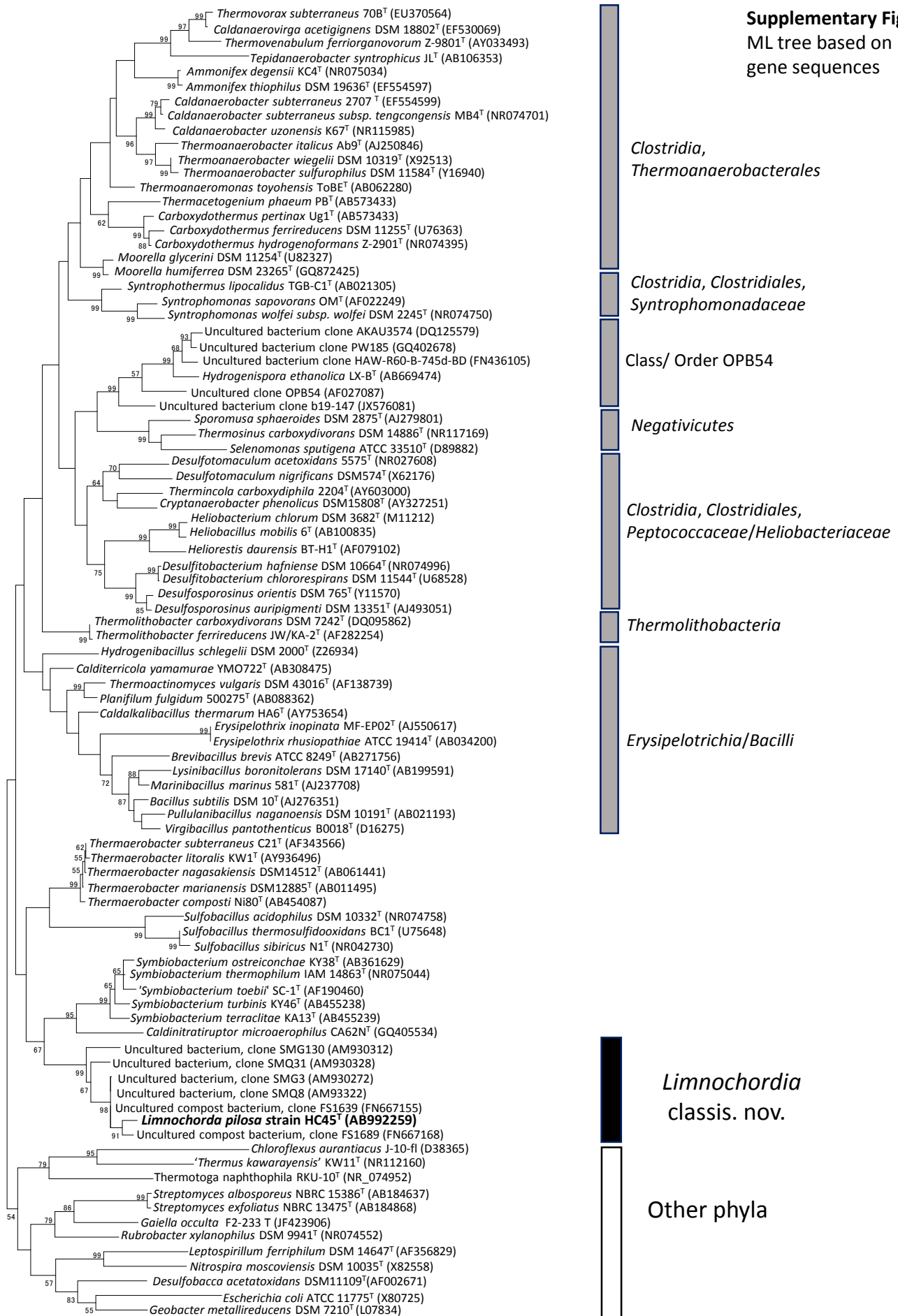
**Supplementary Figure S1:** The culturing procedure of strain HC45<sup>T</sup>.



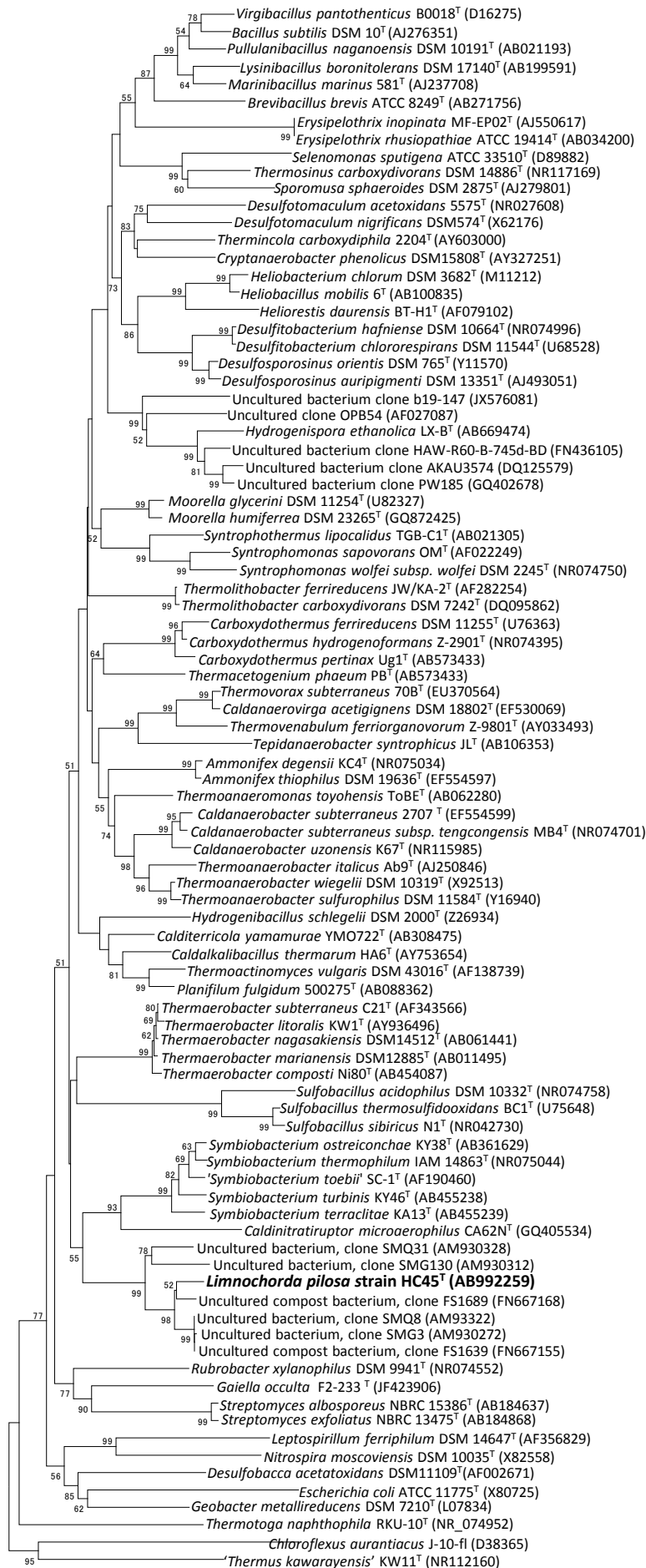
**Supplementary Figure S2:** : Phase-contrast micrograph showing endospore-like structure of the strain HC45<sup>T</sup>.



**Supplementary Figure S3:**  
ML tree based on 16S rRNA  
gene sequences



**Supplementary Figure S4:**  
NJ tree based on 16S rRNA  
gene sequences



*Bacilli 1*

*Erysipelotrichia*

*Negativicutes*

*Clostridia, Clostridiales,  
Peptococcaceae/Heliobacteriaceae*

*Class/ Order OPB54*

*Clostridia, Thermoanaerobacterales 1*

*Clostridia, Clostridiales,  
Syntrophomonadaceae*

*Thermolithobacteria*

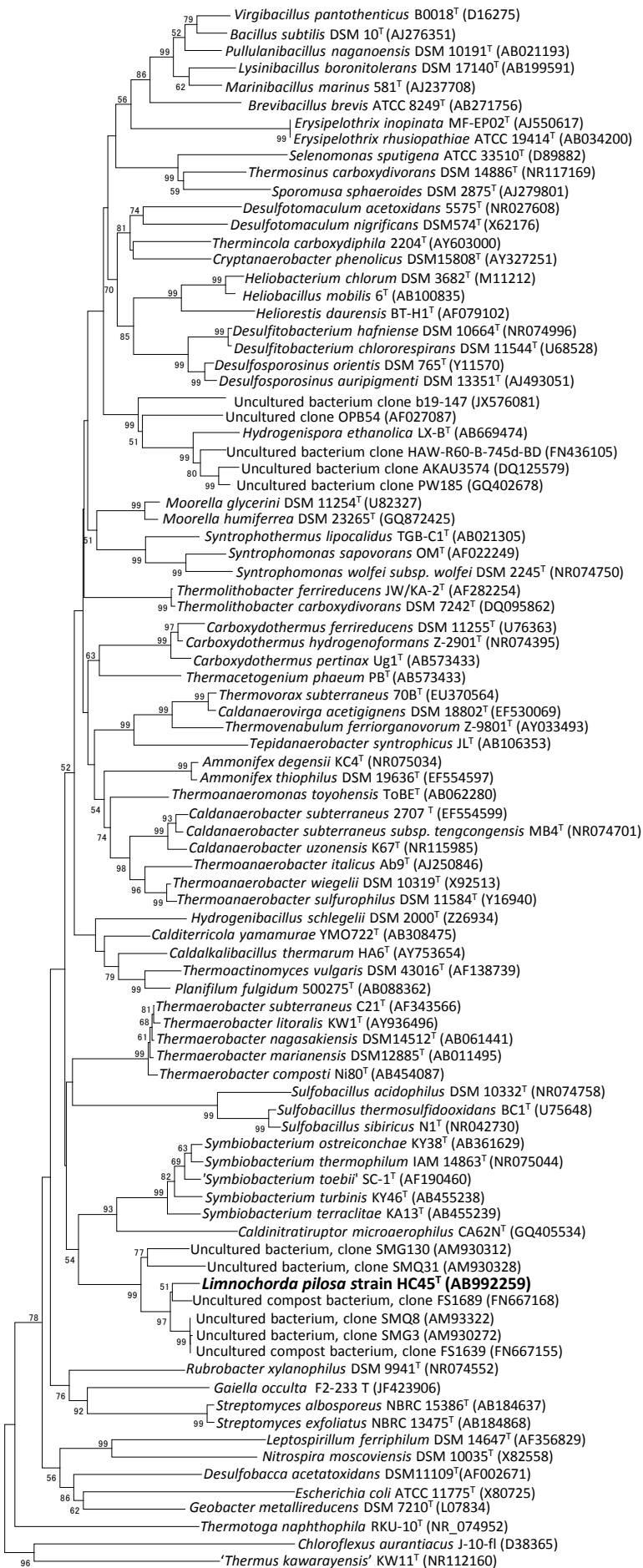
*Clostridia, Thermoanaerobacterales 2*

*Bacilli 2*

*Limnochordia*  
classis. nov.

*Other phyla*

**Supplementary Figure S5:**  
ME tree based on 16S rRNA  
gene sequences



Bacilli 1

Erysipelotrichia

Negativicutes

Clostridia, Clostridiales,  
Peptococcaceae/Heliobacteriaceae

Class/ Order OPB54

Clostridia, Thermoanaerobacterales 1

Clostridia, Clostridiales,  
Syntrophomonadaceae

Thermolithobacteria

Clostridia, Thermoanaerobacterales 2

Bacilli 2

Limnochordia  
classis. nov.

Other phyla

# Supplementary Table S1.

Cellular fatty acid content (percentage of total) of strain HC45<sup>T</sup>

| Fatty acids                | Percent |
|----------------------------|---------|
| C <sub>10:0</sub> 3-OH     | 0.22    |
| iso-C <sub>11:0</sub> 3-OH | 2.76    |
| C <sub>11:0</sub> 2-OH     | 0.40    |
| C <sub>12:0</sub> 2-OH     | 0.26    |
| iso-C <sub>13:0</sub>      | 2.40    |
| iso-C <sub>13:0</sub> 3-OH | 2.85    |
| anteiso-C <sub>13:0</sub>  | 2.84    |
| iso-C <sub>14:0</sub> 3-OH | 0.71    |
| iso-C <sub>14:0</sub>      | 4.57    |
| iso-C <sub>15:0</sub>      | 30.99   |
| iso-C <sub>15:0</sub> 3-OH | 2.84    |
| anteiso-C <sub>15:0</sub>  | 31.65   |
| C <sub>15:0</sub> 2-OH     | 1.77    |
| iso-C <sub>16:0</sub>      | 2.92    |
| C <sub>16:0</sub>          | 4.66    |
| iso-C <sub>17:0</sub>      | 2.47    |
| anteiso-C <sub>17:0</sub>  | 2.17    |
| C <sub>17:0</sub>          | 1.14    |
| C <sub>18:1</sub> ω 9c     | 0.54    |
| C <sub>18:0</sub>          | 0.62    |
| Summed Feature 3           | 1.22    |

Summed Feature 3 : C<sub>16:1</sub> ω 7c / C<sub>16:1</sub> ω 6c