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# PHYLOGENETIC RELATIONSHIPS BETWEEN *BACILLUS* SPECIES AND RELATED GENERA INFERRED FROM 16S RDNA SEQUENCES

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## ABSTRACT

Neighbor-joining, maximum-parsimony, minimum-evolution, maximum-likelihood and Bayesian trees constructed based on 16S rDNA sequences of 181 type strains of *Bacillus* species and related taxa manifested nine phylogenetic groups. The phylogenetic analysis showed that *Bacillus* was not a monophyletic group. *B. subtilis* was in Group 1. Group 4, 6 and 8 respectively consisted of thermophiles, halophilic or halotolerant bacilli and alkaliphilic bacilli. Group 2, 4 and 8 consisting of *Bacillus* species and related genera demonstrated that the current taxonomic system did not agree well with the 16S rDNA evolutionary trees. The position of Caryophanaceae and Planococcaceae in Group 2 suggested that they might be transferred into Bacillaceae, and the heterogeneity of Group 2 implied that some *Bacillus* species in it might belong to several new genera. Group 9 was mainly comprised of the genera (excluding *Bacillus*) of Bacillaceae, so some *Bacillus* species in Group 9: *B. salarius*, *B. qingdaonensis* and *B. thermcloacae* might not belong to *Bacillus*. Four *Bacillus* species, *B. schlegelii*, *B. tusciae*, *B. edaphicus* and *B. mucilaginosus* were clearly placed outside the nine groups.

**Keywords:** *Bacillus* phylogeny; Bayesian inference; Evolutionary trees; 16S rDNA

## INTRODUCTION

In recent years, numerous new species of genus *Bacillus* were reported and at the same time, many new genera of Bacillaceae were established. According to List of Prokaryotic names with Standing in Nomenclature (LPSN, http://www.bacterio.net) (6), of more than 140 *Bacillus* species, there were only 54 species reported before 2000; and of more than 30 genera of Bacillaceae, only six genera were established before 2000. *Bacillus* has long been regarded as a phylogenetic heterogeneous group (1). However, the phylogeny of the new *Bacillus* species and the new genera of Bacillaceae have not been roundly studied.

Numerical classification based on a series of phenetic characters was used for classification of 368 *Bacillus* strains into 79 clusters (23). After 1990, 16S rDNA has been successfully applied in determining phylogenetic

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relationships of the aerobic, endospore-forming bacteria, which played an important role in the creation of several families and genera of Bacillales (7).

Nowadays 16S rDNA is a vital standard for taxonomy of the bacteria. Goto *et al* (9) used partial 16S rDNA sequence for rapid identification of *Bacillus* species. Then Xu and Côté (34) used 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences to infer phylogenetic relationships among *Bacillus* species and related genera. However, the two phylogenetic trees from the above two papers did not seem to be convincing because of less DNA sequences (69 and 40, respectively) and short sequence lengths (1057 bp and 220 bp, respectively). Almost complete 16S rDNA sequences with high quality from recently reported *Bacillus* species are accessible in GenBank, which become ideal data for phylogenetic analyses. Moreover, new softwares (25, 28) executing Bayesian or ML algorithm (11) and personal computer hardwares with high computing capability facilitate further study on phylogeny.

The primary aim of the current investigation was to establish phylogenetic relationships between *Bacillus* species and related genera by reconstructing 16S rDNA phylogenetic trees using several algorithms.

## MATERIALS AND METHODS

*Bacillus* species, type species of the genera of Bacillaceae and type species of some families in Bacillales were selected for the phylogenetic study (LPSN updated date September 04, 2007). The 16S rDNA sequences of the type strains of the bacteria mentioned above were downloaded from the GenBank. If several 16S rDNA sequences from the type strain(s) of the same species were available, the longest one with the least non-AGTC characters would be selected. All the rectifiable ambiguous nucleotides in the selected sequences were corrected according to the homologous sequences searched by BLAST (http://130.14.29.110/BLAST/) and/or other 16S rDNA sequences of the type strain(s).

Nucleotide sequence alignments were made using ClustalX 1.83 (32) and optimized using Tune ClustalX (Hall 2004, http://homepage.mac.com/barryghall/TuneClustalX.html) by modifying multiple alignment parameters. Then Bioedit 7 (10) was used for refining the entire alignment by eye. Calculations of pairwise 16S rDNA sequences similarity were achieved using the EzTaxon server (http://www.eztaxon.org/) (4). *Escherichia coli* was used as the outgroup. The optimal models of nucleotide substitutions were estimated by the program Modeltest 3.7 (22), using hierarchical likelihood ratio tests (hLRT) and the Akaike Information Criterion (AIC).

Neighbor-joining (NJ), maximum-parsimony (MP) and minimum-evolution (ME) analyses were performed with MEGA 4 (29). NJ and ME analyses were performed using the maximum composite likelihood method and 1000 bootstrap replications. Maximum likelihood (ML)-based phylogenetic analyses were performed with RAxML-VI-HPC 4 (http://phylobench.vital-it.ch/raxml-bb/) using default parameters (bootstrap=100). Bayesian trees were inferred using MrBayes 3.1.2 (25) according to the MrBayes 3.1 Manual (2005). All the Markov chain Monte Carlo searches were run with four chains for 4,300,000 generations, with trees being sampled every 100 generations. The first 30000 trees were discarded as "burnin", keeping only trees generated well after those parameters stabilized.

## RESULTS AND DISCUSSION

182 16S rDNA sequences were selected but the 16S rDNA sequence of *B. mycoides* (AB021192) was identical to that of *B. weihenstephanensis* (19 to 1531 bp of AB021199),

so the former was omitted. TrN+I+G (30) and GTR (General time reversible (31)) +I+G models were selected according to hLRT and AIC of Modeltest 3.7, respectively. The Bayesian tree (Fig. 1.) was inferred by the GTR+I+G model while NJ (Fig. 2.) and ME (Fig. 3.) trees were inferred by the TrN+I+G model (Gamma distribution shape parameter = 0.5854).

Although the phylogeny of some bacteria was different among the trees (Fig.1. to Fig. 5.), the phylogeny of most bacteria studied was consistent. Therefore, nine groups could be set up from 181 taxa. Holder and Lewis (11) held that ML and Bayesian approaches were more advantageous than NJ, ME or MP methods, which was supported by the comparison of the five evolutionary trees. The Bayesian analysis of the 16S rDNA data set (181 taxa, 1603 sites) yielded a tree that supported with weak posterior probability (PP=0.5) the monophyly of nine groups (Fig. 1a). In general, the supports for the different groups were stronger in the Bayesian tree than other trees. This was particularly noticeable for Group 6 (PP=1) and Group 7 (PP=0.98) (Fig. 1e). Only in Bayesian tree were the supports (PP) for every group except Group 6 and Group 8 more than 0.5. We did not find any case where the other trees provided much stronger supports than the Bayesian tree for a given node in agreement with the general trend observed in the comparisons among these measures of statistical supports (16). The topology of the ML tree was similar with that of the Bayesian tree and the bootstrap supports of the ML tree were higher than those of NJ, ME or MP trees.

The Bayesian tree demonstrated that *Bacillus* was not a monophyletic group. The species in Group 4, 6 and 9 had similarities in their respective phenotypes while the species in other groups differed much in their phenotypes, which were in agreement with the results of Goto *et al*. (9) and Xu and Côté (34).

Stackebrandt and Swiderski (27) suggested *Bacillus* RNA group 1 (1) should be divided into at least four subgroups; while Fig. 1. demonstrated that the previous RNA group 1 harbored Group 1, 2, 3 and 5. (Note Group 2 also contained the previous RNA group 2). These four groups constituted the core of *Bacillus*, which embraced approximately 65% of the *Bacillus* species.

Group 1 (28 species) contained *B. subtilis*, the type species of *Bacillus*, which was confirmed in other phylogenetic trees (Fig. 1b). Group 2 could be clearly divided into two clusters (Fig. 1c). *B. cereus* cluster included 14 *Bacillus* species (*B. mycoides* not shown in Fig. 1) while *B. insolitus* cluster, i.e. *Bacillus* RNA group 2, contained nine *Bacillus* species and seven other genera including non-spore-forming *Kurthia* and *Caryophanon*. Stackebrandt and Swiderski called this cluster “evolutionary enigma” and “interesting taxonomic problem” (27). If this cluster were not present, Group 1, 2, 3 and 5 would be united. The presence of the complex heterogeneous cluster was consistent with the results of Rheims *et al*. (24); La Duc *et al*. (13) and Zhang *et al*. (37). The heterogeneity of the cluster implied that nine *Bacillus* species might belong to several potential genera in order to make classification consistent with phylogeny. For example, *B. insolitus* and *B. silvestris* would have to be described as novel genera (27).

Group 3 (32 species) contained more species than any other groups (Fig. 1d), but it was not present in MP tree and less supported in NJ or ME tree (bootstrap proportion, BP<0.1). Group 5 was a minor group including eight species, which was accommodated in Group 1 in the NJ or MP trees. In the ME tree, Group 5 was closely related to Group 1 while in the ML or Bayesian tree, it was related to Group 2. It followed that Group 5 was an individual group that could not be merged into Group 1 or 2.

Group 4 consisted of thermophilic bacteria (Fig. 1b). The 16S rDNA sequences of *B. thermantarcticus* and *Geobacillus* *thermoglucosidasius* ATCC 43742$^T$ (X60641) showed a similarity of 98.3%. According to the temperature and pH range suitable for growth and G + C content (17, 18), *B. thermantarcticus* might be transferred to *Geobacillus*. However, other four *Bacillus* species in this group, *B. methanolicus*, *B. aeolius*, *B. alveayuensis* and *B. smithii* could be obviously separated from *Geobacillus* by the low 16S rDNA similarities of 92%. The presence of this Group 4 was consistent with the rRNA group 5 (1).

Group 6 (18 species) (Fig. 1e) was composed of halophilic or halotolerant *Bacillus* species except *B. macyae* (26). The presence of this Group 6 was consistent with the rRNA group 6 (19), which was also supported by the result of Ghosh *et al.* (8). Group 7 (7 species) was entirely composed of new published *Bacillus* species. The MP, ME and NJ trees demonstrated the close relationship between Group 6 and 7, but the two groups were entirely separated in the Bayesian or ML tree (Fig. 1e, Fig. 5). Not all the species in Group 7 were halotolerant except *B. hwajinpoensis* and *B. decolorationis*, which were included in Group 6 according to Yoon *et al.* (36). Nevertheless, our Bayesian tree and ML tree confirmed the position of the two halotolerant species was in Group 7 in agreement with the result of Nowlan *et al*. (21).

The 16S rDNA sequences of the type strains in Group 8 and 9, which had distinct insert sequences between 89 to 90 bp (*B. subtilis* AB042061 numbering), showed marked differences from those in other groups. Group 8 consisted of alkaliphilic and halotolerant bacteria (Fig. 1e) except that *B. mannanilyticus* was not halotolerant (20). *Caldalkalibacillus thermarum* was a thermophile and a peculiar member of Group 8, of which the 16S rDNA showed similarity of less than 92% to those of other *Bacillus* species in Group 8. Group 9 mainly consisted of the genera (excluding *Bacillus*) of Bacillaceae (Fig. 1f), but it had four *Bacillus* species: *B. taeanensis*, *B. salarius*, *B. qingdaonensis* and *B. thermcloacae*. The former three species were included in Group 8 using NJ methods by Lim *et al*. (14, 15) and Wang *et al*. (33), for the feasible reason that the authors did not use enough 16S rDNA sequences of the type strains of Group 9 for phylogenetic analysis. Therefore, the taxonomic positions of the four species were doubtful. The low 16S rDNA similarities (less than 92.5%) between the latter three species and their respective closest relatives in *Bacillus* suggested the latter three species were worthy to be reclassified.

There were four *Bacillus* species outside the nine groups in the 181 taxa phylogenetic trees (Fig. 1a). *B. schlegelii* and *B. tusciae* were thermophilic and facultatively chemolithoautotrophic bacteria with high G + C content of the genome (2). Their 16S rDNA sequences respectively showed a very low level of similarity (less than 90%) with respect to other 180 sequences, which demonstrated that *B. schlegelii* and *B. tusciae* might well belong to two as-yet-undescribed new genera. This opinion showed agreement with the views expressed by Stackebrandt and Swiderski (27) who held that *B. schlegelii*, *B. tusciae* and *B. thermcloacae* were potential new genera. *B. edaphicus* and *B. mucilaginosus* were always clustered with *Paenibacillus polymyxa*, and their relatively high 16S rDNA sequence similarity values (96-97%) to the nearest relatives (*P. elgii* and *P. chinjuensis*, respectively) indicated that *B. edaphicus* and *B. mucilaginosus* might be transferred into *Paenibacillus*. The facts mentioned above revealed that at least nine *Bacillus* species (one in Group 4, four in Group 9 and four outside 9 groups) might not be really *Bacillus* species. Their phylogenetic positions would not be determined pending further polyphasic taxonomic studies.

In the NJ and ME trees, *Ureibacillus*, *B. thermcloacae* and *Exiguobacterium* formed a clade with a bootstrap value of less than 50%; while in the ML, MP and Bayesian trees, *Ureibacillus* was in Group 2 and the latter two were in Group

9, where their phylogenetic positions were supported by BP or PP of more than 50%. The phylogeny of five species was uncertain (Table 1). Low 16S rDNA sequences similarities (<93%) were found between *Sporolactobacillus inulinus* and other 181 taxa. *S. inulinus* showed scanty growth in air, was catalase-negative and not thermophilic (12), which manifested different phenotypes from the species of Group 7 or Group 4. Moreover, in the Bayesian and ML trees, BP or PP (both less than 0.5) could not ensure its position between Group 7 and 9. *B. infernus*, *B. thermoamylovorans*, *B. coagulans* and *B. azotoformans* were in their respective groups of the Bayesian tree with PP of more than 0.5 but their positions could not be supported by BP of less than 50% in the NJ, ME, MP or ML tree. For example, the former three thermophilic bacteria were included in Group 4 with BP of less than 35%. Therefore, their positions in the Bayesian tree were more credible. Originally, *B. infernus* was clustered with *B. methanolicus* (3) and *B. thermoamylovorans* was grouped with *Geobacillus* and *Saccharococcus* (5), but bootstrap analyses were not done in the studies.

The evolutionary relationships among the nine groups differed much in the five phylogenetic trees. However, it was noteworthy that Group 8 was always an outlier of the other eight groups. Because of relatively high PP of more than 0.5, the phylogenetic relationships illustrated by the Bayesian tree, which agreed well with the ML tree, were more believable. In the five evolutionary trees, the phylogeny of the genera in Bacillales did not conform to the current well-known taxonomic system (7) (Table 2). The genera in Bacillaceae except *Bacillus* were mainly in Group 9, 2 and 4, while *Vulcanibacillus* was outside the nine groups, suggesting that it should be removed from Bacillaceae. Caryophanaceae and Planococcaceae (represented by *Kurthia*) were clustered in Group 2, confirming the result of Yoon *et al.* (35) and implying that they might be accommodated in Bacillaceae.

**Table 1** Disputable positions of 5 species in 5 phylogenetic trees.

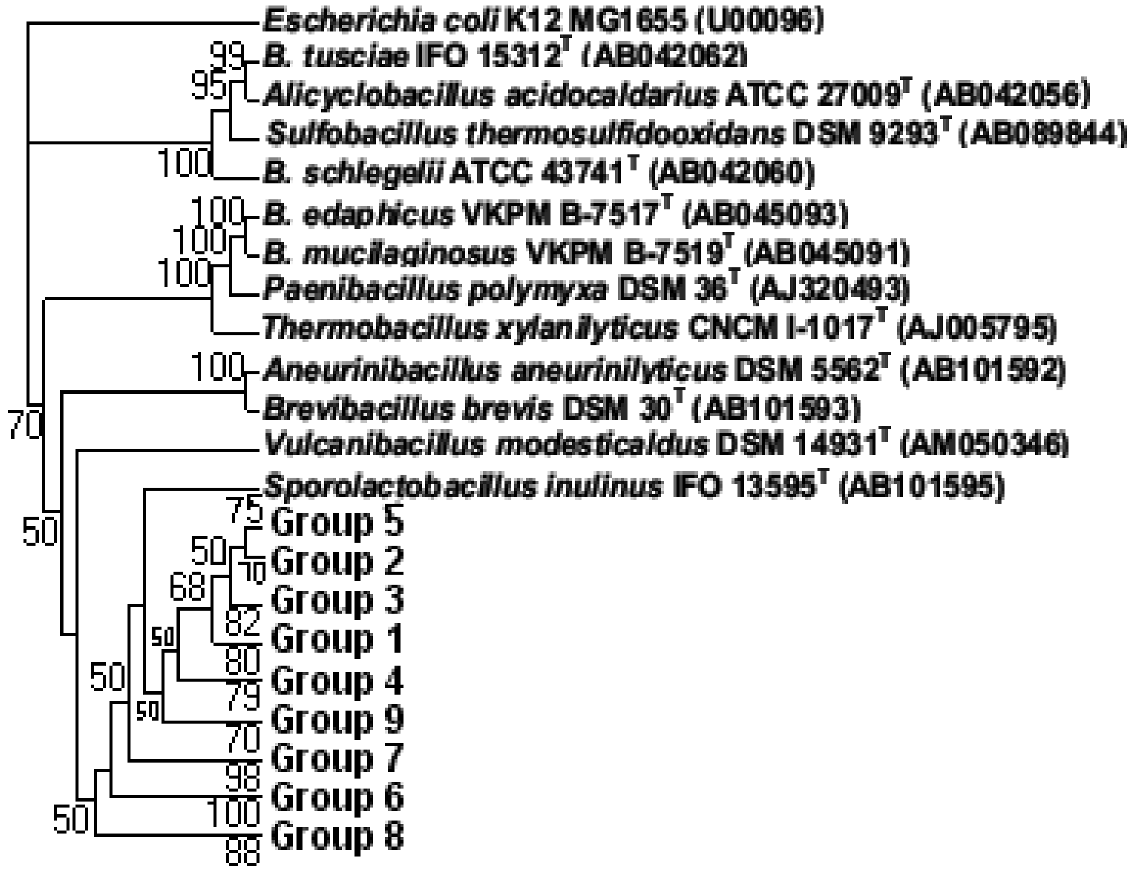
| | Bayesian | ML | ME | NJ | MP |
|---|---|---|---|---|---|
| *B. infernus* | Group 3 | Group 3 | Group 4 | Group 3 | Group 4 |
| *B. thermoamylovorans* | Group 4 | Group 1 | Group 4 | Group 4 | Group 1 |
| *B. coagulans* | Group 1 | Group 1 | Group 4 | Group 4 | Group 1 |
| *B. azotoformans* | Group 5 | Group 2 | Group 3 | Group 3 | Group 3 |
| *S. inulinus* | N[a] | N[a] | Group 7 | Group 4 | Group 7 |

[a] N, Not included in any group

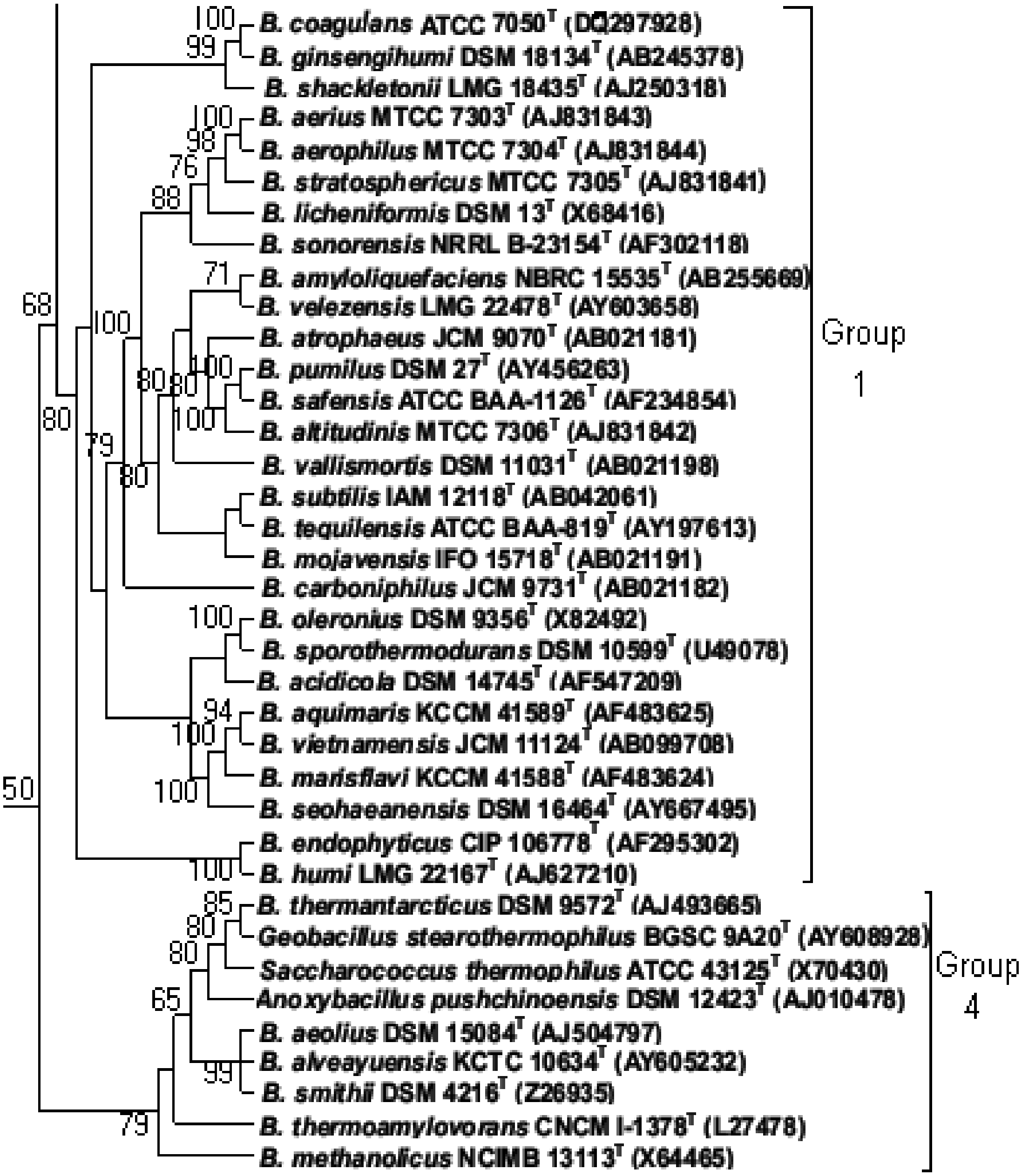
**Table 2** The relationship between obtained *Bacillus* species phylogeny and the current taxonomy.

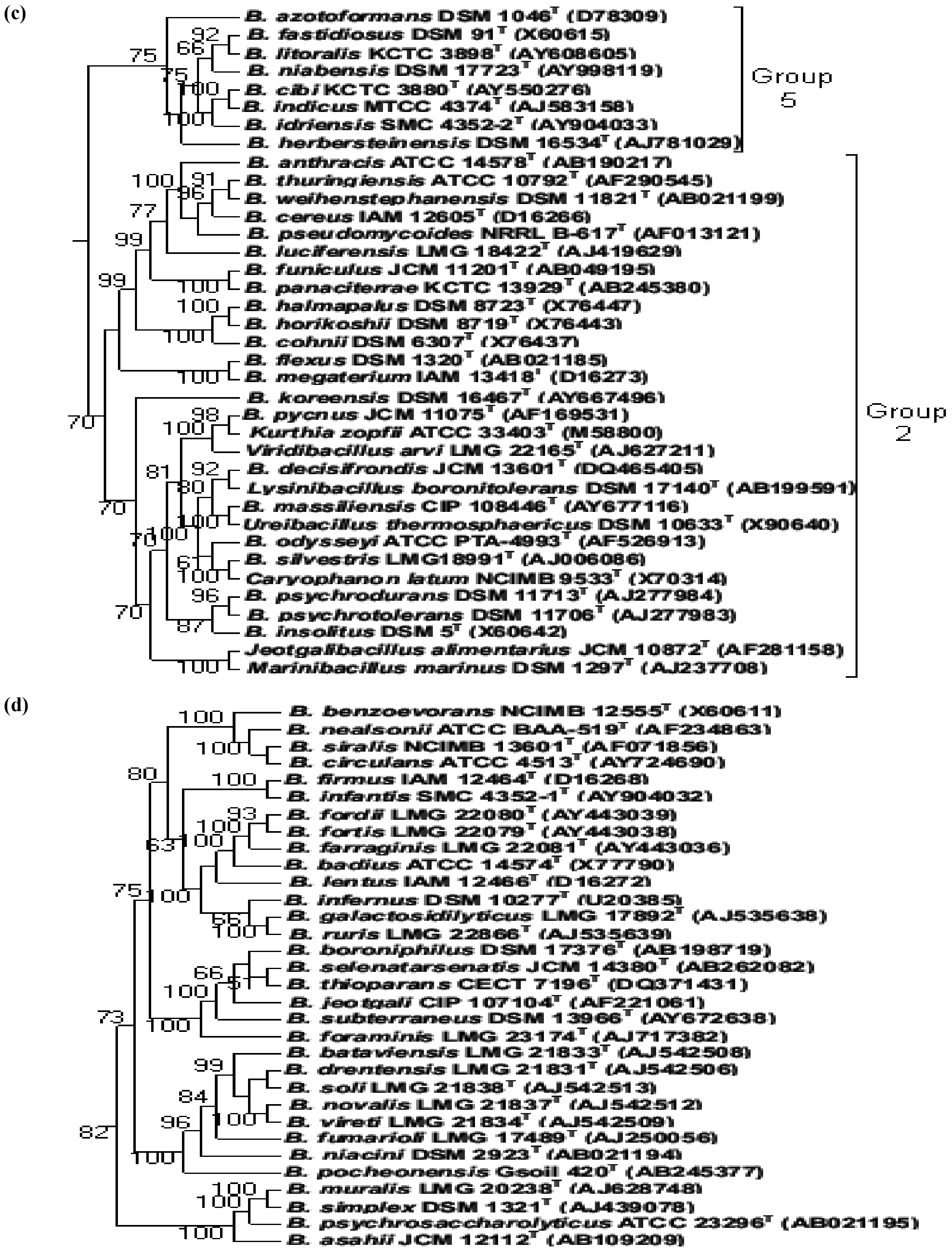
| Group number | Composition |
|---|---|
| 1 | *Bacillus* |
| 2 | 5 genus from Bacillaceae,1 genus from Caryophanaceae, 1 genus from Planococcaceae |
| 3 | *Bacillus* |
| 4 | 4 genus belong to Family Bacillaceae |
| 5 | *Bacillus* |
| 6 | *Bacillus* |
| 7 | *Bacillus* |
| 8 | *Bacillus* and *Caldalkalibacillus* (belong to Family Bacillaceae) |
| 9 | 25 genus belong to Family Bacillaceae |
| Outside 9 groups | 2 genus from Alicyclobacillaceae, 4 genus from Paenibacillaceae, 2 genus from Bacillaceae |

**(a)**



**(b)**


100
99
*B. coagulans* ATCC 7050$^T$ (DQ297928)
*B. ginsengihumi* DSM 18134$^T$ (AB245378)
*B. shackletonii* LMG 18435$^T$ (AJ250318)
100
98
76
88
*B. aerius* MTCC 7303$^T$ (AJ831843)
*B. aerophilus* MTCC 7304$^T$ (AJ831844)
*B. stratosphericus* MTCC 7305$^T$ (AJ831841)
*B. licheniformis* DSM 13$^T$ (X68416)
*B. sonorensis* NRRL B-23154$^T$ (AF302118)
71
*B. amyloliquefaciens* NBRC 15535$^T$ (AB255669)
*B. velezensis* LMG 22478$^T$ (AY603658)
68
100
*B. atrophaeus* JCM 9070$^T$ (AB021181)
100
80
80
*B. pumilus* DSM 27$^T$ (AY456263)
*B. safensis* ATCC BAA-1126$^T$ (AF234854)
80
100
*B. altitudinis* MTCC 7306$^T$ (AJ831842)
79
*B. vallismortis* DSM 11031$^T$ (AB021198)
80
*B. subtilis* IAM 12118$^T$ (AB042061)
*B. tequilensis* ATCC BAA-819$^T$ (AY197613)
*B. mojavensis* IFO 15718$^T$ (AB021191)
*B. carboniphilus* JCM 9731$^T$ (AB021182)
100
*B. oleronius* DSM 9356$^T$ (X82492)
*B. sporothermodurans* DSM 10599$^T$ (U49078)
*B. acidicola* DSM 14745$^T$ (AF547209)
94
100
*B. aquimaris* KCCM 41589$^T$ (AF483625)
*B. vietnamensis* JCM 11124$^T$ (AB099708)
*B. marisflavi* KCCM 41588$^T$ (AF483624)
50
100
*B. seohaeanensis* DSM 16464$^T$ (AY667495)
*B. endophyticus* CIP 106778$^T$ (AF295302)
100
*B. humi* LMG 22167$^T$ (AJ627210)
Group 1
85
80
80
*B. thermantarcticus* DSM 9572$^T$ (AJ493665)
*Geobacillus stearothermophilus* BGSC 9A20$^T$ (AY608928)
*Saccharococcus thermophilus* ATCC 43125$^T$ (X70430)
65
*Anoxybacillus pushchinoensis* DSM 12423$^T$ (AJ010478)
*B. aeolius* DSM 15084$^T$ (AJ504797)
*B. alveayuensis* KCTC 10634$^T$ (AY605232)
99
*B. smithii* DSM 4216$^T$ (Z26935)
79
*B. thermoamylovorans* CNCM I-1378$^T$ (L27478)
*B. methanolicus* NCIMB 13113$^T$ (X64465)
Group 4


(c)
B. azotoformans DSM 1046T (D78309)
B. fastidiosus DSM 91T (X60615)
B. litoralis KCTC 3898T (AY608605)
B. niabensis DSM 17723T (AY998119)
B. cibi KCTC 3880T (AY550276)
B. indicus MTCC 4374T (AJ583158)
B. idriensis SMC 4352-2T (AY904033)
B. herbersteinensis DSM 16534T (AJ781029)
Group 5
B. anthracis ATCC 14578T (AB190217)
B. thuringiensis ATCC 10792T (AF290545)
B. weihenstephanensis DSM 11821T (AB021199)
B. cereus IAM 12605T (D16266)
B. pseudomycoides NRRL B-617T (AF013121)
B. luciferensis LMG 18422T (AJ419629)
B. funiculus JCM 11201T (AB049195)
B. panaciterrae KCTC 13929T (AB245380)
B. halmapalus DSM 8723T (X76447)
B. horikoshii DSM 8719T (X76443)
B. cohnii DSM 6307T (X76437)
B. flexus DSM 1320T (AB021185)
B. megaterium IAM 13418T (D16273)
B. koreensis DSM 16467T (AY667496)
B. pycnus JCM 11075T (AF169531)
Kurthia zopfii ATCC 33403T (M58800)
Viridibacillus arvi LMG 22165T (AJ627211)
B. decisifrondis JCM 13601T (DQ465405)
Lysinibacillus boronitolerans DSM 17140T (AB199591)
B. massiliensis CIP 108446T (AY677116)
Ureibacillus thermosphaericus DSM 10633T (X90640)
B. odysseyi ATCC PTA-4993T (AF526913)
B. silvestris LMG18991T (AJ006086)
Caryophanon latum NCIMB 9533T (X70314)
B. psychrodurans DSM 11713T (AJ277984)
B. psychrotolerans DSM 11706T (AJ277983)
B. insolitus DSM 5T (X60642)
Jeotgalibacillus alimentarius JCM 10872T (AF281158)
Marinibacillus marinus DSM 1297T (AJ237708)
Group 2
75
92
66
75
100
100
100
91
96
77
99
99
100
100
100
100
70
98
100
92
81
80
100
70
100
67
100
96
70
87
100
(d)
B. benzoevorans NCIMB 12555T (X60611)
B. nealsonii ATCC BAA-519T (AF234863)
B. siralis NCIMB 13601T (AF071856)
B. circulans ATCC 4513T (AY724690)
B. firmus IAM 12464T (D16268)
B. infantis SMC 4352-1T (AY904032)
B. fordii LMG 22080T (AY443039)
B. fortis LMG 22079T (AY443038)
B. farraginis LMG 22081T (AY443036)
B. badius ATCC 14574T (X77790)
B. lentus IAM 12466T (D16272)
B. infernus DSM 10277T (U20385)
B. galactosidilyticus LMG 17892T (AJ535638)
B. ruris LMG 22866T (AJ535639)
B. boroniphilus DSM 17376T (AB198719)
B. selenatarsenatis JCM 14380T (AB262082)
B. thioparans CECT 7196T (DQ371431)
B. jeotgali CIP 107104T (AF221061)
B. subterraneus DSM 13966T (AY672638)
B. foraminis LMG 23174T (AJ717382)
B. bataviensis LMG 21833T (AJ542508)
B. drentensis LMG 21831T (AJ542506)
B. soli LMG 21838T (AJ542513)
B. novalis LMG 21837T (AJ542512)
B. vireti LMG 21834T (AJ542509)
B. fumarioli LMG 17489T (AJ250056)
B. niacini DSM 2923T (AB021194)
B. pocheonensis Gsoil 420T (AB245377)
B. muralis LMG 20238T (AJ628748)
B. simplex DSM 1321T (AJ439078)
B. psychrosaccharolyticus ATCC 23296T (AB021195)
B. asahii JCM 12112T (AB109209)
100
100
80
100
93
100
63
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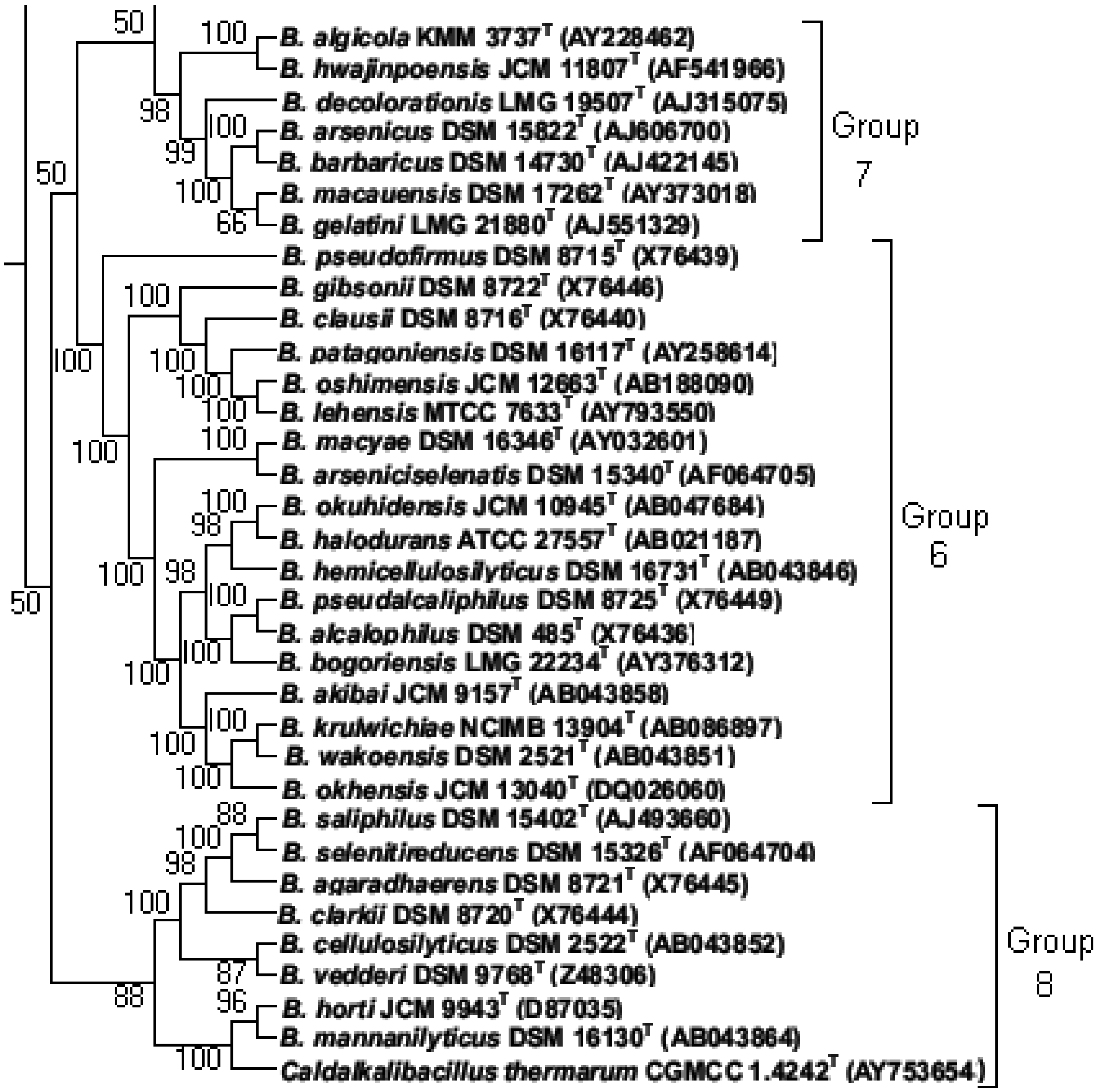
(e)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B. bogoriensis LMG 22234T (AY376312)
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B. saliphilus DSM 15402T (AJ493660)
B. selenitireducens DSM 15326T (AF064704)
B. agaradhaerens DSM 8721T (X76445)
B. clarkii DSM 8720T (X76444)
B. cellulosilyticus DSM 2522T (AB043852)
B. vedderi DSM 9768T (Z48306)
B. horti JCM 9943T (D87035)
B. mannanilyticus DSM 16130T (AB043864)
Caldalkalibacillus thermarum CGMCC 1.4242T (AY753654)
Group 7
Group 6
Group 8
50
50
98
100
99
100
100
66
50
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100
100
87
88
96
100

**(f)**

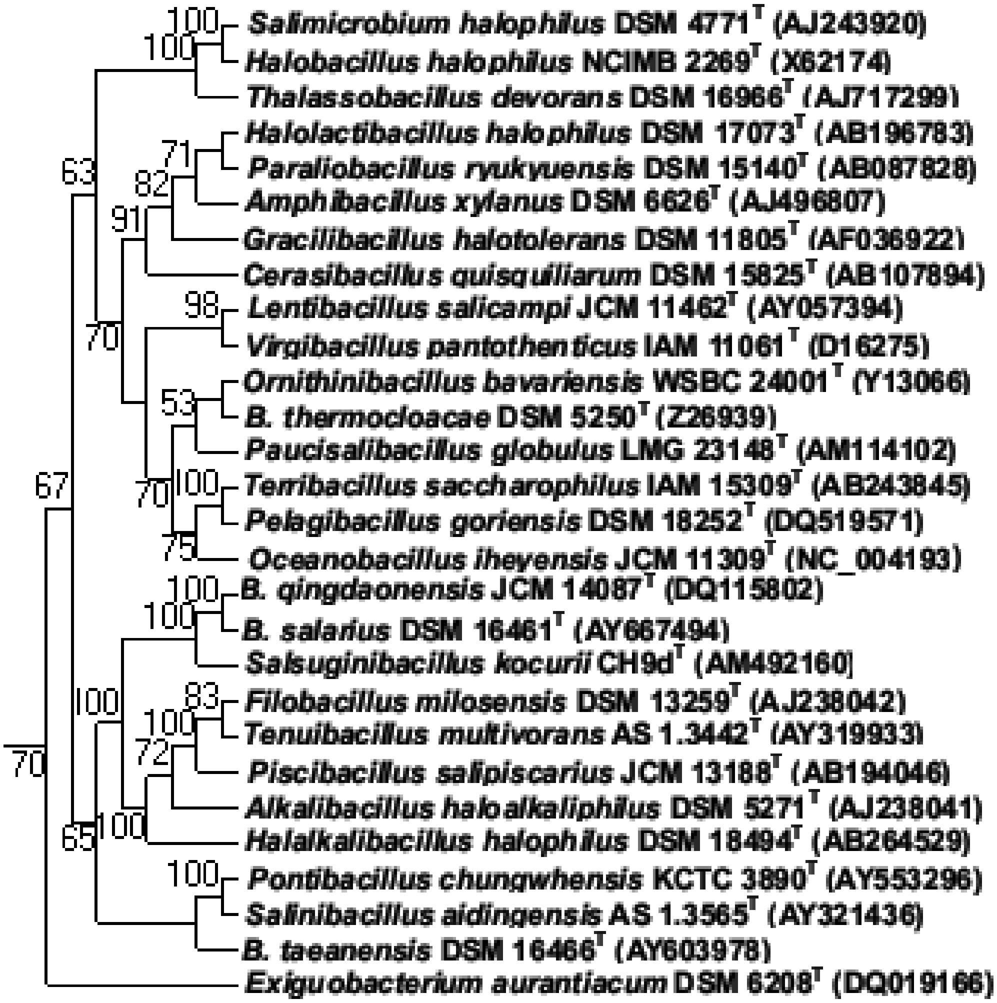

**Figure 1.** Bayesian cladistic tree constructed with the 16S rDNA sequences of the type strains of *Bacillus* species and related genera (181 taxa, 1603 sites; GTR+I+G plus covarion model) demonstrating 9 groups (a), and the species in every group were illustrated as (b) Group 1 and 4, (c) Group 2 and 5, (d) Group 3, (e) Group 6, 7 and 8, and (f) Group 9. Bootstrap confidence levels greater than 50% are indicated at the internodes.

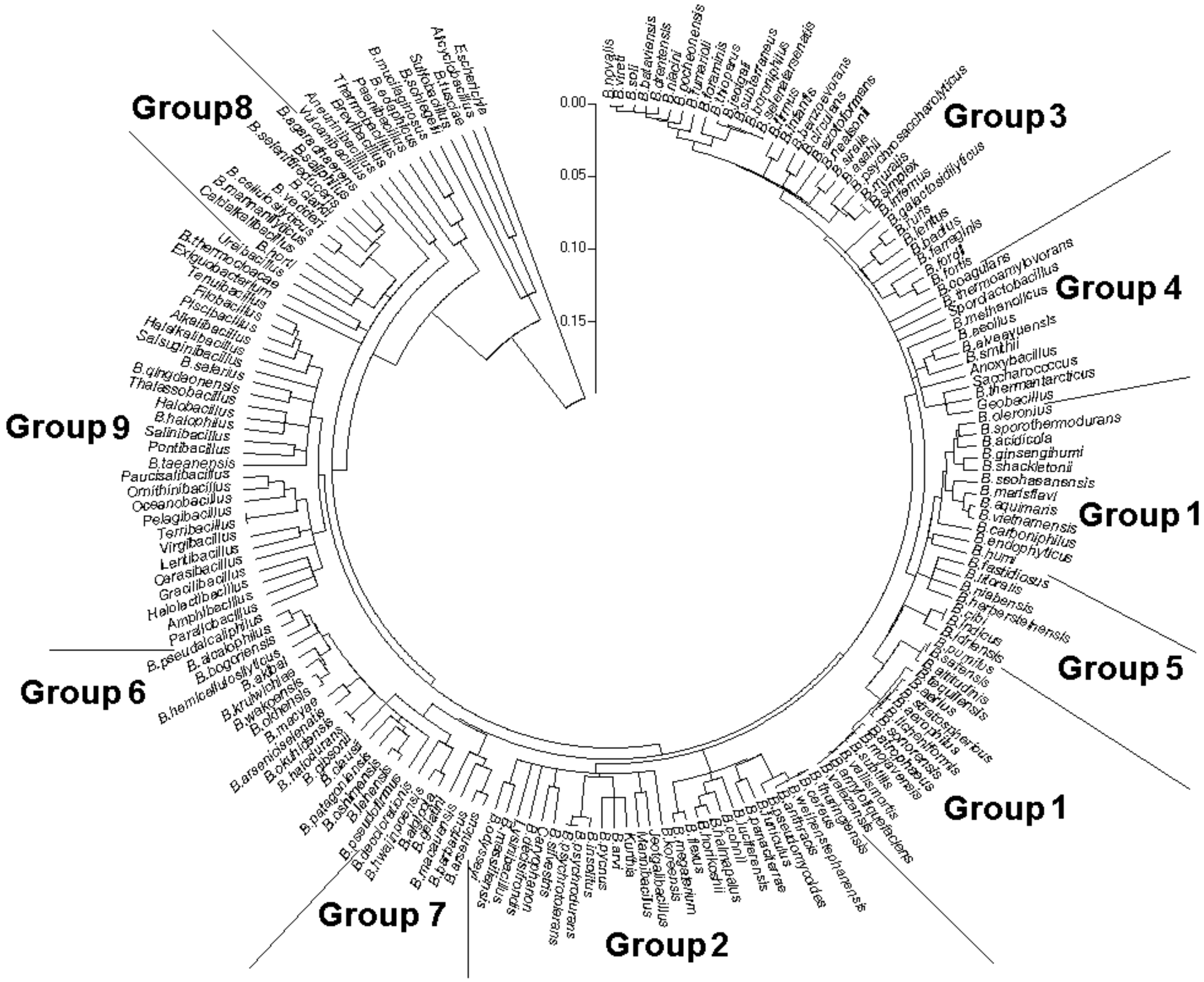

**Figure 2.** 16S rDNA neighbor-joining tree (based on 1000 bootstrap replications). The differences in the composition bias among sequences were considered in evolutionary comparisons. Alignment gaps and missing data were eliminated in pairwise sequence comparisons. Bar, 0.05 changes per nucleotide position.

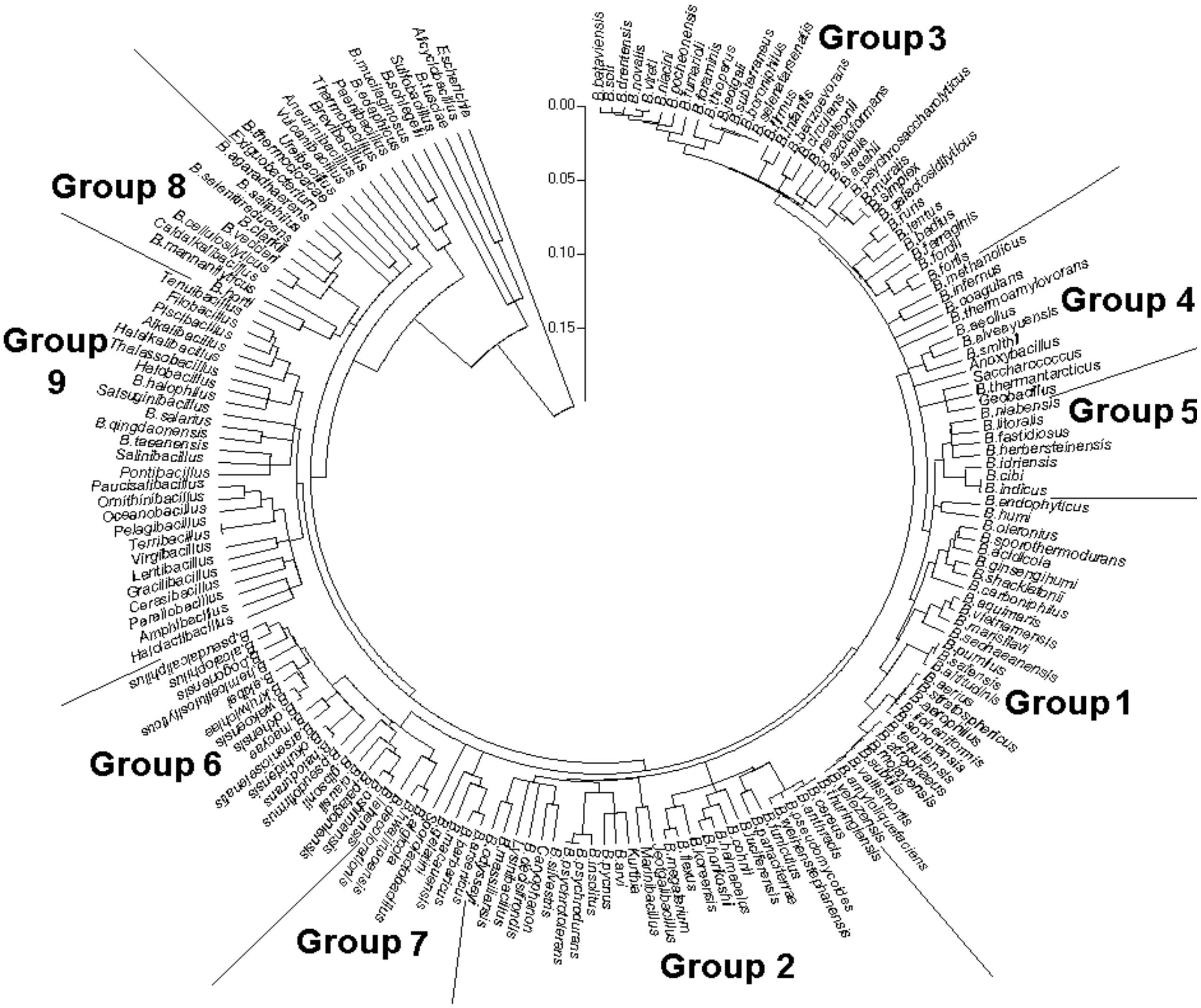

**Figure 3.** 16S rDNA minimum evolution tree (based on 1000 bootstrap replications). The tree was searched using the Close-Neighbor-Interchange algorithm at a search level of 2. The differences in the composition bias among sequences were considered in evolutionary comparisons. Alignment gaps and missing data were eliminated in pairwise sequence comparisons. Bar, 0.05 changes per nucleotide position.

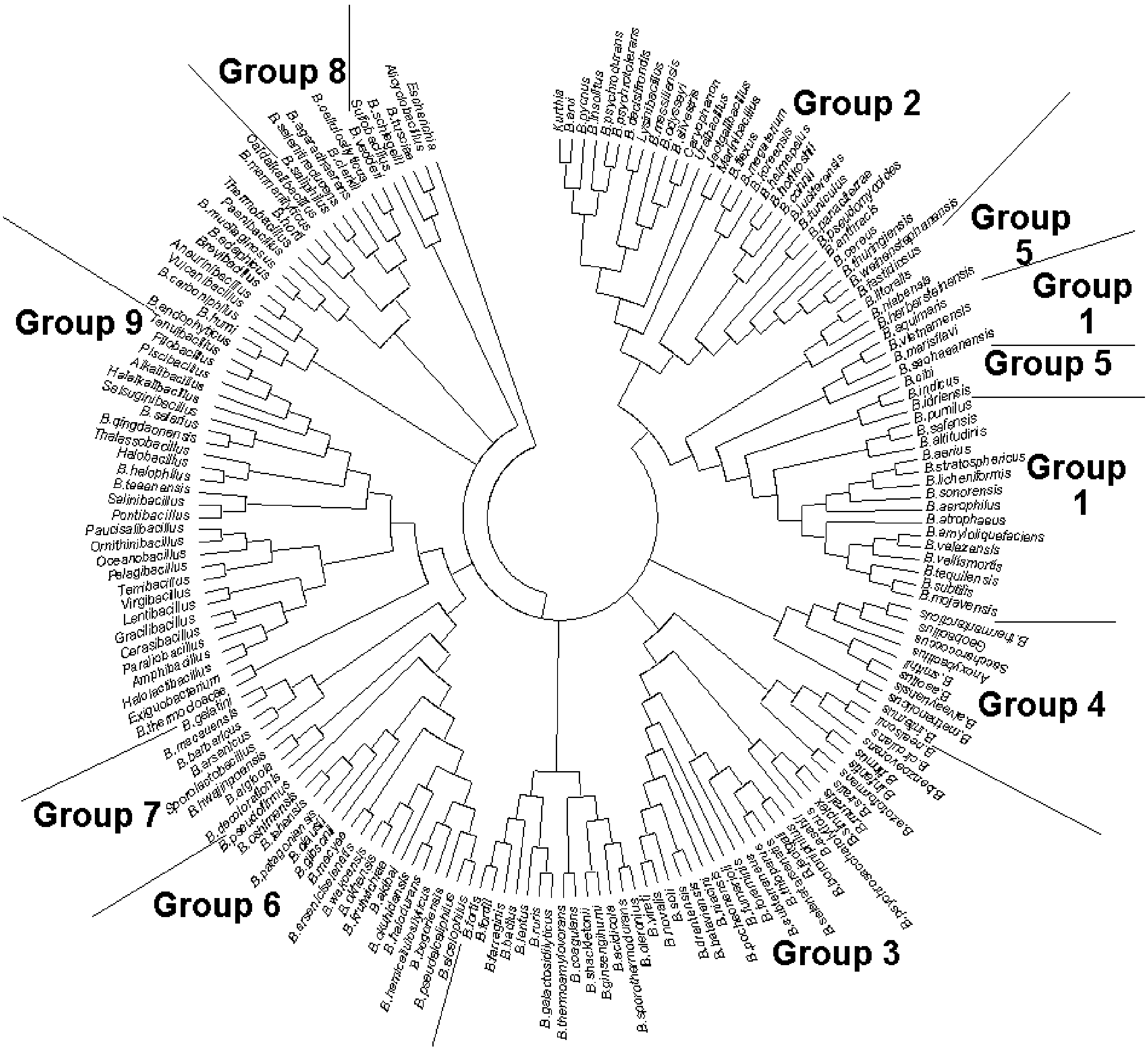

**Figure 4.** The maximum-parsimony phylogenetic tree derived from 16S rDNA sequences. The tree (based on 1000 bootstrap replications) was obtained using the Close-Neighbor-Interchange algorithm with search level 3 in which the initial trees were obtained with the random addition of sequences (10 replicates). All alignment gaps were treated as missing data. *Escherichia coli* was used as the outgroup.

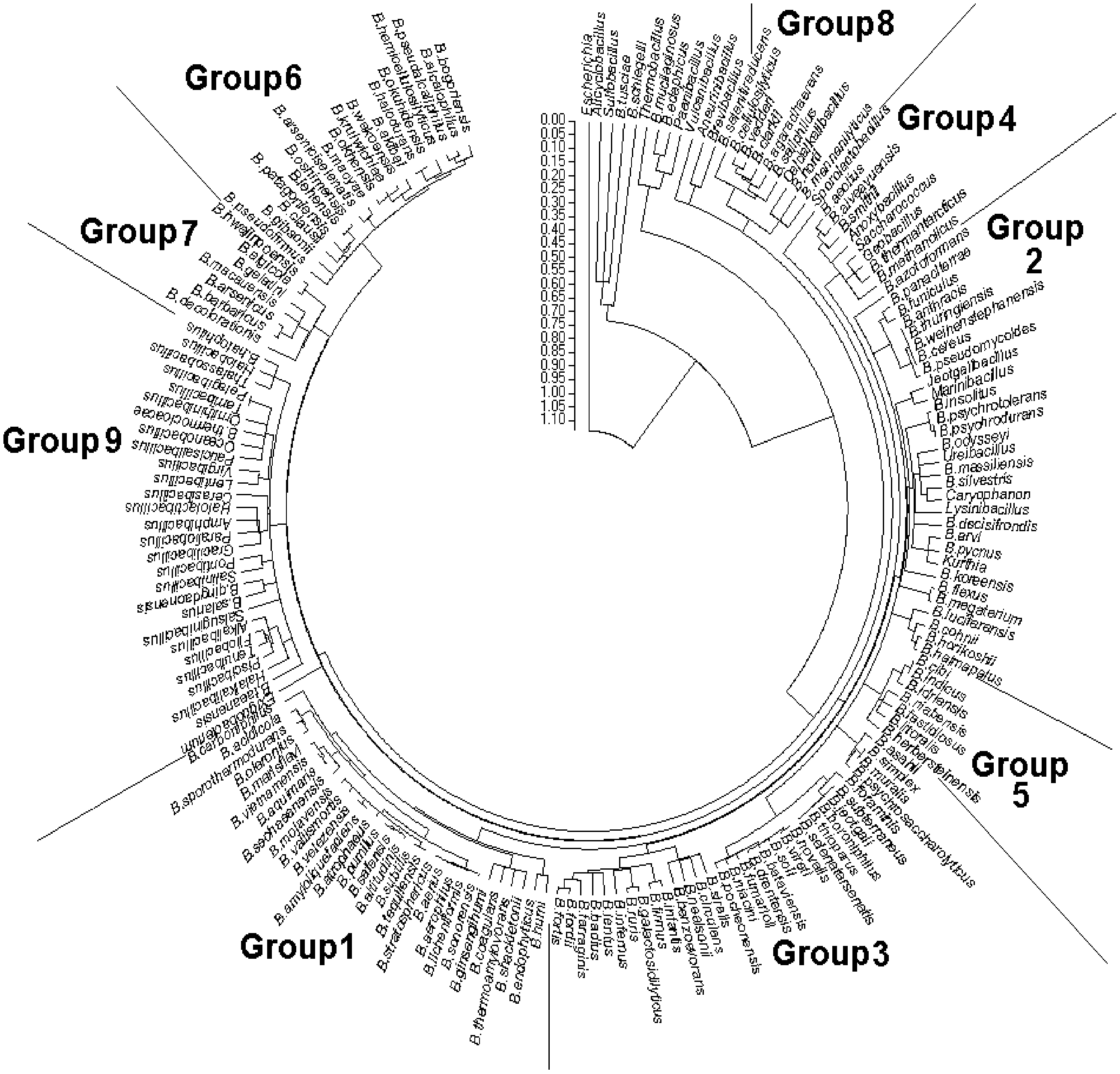

**Figure 5.** 16S rDNA maximum likelihood tree (based on 100 bootstrap replications). The tree was searched using RAxML-VI-HPC version 4.0.0 (Stamatakis, 2007). RAxML executed 100 rapid bootstrap inferences and thereafter a thorough ML search with GTR model of nucleotide substitution. All free model parameters was estimated by RaxML. Bar, 0.01 change per nucleotide position.

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## RESUMO

### Relações filogenéticas entre espécies de *Bacillus* e gêneros relacionados baseadas em sequencias 16S rDNA

Árvores utilizando os métodos de neighbor-joining, máxima parcimônia, evolução mínima, máxima verossimilhança e bayesiana, construídas baseadas em seqüências de rDNA 16S de 181 linhagens-tipo de espécies de *Bacillus* e taxa relacionados, mostraram a formação de nove grupos filogenéticos. A análise filogenética mostrou que *Bacillus* não é um grupo monofilético. *B. subtilis* se colocou no Grupo 1. Grupos 4, 6 e 8, respectivamente, consistiram de bacilos termofílicos, halofílicos ou halotolerantes e alcalifílicos. Grupos 2, 4 e 8 consistindo de espécies de *Bacillus* e gêneros relacionados demonstraram que o sistema taxonômico corrente não concorda perfeitamente com as árvores evolucionárias por rDNA 16S. A posição de Caryophanaceae e Planococcaceae no Grupo 2 sugere que estes podem ser transferidos para Bacillaceae, e a heterogeneidade do Grupo 2 implica em que algumas espécies de *Bacillus* neste grupo podem pertencer a vários novos gêneros. O Grupo 9 foi principalmente composto de gêneros de Bacillaceae (excluindo *Bacillus*), portanto algumas espécies de *Bacillus* no Grupo 9: *B. salarius*, *B. qingdaonensis* e *B. thermcloacae* podem não pertencer a *Bacillus*. Quatro espécies de *Bacillus*, *B. schlegelii*, *B. tusciae*, *B. edaphicus* e *B. mucilaginosus* foram claramente colocadas fora dos nove grupos.

**Palavras-chave:** filogenia de *Bacillus*, inferência Bayesiana, árvores evolucionárias, 16S rDNA

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