

Order *Bacillales*

Subjects: Microbiology

Contributor: Sharareh Harirchi, Taner Sar, Mohaddaseh Ramezani, Habibu Aliyu, Zahra Etemadifar, Seyed Ali Nojoumi, Fatemeh Yazdian, Mukesh Kumar Awasthi, Mohammad J. Taherzadeh

Bacillales (later heterotypic synonyms of *Caryophanales*) is the most productive order of the phylum *Firmicutes*. The enormous diversity of the order, which includes numerous genera and species. The Order *Bacillales*, the type order of the class *Bacilli*, was approved in the list of bacterial names in 1980.

Keywords: Bacillales ; Caryophanales ; taxonomy ; biotechnology

1. Introduction

Bacillales (later heterotypic synonyms of *Caryophanales*) is the most productive order of the phylum *Firmicutes*. The enormous diversity of the order, which includes numerous genera and species, resulted in scanty detailed studies of the diverse taxa. Based on 16S rRNA gene sequencing analysis, phylogenomics, and other approaches, *Bacillales* is delineated to include ten validly published families ^[1]. Of these, the family *Bacillaceae* comprises certain strains capable of surviving under various conditions, which, from an anthropogenic perspective, are considered extreme, including high or low temperatures and pH ranges and high salt concentrations. In addition to these extremes individually, some members of *Bacillaceae* can survive under multiple combinations of the above extremes and, hence, are considered polyextremophiles ^[2]. The ability to withstand harsh conditions placed members of the order *Bacillales* at the center of interest for various industrial applications, and ^[3] up to now, additional biotechnologically relevant insights are being gained, even among the well-known species and strains.

2. Order Bacillales

2.1. Taxonomy

In the revised roadmap of the phylum *Firmicutes*, Ludwig et al. 2009 delineated the *Bacillales* Prévot 1953 (Approved Lists 1980), the type order of the class *Bacilli*, to include eight families ^{[4][5]}. This followed the removal of two families, including *Caryophanaceae* Peshkoff 1939 (Approved Lists 1980) and the reassignment of its type genus, *Caryophanon*, to *Planococcaceae* Krasilnikov 1949 (Approved Lists 1980), despite the priority of the former family ^{[4][6]}. Of note, the authors also questioned the descriptive validity of the name *Caryophanaceae*, thereby virtually promoting the propagation of the name *Bacillales* Prévot 1953 (Approved Lists 1980). The Approved Lists 1980 included the above families and the orders *Caryophanales* Peshkoff 1939 and *Bacillales* Prévot 1953 ^[6]. As highlighted recently by Tindall 2019, the list did not provide a clear recommendation for the assignment of taxa above the genus level and, since only one name could correctly represent the order, considering appropriate rules of the International Code of Nomenclature of Prokaryotes. Considering the priority of *Caryophanales* Peshkoff 1939 over *Bacillales* Prévot 1953 and the inclusion of the nomenclatural types of both, *Caryophanon* Peshkoff 1939 and *Bacillus* Cohn 1872, respectively, in the same order, the correct name of the order is *Caryophanales* Peshkoff 1939 ^{[7][8]}. Thus, *Bacillales* Prévot 1953 (Approved Lists 1980) represents the later heterotypic synonyms of *Caryophanales* Peshkoff 1939 ^{[6][9]}.

The Order *Bacillales*, the type order of the class *Bacilli*, was approved in the list of bacterial names in 1980 ^{[2][9]}. Bergey's Manual of Systematic Bacteriology has completely described this order completely based on 16S rRNA gene sequencing analysis and other polyphasic approaches such as chemotaxonomy and phenotypic methods ^{[2][10]}. Recently, based on the rules provided by The International Code of Nomenclature of Prokaryotes (ICNP), the name *Caryophanales* should be used instead of the name *Bacillales*. Since these names are heterotypic synonyms, however, the name approved first has the priority to be used. In this case, the name *Caryophanales* Peshkoff 1933 was approved prior to the name *Bacillales* Prévot 1953. Hence, the name *Caryophanales* Peshkoff 1933 is the correct name for the order *Bacillales* ^[8].

As with other taxa, the previous classification of the order *Bacillales* relied heavily on 16S rRNA gene sequences, resulting in noticeable anomalies. For example, several spores- and non-spore-forming families and genera group together,

suggesting that a single gene marker does not provide sufficient resolving power for delineating the order *Bacillales* [11]. Recent phylogenomic approaches, notably the work of de Maayer et al. 2019, attempted to resolve the evolutionary relationship among strains affiliated to the order based on comparative genomics [11][12].

Despite referencing the later heterotypic synonym, *Bacillales*, the authors proposed eleven distinct families in the order *Bacillales* and an unplaced group (Table 1).

The allocation of the Incertae sedis families agrees with the phylogenetic outline in volume 3 of Bergey's Manual of Systematic Bacteriology [2][9]. Although *Bacillaceae* is the most imposing and well-known family, *Listeriaceae* consisting of *Listeria* and *Brochothrix* is also noteworthy. Moreover, *Pasteuriaceae*, some genera of the family *Paenibacillaceae* such as *Gorillibacterium* or *Brevibacillus*, and *Staphylococcus* have clinical and pathological importance [2][13]. Based on a consensus phylogenomic strategy, de Maayer et al. resolved the observed anomalies in the family *Bacillaceae*. For instance, although the genus *Staphylococcus* comprises pathogenic strains previously grouped in the family *Micrococcaceae*, the phylogenetic and molecular analysis did not reveal any close relationships between them, resulting in the proposal for a new family *Staphylococcaceae* [11]. However, the precise identification of its members at the species level needs arduous efforts and may fail if based only on phenotypic approaches [14]. Another heterogeneous and polyphyletic family in this order is *Planococcaceae*, with many phylogenetic works focused on the history of its evolution, but its demarcation remains unresolved [11][15][16]. The classification in this family is based on 16S rRNA gene nucleotide signature, phenotypic characteristics, and observed branching in the drawn phylogenetic trees. However, these methods do not have enough resolving power at the genus and species level and cause significant overlap with other species of the families *Bacillaceae* and Incertae sedis 19 [11]. It is noteworthy that *Planococcaceae* represents the later heterotypic synonym of *Caryophanaceae*, as highlighted earlier by Tindal [8] and recently supported by Gupta and Patal [17].

Below the family level, the 16s rRNA gene-based classification of the genus *Bacillus* and especially the *Cereus* clade also represents some challenges [18]. This method remains the gold standard for assigning microbial strains to various taxa due to its low-cost and reproducibility between laboratories worldwide, providing people with an overview of the microbial strains for further research. At present, the *Cereus* clade comprises *B. cereus*, *B. anthracis*, *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus cytotoxicus*, and *Bacillus toyonensis*. Different phylogenomics approaches have clarified the interrelationship of the otherwise incoherent genus *Bacillus* [18][19][20]. Gupta et al. 2020 also showed that a subset of the core proteins of *Bacillus* species, including concatenated proteins GyrA_B-RpoB-C and PolA-UvrD, provide consistent clustering strain in the genus [14][21]. In addition to whole genome approaches, which provide consistent means of identifying and classifying *Bacillales* at different taxonomic ranks, relevant techniques for identifying industrially important strains may be pertinent toward effective industrial deployment [11][22][23].

Table 1. List of families assigned to the order *Bacillales* [2][8][9][11][17][20][24][25].

Family Name	Proposed by	IJSEM ¹ Validation List No. (Year)	Type Genus	Involved Valid and Invalid Genera Until 2022 in LPSN ²	Valid Genera with Correct Name until 2022 in LPSN
<i>Alicyclobacillaceae</i>	da Costa and Rainey 2010	132 (2010)	<i>Alicyclobacillus</i>	5	5
<i>Bacillaceae</i>	Fischer 1895	(1980)	<i>Bacillus</i>	134	117
<i>Caryophanaceae</i>	Peshkoff 1939	(1980)	<i>Caryophanon</i>	26	19
<i>Desulfuribacillaceae</i>	Sorokin et al. 2021	200 (2021)	<i>Desulfuribacillus</i>	1	1
<i>Listeriaceae</i>	Ludwig et al. 2010	132 (2010)	<i>Listeria</i>	3	2
<i>Paenibacillaceae</i>	De Vos et al. 2010	132 (2010)	<i>Paenibacillus</i>	19	16
<i>Pasteuriaceae</i>	Laurent 1890	(1980)	<i>Pasteuria</i>	1	1
<i>Sporolactobacillaceae</i>	Ludwig et al. 2010	132 (2010)	<i>Sporolactobacillus</i>	5	5
<i>Staphylococcaceae</i>	Schleifer and Bell 2010	132 (2010)	<i>Staphylococcus</i>	12	10

Family Name	Proposed by	IJSEM ¹ Validation List No. (Year)	Type Genus	Involved Valid and Invalid Genera Until 2022 in LPSN ²	Valid Genera with Correct Name until 2022 in LPSN
<i>Thermoactinomycetaceae</i>	Matsuo et al. 2006	(2007)	<i>Thermoactinomyces</i>	25	24
Incertae sedis 46	-	-	-	1	1

¹ International Journal of Systematic and Evolutionary Microbiology. ² List of Prokaryotic names with Standing in Nomenclature.

2.2. General Characteristics

General characteristics of bacteria include a wide range of features such as the shape and arrangement of the bacterial cells, cell wall chemical type, spore formation ability, motility, growth conditions, and resistance and tolerance to antibiotics. [13][26]. Interestingly, the genera of order *Bacillales* are phenotypically diverse. For example, cell shapes change from spherical to filamentous and may be motile by flagella. Oxygen requirement has a broad range from strictly aerobic, microaerophilic, facultative anaerobic, and aerotolerant to strictly anaerobic [2][27]. One of the most important characteristics of the order *Bacillales* is endospore formation, although some exceptions exist [2][3]. However, these exceptions remained to be clarified whether from one side lacking the genes for spore formation, or losing all or some of them during evolution, or alternatively, unfavorable physiological conditions cause unsuccessful spore formation [12]. Furthermore, the cell wall of order *Bacillales* stains Gram-positive for young cells, in general, but some genera react Gram-negatively, such as *Aidingibacillus*, *Aquisalibacillus*, *Bhargavaea*, *Caldibacillus*, *Caryophanon*, *Exiguobacterium*, *Mammaliococcus*, *Novibacillus*, *Sediminibacillus*, and *Thalassorhabdus* [2][9][27][28]. Additionally, most of the genera allocated to this order have menaquinone 7 (MK-7) as their respiratory quinone, while various exceptions have been found [2].

References

1. Tindall, B.J. Names at the rank of class, subclass and order, their typification and current status: Supplementary information to Opinion 79. Judicial Commission of the International Committee on Systematics of Prokaryotes. Int. J. Syst. Evol. Microbiol. 2014, 64, 3599–3602.
2. Vos, P.; Garrity, G.M.; Jones, D.; Krieg, N.R.; Ludwig, W.; Rainey, F.A.; Schleifer, K.; Whitman, W.B. (Eds.) Bergey's Manual of Systematic Bacteriology: Volume 3: The Firmicutes; Springer: New York, NY, USA, 2010.
3. Mandic-Mulec, I.; Stefanic, P.; van Elsas, J.D. Ecology of Bacillaceae. Microbiol. Spectrum. 2015, 3, 1–24.
4. Ludwig, W.; Schleifer, K.-H.; Whitman, W.B. Revised road map to the phylum Firmicutes. Bergey's Man. Syst. Bacteriol. 2009, 2, 1–8.
5. Brenner, D.J.; Krieg, N.R.; Staley, J.T. Appendix 2. Taxonomic outline of the Archaea and Bacteria. In Bergey's Manual of Systematic Bacteriology, 2nd ed.; Garrity, G.M., Staley, J.T., Boone, D.R., Brenner, D.J., Vos, P.D., Goodfellow, M., Krieg, N.R., Rainey, F.A., Schleifer, K., Eds.; Springer International Publishing: New York, NY, USA, 2005; pp. 207–220.
6. Skerman, V.B.D.; McGowan, V.; Sneath, P.H.A. Approved lists of bacterial names. Int. J. Syst. Bacteriol. 1980, 30, 225–420.
7. Parker, C.T.; Tindall, B.J.; Garrity, G.M. International Code of Nomenclature of Prokaryotes. Int. J. Syst. Evol. Microbiol. 2019, 69, S1–S111.
8. Tindall, B.J. When treated as heterotypic synonyms the names Caryophanaceae Peshkoff 1939 (Approved Lists 1980) and Caryophanales Peshkoff 1939 (Approved Lists 1980) have priority over the names Planococcaceae Krasil'nikov 1949 (Approved Lists 1980) and Bacillales Prévot 1953 (Approved Lists 1980) and Bacillales Prévot 1953 (Approved Lists 1980), respectively. Int. J. Syst. Evol. Microbiol. 2019, 69, 2187–2195.
9. NamesforLife Bacterial and Archaeal Nomenclature. Available online: <https://www.namesforlife.com/search> (accessed on 20 August 2022).
10. Vandamme, P.; Pot, B.; Gillis, M.; de Vos, P.; Kersters, K.; Swings, J. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 1996, 60, 407–438.
11. De Maayer, P.; Aliyu, H.; Cowan, D.A. Reorganising the order Bacillales through phylogenomics. Syst. Appl. Microbiol. 2018, 42, 178–189.

12. Fritze, D. Taxonomy of the Genus *Bacillus* and Related Genera: The Aerobic Endospore-Forming Bacteria. *Phytopathology* 2004, 94, 1245–1248.
13. Madigan, M.; Bender, K.; Buckley, D.; Sattley, W.; Stahl, D. *Brock Biology of Microorganisms*; Pearson: London, UK, 2014.
14. Gherardi, G.; Di Bonaventura, G.; Savini, V. Chapter 1-Staphylococcal Taxonomy, in *Pet-To-Man Travelling Staphylococci*; Savini, V., Ed.; Academic Press: Cambridge, MA, USA, 2018; pp. 1–10.
15. Yoon, J.H.; Kang, S.S.; Lee, K.C.; Lee, E.S.; Kho, Y.H.; Kang, K.H.; Park, Y.H. *Planomicrobium koreense* gen. nov., sp. nov., a bacterium isolated from the Korean traditional fermented seafood jeotgal, and transfer of *Planococcus okeanoikoites* (Nakagawa et al. 1996) and *Planococcus mcmeekinii* (Junge et al. 1998) to the genus *Planomicrobium*. *Int. J. Syst. Evol. Microbiol.* 2001, 51, 1511–1520.
16. Yarza, P.; Ludwig, W.; Euzéby, J.; Amann, R.; Schleifer, K.-H.; Glöckner, F.O.; Rosselló-Móra, R. Update of the All-Species Living Tree Project based on 16S and 23S rRNA sequence analyses. *Syst. Appl. Microbiol.* 2010, 33, 291–299.
17. Gupta, R.S.; Patel, S. Robust Demarcation of the Family Caryophanaceae (*Planococcaceae*) and Its Different Genera Including Three Novel Genera Based on Phylogenomics and Highly Specific Molecular Signatures. *Front. Microbiol.* 2020, 10, 2821.
18. Maughan, H.; van der Auwera, G. *Bacillus* taxonomy in the genomic era finds phenotypes to be essential though often misleading. *Infect. Genet. Evol.* 2011, 11, 789–797.
19. Ehling-Schulz, M.; Lereclus, D.; Koehler, T.M. The *Bacillus cereus* Group: *Bacillus* Species with Pathogenic Potential. *Microbiol. Spectr.* 2019, 7.
20. Patel, S.; Gupta, R.S. A phylogenomic and comparative genomic framework for resolving the polyphyly of the genus *Bacillus*: Proposal for six new genera of *Bacillus* species, *Peribacillus* gen. nov., *Cytobacillus* gen. nov., *Mesobacillus* gen. nov., *Neobacillus* gen. nov., *Metabacillus* gen. nov. and *Alkalihalobacillus* gen. nov. *Int. J. Syst. Evol. Microbiol.* 2020, 70, 406–438.
21. Gaballa, A.; Cheng, R.A.; Trmcic, A.; Kovac, J.; Kent, D.J.; Martin, N.H.; Wiedmann, M. Development of a database and standardized approach for *rpoB* sequence-based subtyping and identification of aerobic spore-forming *Bacillales*. *J. Microbiol. Methods* 2021, 191, 106350.
22. Parks, D.H.; Chuvpochina, M.; Waite, D.W.; Rinke, C.; Skarszewski, A.; Chaumeil, P.-A.; Hugenholtz, P. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat. Biotechnol.* 2018, 36, 996–1004.
23. Whitman, W.B. Genome sequences as the type material for taxonomic descriptions of prokaryotes. *Syst. Appl. Microbiol.* 2015, 38, 217–222.
24. Gupta, R.S.; Patel, S.; Saini, N.; Chen, S. Robust Demarcation of 17 Distinct *Bacillus* Species Clades, Proposed as Novel *Bacillaceae* Genera, by Phylogenomics and Comparative Genomic Analyses: Description of *Robertmurraya kyonggiensis* Sp. Nov. And Proposal for an Emended Genus *Bacillus* Limiting It Only to the Members of the *Subtilis* and *Cereus* Clades of Species. *Int. J. Syst. Evol. Microbiol.* 2020, 70, 5753–5798.
25. Parte, A.C.; Carbasse, J.S.; Meier-Kolthoff, J.; Reimer, L.C.; Göker, M. List of Prokaryotic Names with Standing in Nomenclature (Lpsn) Moves to the Dsmz. *Int. J. Syst. Evol. Microbiol.* 2020, 70, 5607–5612.
26. Tille, P.M. *Bailey & Scott's Diagnostic Microbiology*; Elsevier: Amsterdam, The Netherlands, 2014.
27. Harwood, C.R. *Bacillus*; Springer: New York, NY, USA, 2013.
28. Driks, A.; Eichenberger, P. *The Bacterial Spore: From Molecules to Systems*; ASM Press: Washington, DC, USA, 2016.