

25 The Family *Planococcaceae*

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Abstract

Planococcaceae, a family within the order *Bacillales*, embraces 14 genera: *Bhargavaea*, *Caryophanon*, *Chryseomicrobium*, *Filibacter*, *Jeotgalibacillus*, *Kurthia*, *Paenisporosarcina*, *Planococcus*, *Planomicrobium*, *Rummeliibacillus*, *Solibacillus*, *Sporosarcina*, *Ureibacillus*, and *Viridibacillus*. Members of the family are Gram-variable, spore forming or nonspore forming, and motile or nonmotile; morphology varies from trichomes in case of *Caryophanon*, filamentous in case of *Filibacter* to rods or rod-cocci, or spherical rods in case of other genera. Diagnostic amino acid in the peptidoglycan is L-lysine with a peptidoglycan variation of A4 α type. Most dominating fatty acids of the family are iso-C_{15:0} or anteiso-C_{15:0} or iso-C_{16:0} or C_{16:1(ω11c)} or anteiso-C_{17:0} or C_{16:1(ω7c)} alcohol. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine and unidentified phospholipids and glycolipids except in case of *Jeotgalibacillus* are the predominant lipids. The G+C values of DNA for the family *Planococcaceae* ranges from 34 to 54 %. Phylogenetically a member of *Firmicutes* group and is closely related to *Bacillaceae*.

Short Description of the Family

Taxonomy, Historical, and Current

Plan.o.coc.ca'ce.ae. N.L. masc. n. *Planococcus*, type genus of the family; L. suff. *-aceae*, ending denoting family; N.L. fem. pl. n. *Planococcaceae*, the *Planococcus* family. The family was described by Krasil'nikov in 1949.

Phylogenetically the family *Planococcaceae* is a member of the order *Bacillales* (Prévot 1953) and the phylum is *Firmicutes*. *Planococcaceae* embraces the type genus *Planococcus* (Migula 1894; emend. Nakagawa et al. 1996; emend. Yoon et al. 2010b), and other genera are *Bhargavaea* (Manorama et al. 2009; emend. Verma et al. 2012), *Caryophanon* (Peshkoff 1939), *Chryseomicrobium* (Arora et al. 2011), *Filibacter* (Maiden and Jones 1985), *Jeotgalibacillus* (Yoon et al. 2001a), *Kurthia* (Trevisan 1885), *Paenisporosarcina* (Krishnamurthi et al. 2009b), *Planomicrobium* (Yoon et al. 2001b), *Rummeliibacillus* (Vaishampayan et al. 2009), *Solibacillus* (Krishnamurthi et al. 2009a), *Sporosarcina* (De Vos et al. 2009; Kluyver and van Niel 1936; emend. Yoon et al. 2001c), *Ureibacillus*

(Fortina et al. 2001), and *Viridibacillus* (Albert et al. 2007) (<http://www.bacterio.cict.fr/classifgenerafamilies.html#Planococcaceae>). Most of the genera are Gram-positive except the genera *Filibacter* (Maiden and Jones 1984) and *Ureibacillus* (Fortina et al. 2001), which stained Gram-negative. Besides these, the genera *Jeotgalibacillus* (Yoon et al. 2001a) and *Planomicrobium* (Yoon et al. 2001b) are Gram-variable, where the older cells stained Gram-positive and the younger cells stained Gram-negative. Morphological forms vary from tri-chomes (curved to straight multicellular rods) in case of *Caryophanon* (Peshkoff 1939), filaments (composed of cylindrical straight or curved) in case of *Filibacter* (Maiden and Jones 1984), to rods or rod-cocci (coccoid when the cells are old) in case of other genera. Most of the genera are motile, except *Bhargavaea* (Manorama et al. 2009), *Chryseomicrobium* (Arora et al. 2011), and *Paenisporosarcina* (Krishnamurthi et al. 2009b), and the motility is due to a single or two or peritrichous flagella or by gliding in case of *Filibacter*. Members of the family are aerobic to facultatively anaerobic and produce acid from a variety of carbohydrates. Cross-linking of the peptidoglycan is by the A4 α type; diagnostic amino acid is L-lysine and the interpeptide bridges contain either aspartic acid or glutamic acid or in some cases the linkage is direct. Iso-methyltetradecanoic acid (iso-C_{15:0}) or anteiso-methyltetradecanoic acid (anteiso-C_{15:0}) or both are the major fatty acids present in all the genera, and other prominent fatty acids are iso-C_{16:0}, C_{16:1}(ω 11c), anteiso-C_{17:0}, and C_{16:1}(ω 7c) alcohol. Menaquinone MK-7 is the predominant isoprenoid quinone, but MK-6 and MK-8 are also present in some genera. Polar lipids present were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylserine, and unidentified phospholipids and glycolipids except in case of *Jeotgalibacillus* (Yoon et al. 2001a) where most of the lipids are aminophospholipids. The G+C values of DNA for the family *Planococcaceae* ranges from 34 to 54 %. Characteristics that differentiate the genera of the family *Planococcaceae* are listed in [Table 25.1](#).

Phylogenetic Structure of the Family and Its Genera

According to the phylogenetic affiliation of the type strains of *Firmicutes* in the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2008; Yarza et al. 2010; Munoz et al. 2011), the family *Planococcaceae* is closely related to *Bacillaceae* and *Bacillaceae* 3, moderately related to a broad group containing the families *Staphylococcaceae*, *Listeriaceae*, *Carnobacteriaceae* 1, *Carnobacteriaceae* 2, *Enterococcaceae*, *Aerococcaceae*, *Streptococcaceae*, *Lactobacillaceae*, and *Leuconostocaceae* (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>) ([Fig. 25.1](#)). Among the *Bacillaceae*, the closely related groups are *Bacillus aquimaris*, *B. vietnamensis*, *B. marisflavi*, *B. coahuilensis*, and *B. seohaeanensis* (Yarza et al. 2010) ([Fig. 25.1](#)). Families *Bacillaceae* 2, *Bacillaceae* 1, and *Sporolactobacillaceae* are deeply rooted and distantly related. Other families of the order *Bacillales* such as *Paenibacillaceae*,

Thermoactinomycetaceae, *Alicyclobacillaceae*, *Tenericutes*, *Acidithiobacillaceae*, and *Clostridiaceae* and genera *Ammoniphilus*, *Oxalophagus*, *Caldalkalibacillus*, *Microaerobacter*, *Tumebacillus*, *Alicyclobacillus*, *Calditerricola*, and *Thermicanus* belonging to the family *Bacillaceae* formed sister clades (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>) ([Fig. 25.1](#)).

Phylogenetic reconstruction of the family *Planococcaceae* based on 16S rRNA gene sequences using the neighbor-joining algorithm (NJ), (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>) resulted in the formation of a monophyletic branch for the family *Planococcaceae* ([Fig. 25.2](#)). However, phylogenetic tree created by Yarza et al. (2010), using maximum likelihood (ML) (tree not shown), resulted in formation of *Jeotgalibacillus* as a separate sister clade in the family *Planococcaceae*, and the genus *Jeotgalibacillus* appeared to be closely related to *Listeriaceae*. Phylogenetic analyses of *Planococcaceae* were also performed using minimum evolution (ME) and maximum parsimony (DNAPARS), and the topology of the phylogenetic trees was similar to NJ. In the phylogenetic analyses of the family *Planococcaceae*, the genera *Bhargavaea*, *Caryophanon*, *Chryseomicrobium*, *Jeotgalibacillus*, *Kurthia*, *Paenisporosarcina*, *Rummeliibacillus*, *Solibacillus*, *Ureibacillus*, and *Viridibacillus* formed distinct clades. The remaining genera, *Planococcus*, *Planomicrobium*, *Filibacter*, and *Sporosarcina*, did not resolve into distinct clades ([Fig. 25.3](#)). Phylogenetic analysis of the genera *Planococcus*, *Marinococcus*, and *Sporosarcina* and their relationships to members of the genus *Bacillus* were also attempted by Farrow et al. (1992).

Genus *Planococcus* was described by Migula (1894) based on phylogenetic and chemotaxonomic characteristics. Subsequently, Yoon et al. (2001b) transferred several species of the genus *Planococcus* to a new genus *Planomicrobium* based on cell morphology and phylogeny. Genus *Planococcus* forms two major clades with clade 1 including the species *P. antarcticus* (Reddy et al. 2002), *P. kocurii* (Hao and Komagata 1985), *P. donghaensis* (Choi et al. 2007), and *P. halocryophilus* (Mykytczuk et al. 2012), and clade 2 contains *P. rifietensis* (Romano et al. 2003), *P. citreus* (Migula 1894), *P. columbae* (Suresh et al. 2007), *P. maritimus* (Yoon et al. 2003), *P. plakortidis* (Kaur et al. 2012), and *P. maitriensis* (Alam et al. 2003) ([Fig. 25.3](#)). Out of the 11 species described, under the genus *Planococcus*, *P. salinarum* (Yoon et al. 2010b) formed a separate branch and is closely related to clade 1 of *Planococcus*.

Planococcus is closely related to *Planomicrobium*, but it can be differentiated from the nearest phylogenetic neighbor, on the basis of 16S rRNA gene signature nucleotides. Signature nucleotides were identified based on their presence in all the species of the particular genus and were not present in more than four genera of the family *Planococcaceae* in the 16S rRNA gene. In the genus, *Planococcus* T and A are conserved at positions 183 and 190, respectively (Dai et al. 2005) whereas in *Planomicrobium* C and G are present, respectively, at identical positions. Besides these, the genus *Planococcus* contains C (170); T (192); T, T, G, C, G, and G (between 192 and 209); C (209); T (210); T (212); A (217); G (218); C, T, and G (between 218 and 220); A (220);

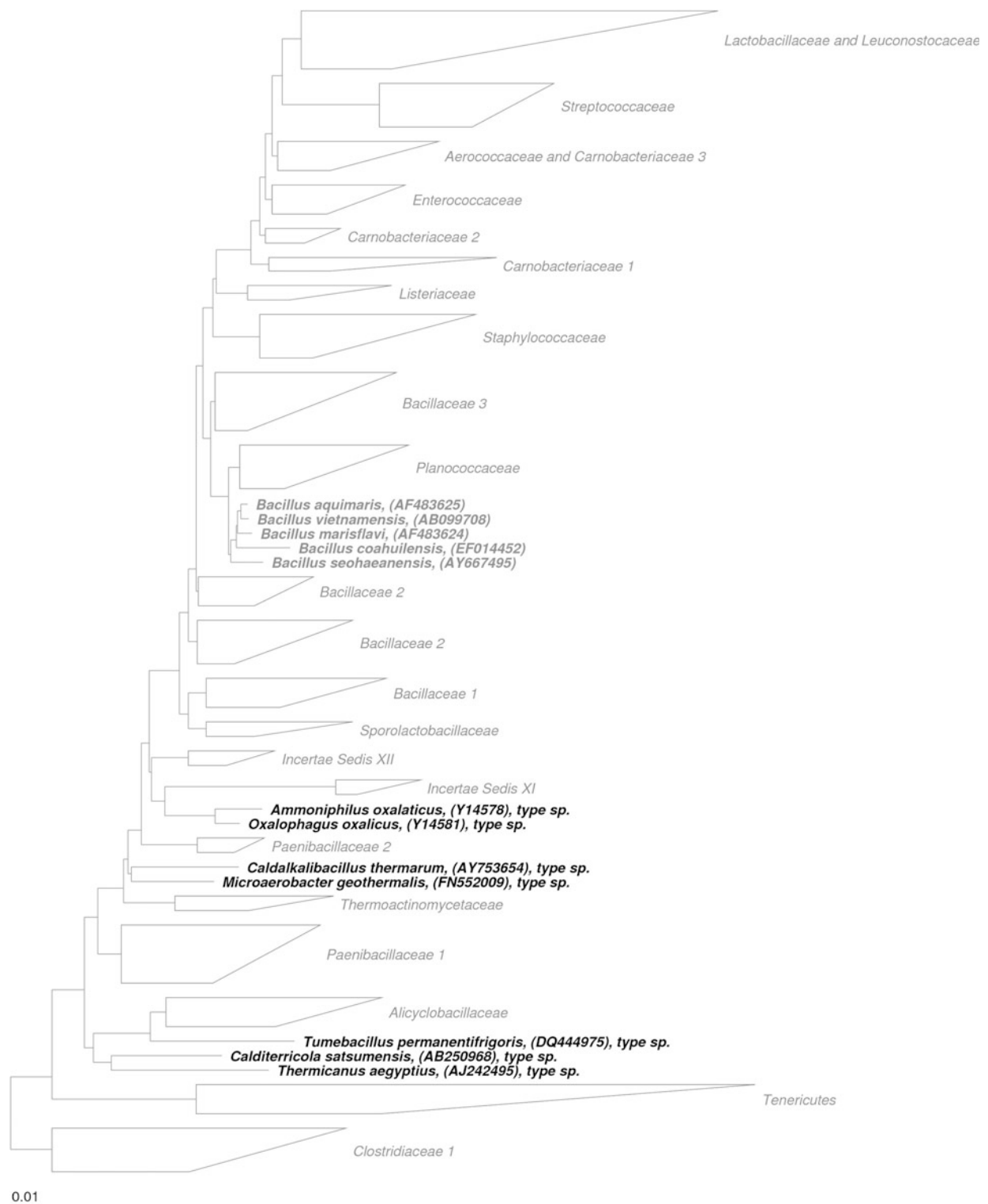
Table 25.1

Diagnostic characteristics that differentiate the genera of the family Planococcaceae^a. 1. *Bhargavaea* (Manorama et al. 2009; Verma et al. 2012); 2. *Caryophanon* (Peshkoff 1939); 3. *Chryseomicrobium* (Arora et al. 2011); 4. *Filibacter* (Maiden and Jones 1984); 5. *Jeotgalibacillus* (Yoon et al. 2001a); 6. *Kurthia* (Trevisan 1885); 7. *Paenisporosarcina* (Krishnamurthi et al. 2009b); 8. *Planococcus* (Migula 1894); 9. *Planomicrobium* (Yoon et al. 2001b); 10. *Rummelibacillus* (Vaishampayan et al. 2009); 11. *Solibacillus* (Krishnamurthi et al. 2009a); 12. *Sporosarcina* (Kluyver and van Niel 1936); 13. *Ureibacillus* (Fortina et al. 2001); 14. *Viridibacillus* (Albert et al. 2007)

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Morphology	Rod to coccoid	Trichome rods	Rods	Filamentous, composed of cylindrical cells, straight or curved	Rods	Rods	Rod/coccus	Cocci/short rods/rods	Cocci/short rods/rods	Rods	Rods	Rods or sphere	Rods	Rods
Spore formation	–	–	–	–	+	–	+	–	–	+	+	+	+	+
Endospore shape	–	–	–	–	Ro/el	–	Ro	–	–	Ro	Ro	Ro	Sp	Ro
Gram-stain	+	+	+	–	v	+	+	+v	v	+	+	+	–	+
Motility	nm	m	nm	m, gliding	m	m/nm	nm	m/nm	m	m	m	m	m	m
Flagella type	–	PT	–	–	PT	PT	–	One or two flagella	Single flagellum/PT	NR	PT	Single or PT	PT	NR
Peptidoglycan type	A4 α	A4 α	A4 α	A4 α	A1 α	A4 α	A4 α	A4 α	A4 α	A4 α	A4 α	A4 α	A4 α	A4 α
Diagnostic peptidoglycan amino acids	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Asp	L-Lys-D-Glu	L-Lys	L-Lys-D-Asp	L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Glu or L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-L-Gly-D-Glu	L-Lys-D-Asn	L-Lys-D-Glu or L-Lys-D-Asp
Major polar lipids	PG, DPG	NR	PG, DPG, PE	NR	APL-1, APL-2, APL-3, APL-4	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE, PS	PG, DPG, PL, GL	NR	PG, DPG, PL, GL	PG, DPG, PE
Major fatty acids	Iso-C _{15:0} , iso-C _{16:0} , anteiso-C _{15:0} , C _{15:0}	Iso-C _{15:0} , C _{16:1(ω11c)}	Iso-C _{15:0} , iso-C _{16:0} , C _{16:1(ω7c)} , OH ₁	Anteiso-C _{15:0} , anteiso-C _{17:0}	Iso-C _{15:0} , C _{15:0}	Iso-C _{15:0} , anteiso-C _{15:0} , C _{15:0} , anteiso-C _{16:0} , C _{16:1(ω7c)} , OH	Iso-C _{15:0} , anteiso-C _{15:0} , C _{15:0} , anteiso-C _{16:0} , C _{16:1(ω7c)} , OH	Iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0} , C _{16:1(ω7c)} , OH	Iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0} , C _{16:1(ω7c)} , OH	Iso-C _{15:0} , anteiso-C _{15:0} , iso-C _{16:0} , C _{16:1(ω7c)} , OH	PG, DPG, PE, PS	Anteiso-C _{15:0} , C _{15:0}	Iso-C _{15:0} , iso-C _{17:0}	Iso-C _{15:0} , anteiso-C _{15:0} , C _{15:0}
Menaquinone	MK-6, MK-8	MK-6	MK-6, MK-7, MK-7(H ₂), MK-8	MK-7	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7	MK-7	MK-7	MK-7	MK-7, MK-8
DNA G+C content	50.2–53.7	41–46	53.4	44.0	44	36–38	46	39–52	35–47	34.3	39.3	40–42	35–45	35–40.4

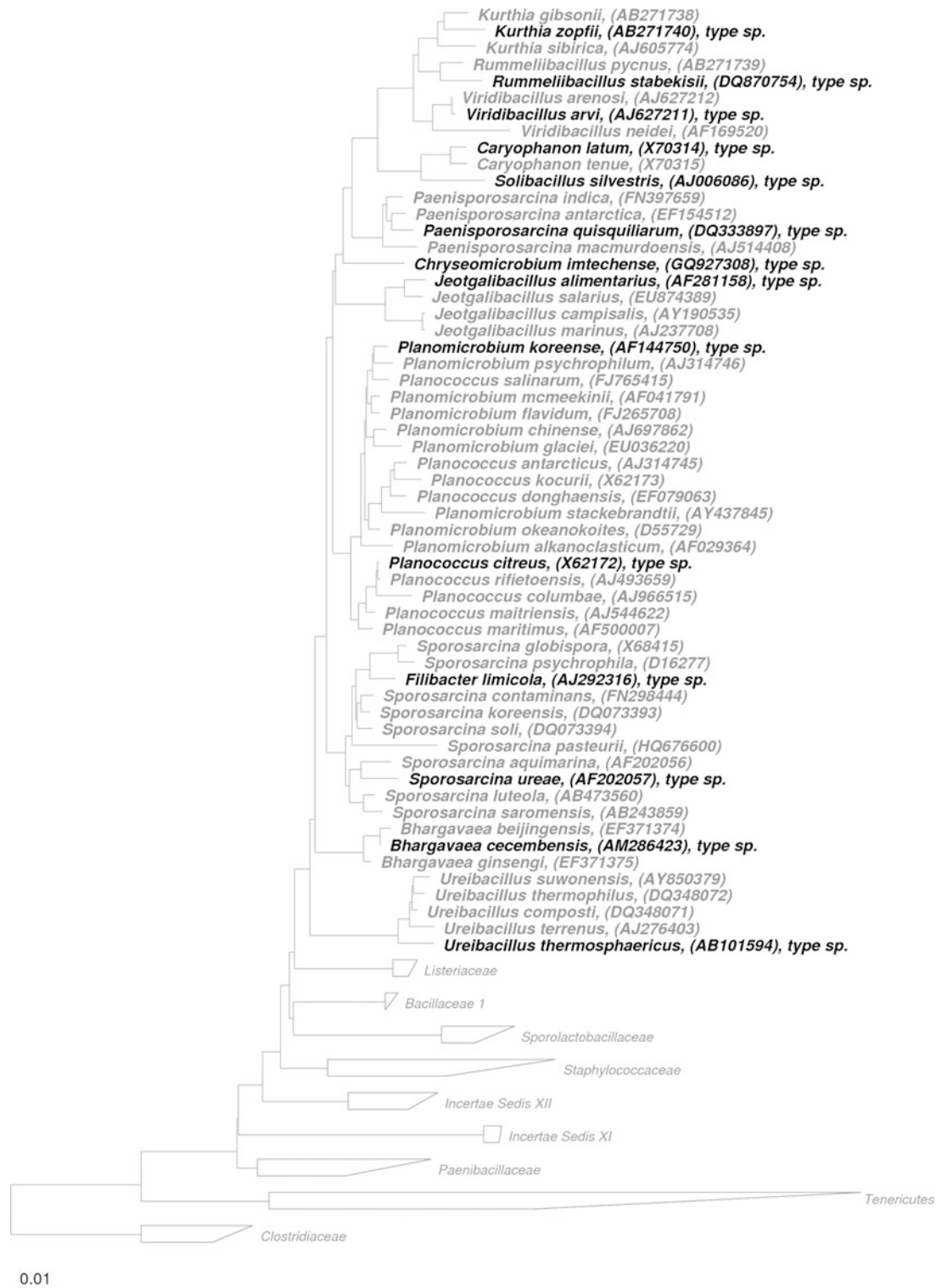
^aSome data was also obtained from *Bergey's Manual of Systematic Bacteriology*, vol. 3

Ro round, Sp spherical, el ellipsoid, + positive, – negative, v variable, nm nonmotile, m motile, PT peritrichous, NR not reported, L-Lys L-lysine, d-Glu D-glutamic acid, d-Asp D-aspartic acid, Gly glycine, PG phosphatidylglycerol, DPG diphosphatidylglycerol, PE phosphatidylethanolamine, PS phosphatidylserine, PL unidentified phospholipids, APL unidentified aminophospholipids



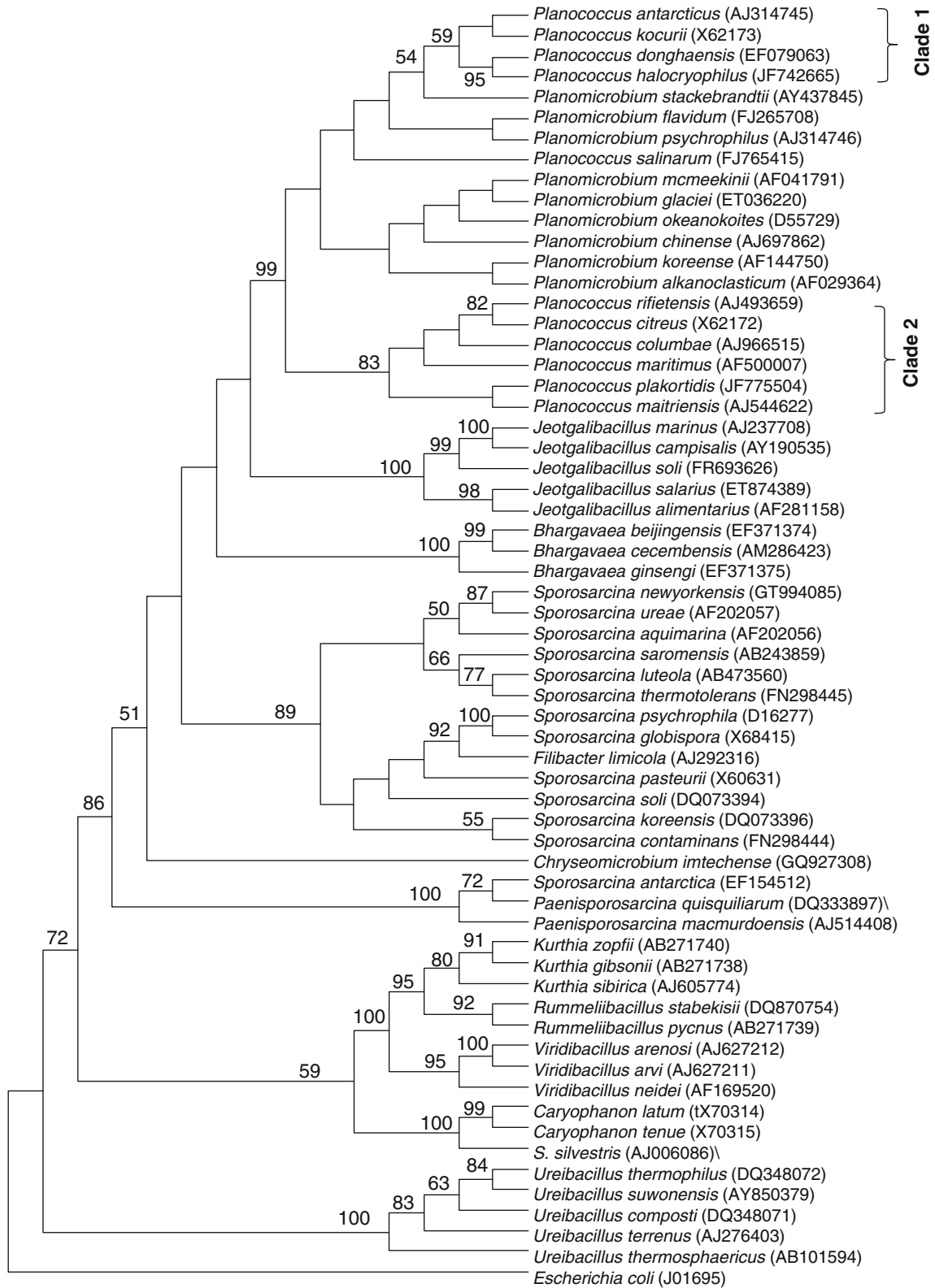
■ Fig. 25.1

Phylogenetic reconstruction of the Order *Bacillales* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>). The tree topology was stabilized with the use of a representative set of nearly 750 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence



■ Fig. 25.2

Phylogenetic reconstruction of the family *Planococcaceae* based on 16S rRNA and created using the neighbor-joining algorithm with the Jukes-Cantor correction. The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>). The tree topology was stabilized with the use of a representative set of nearly 750 high quality type strain sequences proportionally distributed among the different bacterial and archaeal phyla. In addition, a 40 % maximum frequency filter was applied in order to remove hypervariable positions and potentially misplaced bases from the alignment. Scale bar indicates estimated sequence divergence



■ Fig. 25.3

Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationship among the genera of family Planococcaceae. Bootstrap values (expressed as percentages of 1,000 replications) greater than 50 % are given at nodes

C (221); G (222); A (228); T (295); G (397); C (416); C (485); A (496); G (499); C (519); C (520); T (618); A (761); G (1273); and G (1395) as signature nucleotides. The signature nucleotides at positions 210, 217, 618, and 1395 differentiate the genus *Planococcus* from *Planomicrobium*. Besides the above signatures, nucleotides G, C, and G (between 192 and 209) and C, T, and G (between 218 and 220) are highly conserved among the members of *Planococcus* compared to *Planomicrobium*.

Genus *Planomicrobium* has nine species which on phylogenetic analyses form two clades closely related to clade 1 of *Planococcus*. *Planomicrobium mcmeekinii* (Junge et al. 1998), *P. glaciei* (Zhang et al. 2009), *P. okeanoikoites* (ZoBell and Upham 1944), *P. chinense* (Dai et al. 2005), *P. korensae* (Yoon et al. 2001b), and *P. alkanoclasticum* (Engelhardt et al. 2001) formed a tight and a distinct cluster, whereas *P. flavidum* (Jung et al. 2009) and *P. psychrophilum* (Reddy et al. 2002) formed a separate clade closely affiliated with clade 1 of *Planococcus*. Genus *Planomicrobium* contains the following 16S rRNA gene signature nucleotides: C (170), T (192), C (210), C (214), T (212), G (217), A (220), C (221), G (222), A (228), C (295), G (397), C (416), C (485), A (496), G (499), C (519), C (520), C (618), G (1273), and A (1395).

Genus *Bhargavaea* was created by Manorama et al. (2009) and emended by Verma et al. (2012). The phylogenetic analysis of the genus was based on both 16S rRNA and Gyr B genes of three species: *B. cecembensis* (Manorama et al. 2009), *B. beijingensis*, and *B. ginsengi* (Verma et al. 2012). NJ-based phylogenetic analysis indicated that the genus is closely related to *Planococcus*, *Planomicrobium*, and *Jeotgalibacillus* (Fig. 25.3). According to Verma et al. (2012), genus *Bhargavaea* is closely related to the genus *Sporosarcina*. However, the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Munoz et al. 2011) indicated that the genus *Bhargavaea* forms a deeply rooted branching from other members of the family (Fig. 25.2) and its sister clade is the family *Listeriaceae*. Genus *Bhargavaea* contains the following signature nucleotides in its 16S rRNA gene sequence: C (116), T (162), G (163), T (164), A (165), G (206), C (444), C (445), G (451), T (488), C (505), T (1027), A (1034), C (between 1053 and 1054) and G (between 1061 and 1062), A (1068), G (1278), and C (1324). Out of these, the signature nucleotides at positions 116, 206, 444, 451, 488, and 1068 are unique to this genus.

Genus *Caryophanon* was described by Peshkoff (1939) based on its unique cell morphology. Stackebrandt et al. (1987) performed the 16S rRNA oligonucleotide analyses of spore-forming *Bacilli* and nonspore-forming genus *Caryophanon* and found that the oligomers AAUAAG (position 456), AAUCUG, CCCCCG (617), ACAUAG (1002), UCCUAG, CACUCUCG (1132), CCAAUCCG, UACCUUUG (462), UUACCUUG (480), ACUUAUCG, AUUUCUUCG, CUACAAACG, CCAUCCACUG, UAACCCUUUAG, and UAACCUACCUUAUAG (124) are uniquely found in the genus *Caryophanon*. Phylogenetic analysis performed by Farrow et al. (1994) indicated that the genus *Caryophanon* branches out with *Bacillus fusiformis* and *Bacillus sphaericus*. Present phylogenetic analyses based on 16S rRNA gene sequence indicated that the

genus is closely associated with *Solibacillus* and their sister clade contains the genera *Viridibacillus*, *Rummeliibacillus*, and *Kurthia*. Clade represented by above genera form a deeply rooted branching with other members of the family (Fig. 25.3). A comparison of 16S rRNA gene sequences of the family *Planococcaceae* resulted in identification of following signature nucleotides: T (154), T (250), A (444), T (451), G (484), T (487), T (696), G (862), C (878), T (923), A (930), T (1145), C (1146), T (1158), T (1164), G (1179), C (1280), G (1311), T (1313), and G (1317). The nucleotide G at position 1179 is present only in *Caryophanon*.

The genus *Chryseomicrobium* is represented by a single species (*Chryseomicrobium imtechense*) which phylogenetically forms a deep branching from the genera *Planococcus*, *Sporosarcina*, *Jeotgalibacillus*, *Bhargavaea*, and *Planomicrobium* (present analysis; Arora et al. 2011). However, the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Munoz et al. 2011) indicated that the closest genera are *Caryophanon*, *Kurthia*, *Paenisporsarcina*, and *Sporosarcina* (Fig. 25.2). In all the above phylogenetic methods, the rooting is not well supported as the bootstrap values were low (<50 %). Though the phylogeny is based on a single species, it did possess some signature nucleotides in its 16S rRNA, and they are as follows: C (588), A (640), T (652), T (923), A (930), C (1029), C (1050), T (1271), and T (1363) (the positions are with respect to *E. coli* 16S rRNA gene sequence accession number J01695) and C at position 1029 is uniquely present in this genus compared to the other genera of the family.

Genus *Filibacter* was originally described by Maiden and Jones (1984), and 16S rRNA analysis of the lone species *Filibacter limicola* was compared with species of *Bacillus* (Clausen et al. 1985). However, no reports are available on phylogenetic analyses of *Filibacter*. Present analyses and the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Munoz et al. 2011) indicated that the genus is closely related to *Sporosarcina* (Figs. 25.2 and 25.3). Further, its affiliation is supported by the riboprinting analysis (Stackebrandt 2009). A comparison of 16S rRNA gene sequence of *Filibacter* with other members of the family *Planococcaceae* resulted in the identification of A (505), A-T (640–652), A (956), T (1271), A (1272), G (1278), and T (1319) as diagnostic nucleotides, and A (1272) and T (1319) are present only in this genus.

Genus *Jeotgalibacillus* was described by Yoon et al. (2001a), and it is represented, presently, by five species. Phylogenetic analysis of all species of the genus was performed by Cunha et al. (2012), but the study did not compare their relationship with other members of the family, *Planococcaceae*. Phylogenetic analyses using the RaxML 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Munoz et al. 2011) resulted in clustering of the genus with *Caryophanon* and *Kurthia*. However, the RaxNJ 16S rRNA gene tree of the Living Tree Project (Yarza et al. 2010; Munoz et al. 2011) indicated that it forms a clade by itself and is deeply rooted from other members of the family (Fig. 25.2). In our analyses, genus *Jeotgalibacillus* clustered with *Planococcus*, *Planomicrobium*, and *Bhargavaea* in methods NJ (Fig. 25.3) and ME (data not shown), but the bootstrap

values were below 50 %, thus implying a poor phylogenetic relationship with members of *Planococcaceae*. Our analysis identified the nucleotides T (164), A (165), C (169), G (203), T (229), T (294), G (397), C (416), A (486) T (498), C (519), C (520), T (612), C (640) G (652), A (675), G (688), T (732), C (770), A (782), G (862), C (878), C (1037), A (1290), T (1299), and T (1363) as 16S rRNA gene signatures and of these T (732), A (1290), and T (1299) were unique to this genus.

Phylogenetic analyses based on 16S rRNA gene sequence indicated that genus *Kurthia* clusters with the genus *Caryophanon* (● Fig. 25.2). However, our analyses indicated the affiliation of *Kurthia* with the genus *Rummeliibacillus* in the ME, NJ (● Fig. 25.3), and DNA parsimony trees. Genus *Kurthia* is characterized by the presence of the following diagnostic nucleotides in their 16S rRNA gene: T (250), T (266), A (312), T (342), A (363), T (438), T (486), G (490), A (493), A (498), T (586), T (617), A (675), T (696), G (764), A (922), T (931), A (1028), T (1066), A (1144), T (1164), A (1281), T (1291), T (1305), and T (1310).

Genus *Paenisporosarcina* was created by Krishnamurthi et al. (2009b) and emended by Reddy et al. (2013), and presently it contains four species. Phylogeny of the genus based on 16S rRNA gene sequence indicated that the genus forms a separate branch rooted from the clade represented by *Planococcus*, *Planomicrobium*, *Sporosarcina*, *Bhargavaea*, *Jeotgalibacillus*, *Chryseomicrobium*, and *Filibacter* (● Fig. 25.3). In RaxML and RaxNJ 16S rRNA gene trees of the Living Tree (Yarza et al. 2010; Munoz et al. 2011), the phylogenetic affiliation of *Paenisporosarcina* was different, and it formed a clade with *Sporosarcina* (in case of RaxML) and with *Kurthia* and *Caryophanon* (in case of RaxNJ). Genus *Paenisporosarcina* is characterized by the presence of the following diagnostic nucleotides in their 16S rRNA gene: A (159), T (162), T (165), T (211), G (228), C (229), A (252), A (283), G (487), A (684), and C (699), and nucleotides at positions 211 and 283 are unique to the genus.

Genus *Rummeliibacillus* was described by Vaishampayan et al. (2009) and is presently represented by two species, *Rummeliibacillus stabekisii* and *Rummeliibacillus pycnus*. Phylogenetic analyses based on 16S rRNA gene sequence using the methods ME, NJ, and DNA parsimony indicated that it is closely affiliated with the genus *Kurthia* (● Fig. 25.3). This clade is part of a bigger cluster represented by the genera *Caryophanon*, *Solibacillus*, and *Viridibacillus*. Genus *Rummeliibacillus* contains the signature nucleotides C (169), G (203), T (266), A (312), T (342), A (363), T (438), T (486), G (490), A (493), A (498), T (586), T (617), T (735), A (922), T (931), T (1066), T (1158), A (1281), T (1291), T (1305), and T (1310) in its 16S rRNA gene.

Genus *Solibacillus* was carved by Krishnamurthi et al. (2009a) from the genus *Bacillus* and is represented by a single species *Solibacillus silvestris* that was originally described by Rheims et al. (1999). Our 16S rRNA genes sequence-based analyses using the phylogenetic methods ME, NJ (● Fig. 25.3), and DNA parsimony indicated that *Solibacillus* and *Caryophanon* cluster together as part of the clade represented by *Kurthia*, *Rummeliibacillus*, and *Viridibacillus*. Genus contains the 16S rRNA gene signature nucleotides T, A, T, A, T, G, T, A, G,

C, T, A, A, A, T, C, and T at positions 106, 113, 154, 444, 451, 484, 612, 782, 862, 878, 923, 930, 1034, 1066, 1145, 1280, and 1305, and nucleotides at positions 106 and 113 are unique to this genus.

Genus *Sporosarcina* was proposed by Kluyver and van Niel (1936) and presently contains 12 species. In RaxML and RaxNJ, 16S rRNA gene trees of the Living Tree (Yarza et al. 2010; Munoz et al. 2011) and our analyses indicated the formation of two major branches within the genus *Sporosarcina* and also contain the genus *Filibacter* within the major clade (● Figs. 25.2 and ● 25.3). Clade represented by *Sporosarcina* is well separated from the genera *Planococcus*, *Planomicrobium*, *Bhargavaea*, and *Jeotgalibacillus* (● Figs. 25.2 and ● 25.3). Genus *Sporosarcina* contains the following signature nucleotides in its 16S rRNA gene sequence: G, G, G, A, T, A, C, G, A, G, T, and T at positions 170, 486, 487, 640, 652, 684, 709, 735, 956, 1035, 1284, and 1415, respectively.

Genus *Ureibacillus* was created by Fortina et al. (2001) with *Ureibacillus thermosphaericus* as the type species and has five species. In the phylogenetic analyses based on RaxML and RaxNJ 16S rRNA gene trees of the Living Tree (Yarza et al. 2010; Munoz et al. 2011), all the species cluster together with a single branching and are related to a major clade represented by all other genera of the family (● Fig. 25.2). Our analysis, based on NJ, NE, and DNA parsimony, indicated a deeply rooted branching of all five species (● Fig. 25.3). Evaluation of 16S rRNA gene sequence with respect to other genera of the family *Planococcaceae* resulted in the identification of T, G, C, G, T/C, C, G, T, A, G, C, C, G, G, A, C, C, C, A, C, A, T, A, A, G, and C at positions 115, 181, 182, 189, 190, 208, 210, 229, 242, 404, 411, 501, 688, 695, 735, 765, 770, 1146, 1193, 1194, 1195, 1198, 1199, 1313, 1317, and 1321, respectively, as the signature nucleotides.

Genus *Viridibacillus* was carved from *Bacillus* by reclassifying three species: *Bacillus arvi*, *Bacillus arenosi*, and *Bacillus neidei* (Albert et al. 2007). Previous studies have not reported the affiliation of *Viridibacillus* with members of the family *Planococcaceae*. Present analysis, based on 16S rRNA gene sequence, indicated that the species are closely related to each other and clustered within the clade represented by *Caryophanon*, *Solibacillus*, *Rummeliibacillus*, and *Kurthia* (● Fig. 25.3). Genus contains the following diagnostic nucleotides in its 16S rRNA gene: A (163), T (251), T (266), A (312), T (342), A (363), T (438), T (486), A (498), T (586), T (612), A (674), A (709), T (931), A (1028), T (1066), A (1281), T (1291), T (1300), A (1301), T (1302), T (1305), and T (1310). Nucleotides at positions 163, 251, and 1302 are unique to this genus.

Molecular Analysis

DNA-DNA Hybridization Studies

DNA-DNA hybridization (DDH) between species of the 14 genera of *Planococcaceae* indicated that similarity values ranged from 13 to <70 %. However, DNA-DNA relatedness was high

within strains of the same species (>70 %) (Adcock et al. 1976). DNA-DNA relatedness between *Caryophanon latum* and *C. tenue* was 13–30 % (Adcock et al. 1976), between *Jeotgali-bacillus* species <21 % (Yoon et al. 2004, 2010a); between *Kurthia* species <41 % (Cherevach et al. 1983); between *Paenisporosarcina quisquiliarum* and *P. macmurdoensis* 18 % (Krishnamurthi et al. 2009b); between *Planococcus* species <62 % (Nakagawa et al. 1996; Suresh et al. 2007; Choi et al. 2007; Mykytczuk et al. 2012; Yoon et al. 2003, 2001b; Kaur et al. 2012); between *Planomicrobium* species <62 % (Mayilraj et al. 2005; Dai et al. 2005; Jung et al. 2009; Zhang et al. 2009; Yoon et al. 2001b); between *Rummeliibacillus stabekisii* and *R. pycnus* 13 % (Vaishampayan et al. 2009); between *Sporosarcina* species <70 % (Yu et al. 2008; Yoon et al. 2001c; Kämpfer et al. 2010; Kwon et al. 2007; Tominaga et al. 2009; Wolfgang et al. 2012; An et al. 2007); between *Ureibacillus* species <52 % (Weon et al. 2007; Fortina et al. 2001; Kim et al. 2006); and between *Viridibacillus* species <42.0 % (Heyrman et al. 2005).

DNA-DNA relatedness experiments were not carried out between a number of novel taxa with their respective nearest neighbor, as the level of 16S rRNA gene sequence similarity between the novel taxa with the nearest neighbor (Reddy et al. 2003; Manorama et al. 2009; Arora et al. 2011; Yoon et al. 2001a; Cunha et al. 2012; Rheims et al. 1999) was less than 96.9 %, which is below the cutoff value (97 %) suggested by Stackebrandt and Goebel (1994) for genomic distinction of species.

The species *Filibacter limicola* which was established by Maiden and Jones (1985) was mainly based on phenotypic characteristics and was not based on either DNA-DNA hybridization or 16S rRNA gene sequence analysis. Phylogenetically *Filibacter limicola* is closer to the species of the genus *Sporosarcina* especially *S. psychrophila* and *S. globispora* (► Figs. 25.2 and 25.3).

Genome Comparison

Whole genome sequencing of nine strains in the family *Planococcaceae* has been reported (NCBI Database). Out of these nine strains, only five strains are completely processed by NCBI GenBank. The whole genome sequence of *Kurthia* sp. JC8E (GOLD ID: Gi13538) is 2,976,740 bp long, has 3,006 genes including one 16S rRNA gene, and has a G+C mol% of 38.22 %. The genome has 2,916 proteins including some antibiotic resistance proteins like glyoxalase/bleomycin resistance protein (providing resistance against bleomycin), several penicillin-binding proteins (providing resistance against penicillin), and lantibiotic ABC transporter ATP-binding protein (providing resistance against lantibiotics). The bacterium may also be resistant to camphor and tellurium due to the presence of camphor resistance CrcB protein and tellurium resistance protein, respectively. NTP pyrophosphohydrolase (including oxidative damage repair enzymes, which hydrolyze noncanonical NTPs) can be used for “house cleaning” work (Galperin et al.

2006). Fatty acid desaturase enzyme was also identified in *Kurthia* sp. JC8E which is responsible for desaturation of fatty acids (Ford 2010).

Another reported whole genome sequence of *Kurthia* sp. JC30 (GOLD ID: Gi13535) is 3,201,696 bp long and has 3,296 genes including one 16S rRNA gene and a G+C mol% of 39.26 %. The genome has 3,201 proteins. As in *Kurthia* sp. JC8E, the *Kurthia* sp. JC30 also has antibiotics resistance proteins like glyoxalase/bleomycin resistance protein and several penicillin-binding proteins. Apart from these two, *Kurthia* sp. JC30 also has other antibiotic resistance proteins like tetracycline resistance protein and teicoplanin resistance associated membrane protein. *Kurthia* sp. JC30 shows the presence of some metal resistance proteins like copper resistance protein and tellurium resistance protein. The annotation of the genome also reports the presence of a lipase enzyme.

The genome of *Planococcus donghaensis* MPA1U2 (GOLD ID: Gi08597) (Choi et al. 2007) is 3,303,464 bp long, has 3,331 genes including one 16S rRNA gene, and has a G+C mol% of 39.68 %. The genome codes for 3,267 proteins including some antibiotics resistance proteins like glyoxalase/bleomycin resistance protein, acriflavin resistance protein (providing resistance against acriflavin), and quinolone resistance protein (providing resistance against quinolone). The bacterium also shows the presence of protein CopC (providing resistance against copper), mercuric resistance operon regulatory protein MerR (providing resistance against mercury), and aluminum resistance protein (providing resistance against aluminum). The annotation of the genome also reports the presence of multiple resistance and pH homeostasis protein; this protein is involved in pH homeostasis and resistance against cholate and Na⁺ (Ito et al. 1999).

The reported whole genome sequence of *Planococcus antarcticus* DSM 14505^T (GOLD ID: Gi17702) (Margolles et al. 2012) is 3,772,109 bp long and has 3,750 genes, including one 16S rRNA gene and a G+C mol% of 42.14 %. The annotation of the genome reports 3,825 proteins. *P. antarcticus* DSM 14505^T has some antibiotic resistance proteins like glyoxalase/bleomycin resistance protein and quinolone resistance protein that have been discussed earlier. The bacterium also has an organic hydroperoxide resistance protein that provides resistance against organic hydroperoxides (Cussiol et al. 2010). The annotation of *P. antarcticus* DSM 14505^T also reports to have many other resistance proteins like tellurium resistance protein, copper resistance protein, aluminum resistance protein, arsenical resistance proteins, mercuric resistance operon regulatory protein MerR, and camphor resistance protein. Abortive infection bacteriophage resistance protein is also present in the genome of the bacterium; this protein is involved in resistance against bacteriophage infections (Fineran et al. 2009).

The whole genome sequence of *Sporosarcina newyorkensis* 2681 (GOLD ID: Gi05603) is 3,627,407 bp long, has 3,899 genes, including one 16S rRNA gene, and has a G+C mol% of 42.14 %. The genome reports the presence of 3,825 proteins. *S. newyorkensis* 2681 also has some antibiotic resistance proteins like glyoxalase/bleomycin resistance protein, quinolone

resistance protein, tetracycline resistance protein, and several penicillin resistance proteins. *S. newyorkensis* 2681 has many other resistance proteins like aluminum resistance protein, copper resistance protein, mercuric resistance operon regulatory protein MerR, tellurite resistance protein, and camphor resistance protein. The genome annotation also reports the presence of bacitracin resistance protein. BCCT family osmoprotectant transporter is also found in the genome of *S. newyorkensis* 2681 (Kuhlmann et al. 2011). The bacterium may also show lantibiotic resistance due to the presence of MutG family lantibiotic protection ABC superfamily in the genome.

All the five whole genome sequences show similarities like presence of antibiotics resistance and metal resistance. Some of them even show resistance against lantibiotics and bacitracin apart from peculiar features like osmotic protection and protection against bacteriophage infections.

Apart from whole genome analyses, there were several other molecular studies targeting a few housekeeping genes and some operons such as *gyrB* gene (gyrase subunit B) in *Bhargavaea beijingensis* and *Bhargavaea ginsengi* (Verma et al. 2012); *secY* (preprotein translocase SecY), *adk* (adenylate kinase), and *map* (methionine aminopeptidase) genes in *Jeotgalibacillus marinus* (AY690426); chitinase gene in *Kurthia gibsonii* and *Kurthia zopfii* (JQ739168; D63702); *tuf* gene (encoding elongation factor Tu) in *Planomicrobium chinense* (AB472778); *DHFR-PRL*, *fokIR*, *fokIM*, *MFokI*, and *RFokI* genes encoding dihydrofolate reductase-Prolactin fusion protein, endonuclease, methyltransferase, methylase, and endonuclease, respectively, in *Planomicrobium okeanokoites* (E02430; Looney et al. 1989; Kita et al. 1989); *cspB* gene (for major cold shock protein), *ctsU*, *ctsV*, *ctsW*, *ctsX*, *ctsY*, *ctsZ*, *ORF-7* genes (encoding cyclic tetrasaccharide-synthesizing enzymes), *ctsX*, *ctsY*, *ctsZ*, *ORF-4* genes (for hypothetical protein, 3- α -isomaltosyltransferase, 6- α -glucosyltransferase, hypothetical protein) in *Sporosarcina globispora* (Schröder et al. 1993; Aga et al. 2002, 2003); urease operon (comprising *ureA*, *ureB*, *ureC*, *ureD*, *ureE*, *ureF*, and *ureG* genes), osmotically regulated ectoine biosynthesis gene cluster (*ectA*, *ectB*, and *ectC* genes) (Kuhlmann and Bremer 2002); *cytC* gene (for cytochrome c553) in *Sporosarcina pasteurii* (X78411, AF361945, AF316874, AJ318066, respectively); *aph* (encoding amino acyl peptidase), *cdd* (encoding cytidine deaminase), *leuB* (3-isopropylmalate dehydrogenase), *pyrP* (uracil permease), *pyrB* (aspartate transcarbamoylase), and *pyrC* (dihydroorotase) genes in *Sporosarcina psychrophila* (Brunialti et al. 2011; AJ237978; AB706401; HQ625362; AY147014); *gyrB* gene (encoding DNA gyrase B) in *Sporosarcina saromensis* (AB243078); *Su-1* gene (encoding small, acid-soluble spore protein), *spoIIIE* gene (sporulation protein SpoIIIE), *sslA* gene (encoding S-layer protein SslA), *tetL* gene (encoding tetracycline efflux pump), *tetM* gene (encoding tetracycline resistance protein), and phenylalanine dehydrogenase gene in *Sporosarcina ureae* (Magill et al. 1990; Chary et al. 2000; Ryzhkov et al. 2007; You et al. 2012; GU584212; AB001031).

Ribotyping

Conventional methods based on polyphasic taxonomy are not always sufficient to delineate strains at the species level. Under these circumstances, additional molecular methods like ribotyping and multilocus sequence typing (MLST) can help to resolve the taxonomic status. Ribotyping involves polymerase chain reaction (PCR) amplification of small subunit ribosomal RNA genes followed by restriction digestion and analysis of the DNA fingerprint of the ribosomal RNA genes. The DNA fingerprint obtained is known as a riboprint pattern. The advantage of this method compared to other typing methods is that this is automated and the catalog of fingerprints is dynamic, as it changes and expands with the addition of each new sample that is processed. The RiboPrinter system combines automation with the power of DNA to not only identify the genus/species of contaminants but also provide deeper, strain-level typing at the same time.

Riboprint patterns have been generated for some members of the family *Planococcaceae* including species of the genus *Kurthia*, *Sporosarcina*, *Filibacter*, and *Ureibacillus*. Comparison of the riboprint patterns of *Filibacter limicola*, *Sporosarcina globispora*, *Sporosarcina pasteurii*, *Sporosarcina psychrophila*, and *Sporosarcina ureae* showed that they are all different. But the pattern of *Sporosarcina psychrophila* and *Sporosarcina globispora* was closely related which is also supported by 16S rRNA gene sequence analysis (Fig. 25.4). Riboprint patterns of all the three type strains of the genus *Kurthia* are different (Fig. 25.5) (Pukall and Stackebrandt 2009), implying that the method could also be used effectively for discriminating at the species level.

Riboprint patterns of *Bacillus thermosphaericus* DSM 10633^T, strains TH29 and TU1A, and *Ureibacillus terrenus* TH9A^T indicated that the riboprint patterns for strains TH9A^T and TU1A were very similar and could thus be considered characteristic of *Ureibacillus terrenus*, and riboprint patterns for strains *Bacillus thermosphaericus* DSM 10633^T and isolate TH29 displayed similar pattern and could thus be considered characteristic of *Bacillus thermosphaericus* (*Ureibacillus thermosphaericus*) (Fortina et al. 2001) (Fig. 25.6).

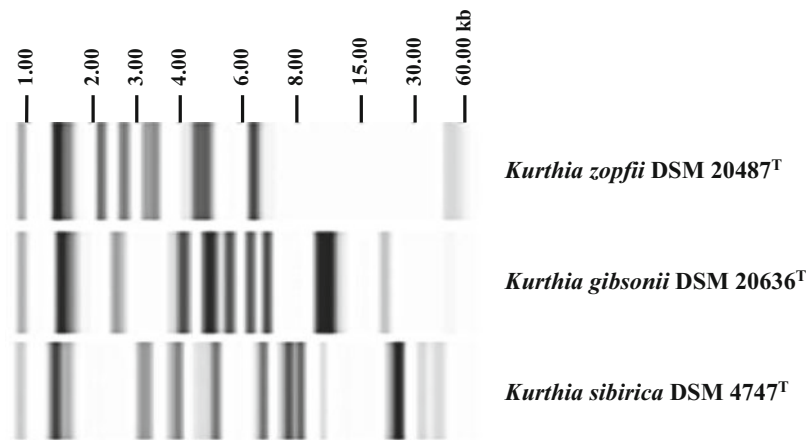
Multilocus Sequence Typing

Multilocus sequence typing (MLST) is an explicit method for characterizing isolates of bacterial strains using the sequences of internal fragments of up to seven housekeeping genes. Using an automated DNA sequencer, internal fragments of about seven genes (~450–500 bp) are sequenced on both strands. Distinct alleles are assigned to each housekeeping gene based on the diversity of the sequence present within a bacterial species. The alleles define the sequence type (ST), and each isolate of a species is explicitly exemplified by a series of multiple integers which match the alleles. Using seven housekeeping loci, it has been



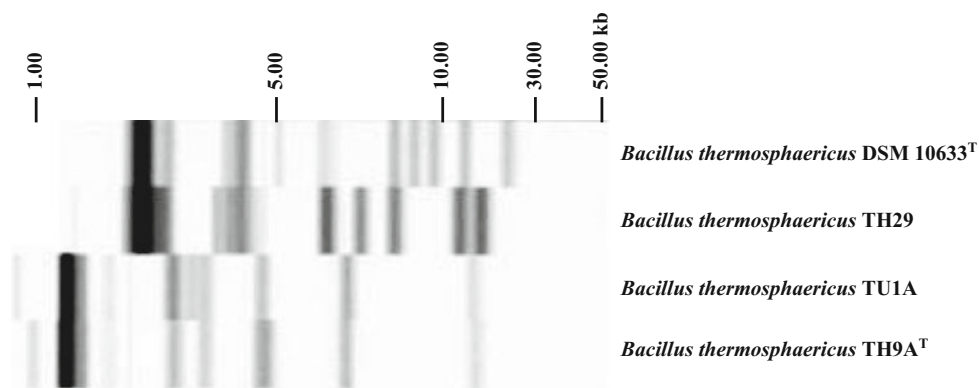
■ Fig. 25.4

Riboprint™ patterns of the DNA of *Filibacter limicola* and the type species of the genus *Sporosarcina*, *Sporosarcina urea* and three closely related species of the genus *Sporosarcina* (Data taken from Stackebrandt (2009))



■ Fig. 25.5

Diversity of normalized *Eco*RI ribotype patterns found within the type strains of the genus *Kurthia* (Data taken from Pukall and Stackebrandt (2009))



■ Fig. 25.6

Diversity of normalized ribotype patterns found within isolates related to strains of *Bacillus thermosphaericus* (Data taken from Fortina et al. (2001))

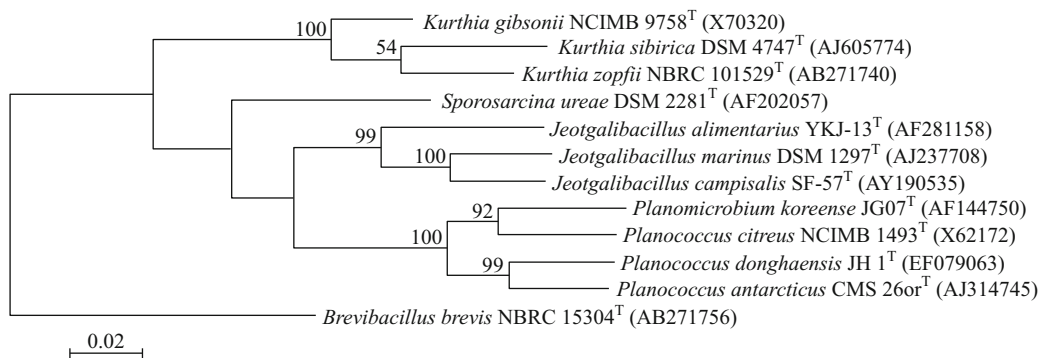


Fig. 25.7

Maximum-likelihood tree generated on the basis of concatenated 16S rRNA and *groEL* gene sequences based on the Tamura-Nei model. The tree with the highest log likelihood (8924.2140) is shown. The tree is drawn to scale, with branch lengths measured as the number of substitutions per site. The analysis involved 11 nucleotide sequences of members of the family *Planococcaceae*. All positions containing gaps and missing data were eliminated. There were a total of 1,906 positions in the final dataset

observed that majority of the bacterial species have ample disparity with respect to the number of alleles per locus, allowing billions of discrete allelic profiles to be distinguished. MLST is based on multilocus enzyme electrophoresis but varies in that it assigns alleles at multiple housekeeping loci directly by sequence information rather than circuitously through the banding pattern of their gene products.

Vema et al. (2012) reclassified *Bacillus beijingensis* and *B. ginsengi* (Qiu et al. 2009) as *Bhargavaea beijingensis* comb. nov. and *Bhargavaea ginsengi* comb. nov. based on the phylogenetic analysis of 16S rRNA gene sequence and *GyrB* amino acid sequence. *Bhargavaea cecembensis*, *Bacillus beijingensis*, and *Bacillus ginsengi* constituted a deeply rooted cluster separated from the clades represented by the genera *Bacillus*, *Planococcus*, *Planomicrobium*, *Sporosarcina*, *Lysinibacillus*, *Viridibacillus*, *Kurthia*, and *Geobacillus*, supporting their placement in the genus *Bhargavaea* (Vema et al. 2012).

The phylogenetic tree constructed with concatenated 16S rRNA and *groEL* gene sequences of 11 members of the family *Planococcaceae* was similar to the clustering observed when only 16S rRNA gene sequences were used for the analysis (Figs. 25.2 and 25.7).

Plasmids

Species of the genera *Bhargavaea* and *Sporosarcina* contain a mobilizable plasmid carrying *tet* (L) gene (You et al. 2012). Both *Bhargavaea* sp. DMV46A and *Bhargavaea* sp. DMV9 contain plasmids pBSDMV46A and pBSDMV9, respectively, with relaxase and tetracycline efflux pump gene in plasmid pBSDMV46A and *rep* gene on plasmid pBSDMV9. Chanda et al. (2010) confirmed that in *Kurthia gibsonii*, resistance to ampicillin, bacitracin, and cefotaxime is due to genes located in

the plasmid. Curing of the plasmid from the *Planococcus* sp. strain S5 showed that plasmid pLS5 was involved in salicylate degradation (Labuzek et al. 2003).

Phages

Bacteriophages specific to the genus *Caryophanon* include Csl_{x13b} (*Myoviridae*, morphotype A1), Csl_{x13a}, Ct_{kas} (*Siphoviridae*, morphotype B1), and øCVL-29 (*Siphoviridae*, morphotype B2) (Bacteriophage Names 2000). These phages have been shown to be either specific to *C. latum* or *C. tenue* or active against both species (Trentini 1978). These bacteriophages produced either clear or turbid center plaques on native isolates of *C. latum* and produced clear plaques on *C. tenue* but were inactive against *C. latum* isolated from cow dung (Peshkoff et al. 1966; 1967). Nauman and Wilkie (1974) isolated a plaque-forming bacteriophage (øCL-29) and a clear plaque mutant (øCLV-29) with the aid of the mutagen *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NG) from *C. latum* (Nauman and Wilkie 1974).

Rocourt et al. (1984) isolated 6/K27 a bacteriophage of *Kurthia zopfii* belonging to the family *Podoviridae* (http://ictvdb.bio-mirror.cn/WIntkey/Images/em_6-K27-HWA.htm). There are no predicted phage-related sequences, transposons, insertion elements, extrachromosomal elements, or pseudogenes in *Planococcus donghaensis* strain MPA1U2 (Pearson and Noller 2011). But *Planococcus antarcticus* DSM 14505^T contains a phage protein in the genome (Margolles et al. 2012). *Sporosarcina newyorkensis* 2681 contains *malR* (gene encoding phage integrase family site-specific recombinase), phage protein, phage methyltransferase, and phage infection protein in the genome (EGQ26312, EGQ26741, EGQ21095, EGQ26766) (Data from the NCBI database, protein).

Phenotypic Analyses

The main features of members of *Planococcaceae* are listed in [▶ Table 25.1](#).

Planococcus (Migula 1894, Emend. Nakagawa et al. 1996; Emend. Yoon et al. 2010)

Planococcus (*Plan.o.coc'cus*. Gr.n. *planos* wanderer; Gr. N. *coccus* a grain, berry; N.L. masc. N. *Planococcus* motile coccus).

The genus *Planococcus* was originally described by Migula (1894) and later on emended twice by Nakagawa et al. (1996) and then by Yoon et al. (2010b). Strains belonging to the genus *Planococcus* can be identified based on the following characteristics: cells are cocci or short rods or rods, motile (possess one or more flagella) or nonmotile, Gram-positive to Gram-variable, nonspore forming, and occur singly, in pairs, in threes, or in tetrads. The color of the cell mass is yellow to orange. Strictly aerobes, halotolerant, catalase positive, and urease negative. The cell wall peptidoglycan is L-Lys-D-Glu with a peptidoglycan variation of A4 α ; the major isoprenoid quinones are MK-6, MK-7, and MK-8; the cellular fatty acids are mainly branched fatty acids and dominated by iso-C_{15:0}, anteiso-C_{15:0}, and iso-C_{16:0}; and predominant polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The G+C content of the DNA is 39–52 mol%. In addition to the above characteristics, the genus *Planococcus* can be differentiated from the nearest phylogenetic neighbor, the *Planomicrobium*, on the basis of 16S rRNA gene signature nucleotides wherein at position 183 and 190, the nucleotides, respectively, T and A are conserved in case of *Planococcus* (Dai et al. 2005) whereas in case of *Planomicrobium* C and G are conserved at positions 183 and 190.

The type species of the genus is *Planococcus citreus* (Migula 1894), and the type strain is ATCC 14404^T = CIP 81.74^T = DSM 20549^T = IFO (now NBRC) 15849^T = JCM 2532^T = LMG 17319 = VKM B-1307^T.

Presently, the genus contains 11 species: *P. antarcticus* (Reddy et al. 2002), *P. citreus* (Migula 1894), *P. columbae* (Suresh et al. 2007), *P. donghaensis* (Choi et al. 2007), *P. halocryophilus* (Mykytzcuk et al. 2012), *P. kocurii* (Hao and Komagata 1985), *P. maitriensis* (Alam et al. 2003), *P. maritimus* (Yoon et al. 2003; Ivanova et al. 2006), *P. plakortidis* (Kaur et al. 2012), *P. rifietoensis* (Romano et al. 2003), and *P. salinarum* (Yoon et al. 2010b). The discriminative characteristics are listed in the [▶ Tables 25.2](#) and [▶ 25.3](#). All the 11 species are negative for growth at pH 5 and positive for growth at pH 7, at 15, 25, and 30 °C, and in 2–7 % NaCl. Except *P. kocurii* (Hao and Komagata 1985) and *P. halocryophilus* (Mykytzcuk et al. 2012), nine species are negative for the following biochemical tests performed using VITEK2-GP plates: cyclodextrin, α -mannosidase, phosphatase, leucine arylamidase, β -glucuronidase, D-sorbitol, urease, polymyxin B resistance, D-galactose, D-ribose, L-lactate alkalization, lactose, methyl β -D-glucopyranoside, pullulan, trehalose,

dihydrolase, and resistance to optochin. Negative for acid production from xylose, trehalose, inositol, adonitol, dulcitol, inulin, sorbitol, rhamnose, lactose, melibiose, and arabinose. Positive for oxidation of D-sorbitol using Biolog GP2 MicroPlates; negative for α -cyclodextrin, β -cyclodextrin, glycogen, inulin, mannan, N-acetyl-D-glucosamine, N-acetyl- β -D-mannosamine, amygdalin, D-arabitol, L-fucose, gentiobiose, myo-inositol, lactose, melezitose, melibiose, methyl α -D-galactoside, methyl β -D-galactoside, methyl α -D-glucoside, methyl β -D-glucoside, methyl α -D-mannoside, L-rhamnose, sedoheptulosan, D-tagatose, xylitol, α -hydroxybutyric acid, p-hydroxyphenylacetic acid, α -ketoglutaric acid, lactamide, D-lactic acid methyl ester, L-lactic acid, D-malic acid, N-acetyl-L-glutamic acid, glycyl-L-glutamic acid, L-pyrroglutamic acid, AMP, D-fructose 6-phosphate, α -D-glucose 1-phosphate, and D-glucose 6-phosphate.

Most of the characteristics for species *P. halocryophilus* (Mykytzcuk et al. 2012) are included in [▶ Tables 25.2](#) and [▶ 25.3](#) except the following: utilizes rhamnose, lactose, pectin, dextrin, acetic acid, gelatin, and gluconic acid but not melibiose, inositol, methyl pyruvate, aminobutyric acid, ketobutyric acid, and bromosuccinic acid. Cells produce acid from rhamnose and L-arabinose but not from inositol, sorbitol, and amygdalin. *P. halocryophilus* is positive for arginine decarboxylase, lysine decarboxylase, and production of H₂S but negative for ornithine decarboxylase and tryptophan deaminase.

Species *P. kocurii* was poorly characterized compared to the other species of the genus. Most of the characteristics are listed in [▶ Tables 25.2](#) and [▶ 25.3](#), and in addition to them, it exhibits following features as characterized by Jung et al. (1998): *P. kocurii* is positive for utilization of propionate, pyruvate, benzoate, α -ketoglutarate, acetate, aspartate, Na-butyrate, glucolic acid, and methanol but not fumarate, glutamate, lactose, L-proline, L-leucine, and N-acetyl-glucosamine.

Bhargavaea (Manorama et al. 2009 Emend. Verma et al. 2012)

Bhargavaea (*Bhar.ga.va'e.a*. N.L. fem. n. *Bhargavaea* named in honor of Pushpa Mittra Bhargava, the renowned Indian biologist).

Genus *Bhargavaea* was described by Manorama et al. (2009) and emended by Verma et al. (2012). The genus presently contains three species: *Bhargavaea cecembensis* (Manorama et al. 2009), *Bhargavaea beijingensis*, and *Bhargavaea ginsengi* (Verma et al. 2012). The latter two species were originally described as *Bacillus beijingensis* and *Bacillus ginsengi* (Qiu et al. 2009) but were transferred to the genus *Bhargavaea* based on their 16S rRNA gene sequence and *GyrB* amino acid sequence.

The emended description of the genus is as follows: cells are Gram-positive, aerobic, nonspore forming, rod to coccoid, and rod shaped that are positive for catalase, oxidase, and lipase

Table 25.2

Diagnostic phenotypic characteristics of species of the genus *Planococcus* (Data from Kaur et al. (2012); Mykytzcuk et al. (2012); Hao and Komagata (1985); Nakagawa et al. (1996); Engelhardt et al. (2001); Yoon et al. (2003); Shivaji (2009)). Strains: 1, *P. plakortidis* AS/ASP6 (II)^{T a}; 2, *P. maritimus* TF-9^{T a}; 3, *P. riffoensis* M8^{T a}; 4, *P. maitriensis* S1^{T a}; 5, *P. citreus* NCIMB 1493^{T a}; 6, *P. salinarum* ISL-16^{T a}; 7, *P. columbae* PgEx11^{T a}; 8, *P. donghaensis* JH1^{T a}; 9, *P. antarcticus* CMS 26ot^{T a}; 10, *P. halocryophilus* Or1^{T b}; 11, *P. kocurii* DSM 20747^{T c}

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Morphological characteristics											
Colony color	Dull yellow/orange	Yellow/orange	Orange	Orange	Orange/yellow	Pale yellow	Orange	Orange	Orange	Bright orange	Orange/yellow
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci/short rods/rods	Cocci	Cocci	Cocci	Cocci	Cocci
Cell arrangement	Pairs or clusters	NR	Single, pairs, tetrads, clumps	Single, pairs, in threes, tetrads	Single, pairs, in threes, tetrads	NR	NR	Single, pairs, in threes, tetrads	Single, pairs, in threes, tetrads	Single or pairs	Single, pairs, in threes
Motility	+	+	+	+	+	-	+	+	+	+	+
Growth characteristics											
pH Range	6-10	5-8	6-10.5	6-12	6-11	6-7.5	6-11	7-8	6-12	6-11	NR
Temperature range	15-37	4-41	5-42	0-30	4-37	4-38	8-42	4-37	0-30	10-37	4-37
37 °C	+	+	+	-	+	+	+	+	-	+	+
42 °C	-	+	+	-	-	-	+	-	-	-	-
NaCl tolerance (%)	7	17	15	12.5	15	13	14	12	12	19	10
10 % NaCl	-	+	+	+	+	+	+	-	+	+	+
15 % NaCl	-	+	+	-	+	-	-	-	-	+	-
Biochemical characteristics											
Methyl red test	+	+	-	-	-	-	-	-	+	NR	-
Voges-Proskauer test	-	-	-	-	-	-	-	+	-	-	-
Indole test	-	-	-	-	-	-	-	-	-	+	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	-	+	-
Nitrate reduction	-	-	-	+	-	-	-	+	+	-	-
Oxidase	+	-	+	-	-	+	+	+	+	+	-
Hydrolysis of											
Starch	-	-	-	-	-	-	-	+	-	-	-
Casein	-	+	-	-	-	+	-	+	-	-	NR
Gelatin	-	+	+	-	+	-	-	+	-	+	V
Aesculin	-	-	-	-	-	-	-	+	-	-	-

Table 25.2 (continued)

Characteristic	1	2	3	4	5	6	7	8	9	10	11
N-acetyl-D-glucosamine	-	-	-	-	-	-	-	-	-	+	-
Lactulose	-	+	+	-	-	-	-	-	-	NRA	NR
Maltose	+	-	-	-	+	-	+	+	-	+	NR
Maltotriose	-	-	-	-	-	-	+	-	-	NR	NR
D-Mannitol	-	-	+	+	+	-	-	+	-	+	NR
D-Mannose	-	-	+	-	+	-	-	+	-	+	NR
3-Methyl D-glucose	-	+	-	-	-	-	-	-	-	NR	NR
Palatinose	-	-	-	+	-	-	-	-	+	NR	NR
D-Psicose	-	+	+	+	-	-	-	+	-	NR	NR
Raffinose	-	+	-	-	-	-	-	-	-	-	NR
D-Ribose	+	+	+	+	+	+	+	+	+	+	-
Stachyose	-	-	-	-	-	-	+	-	-	NR	NR
Salicin	-	+	-	-	+	+	-	-	+	NR	NR
Sucrose	+	-	-	-	+	-	+	-	-	+	-
Trehalose	-	-	-	-	-	-	-	+	-	+	NR
Turanose	-	-	-	-	-	-	-	+	-	NR	NR
Tween 40	+	-	-	-	-	+	-	+	-	+	NR
Tween 80	+	-	-	-	-	+	+	-	-	-	-
D-Xylose	+	-	+	-	-	-	-	+	+	NR	NR
Acetic acid	+	-	+	+	+	+	-	-	+	NR	NR
β -Hydroxybutyric acid	-	-	-	-	-	+	-	+	-	NR	NR
γ -Hydroxybutyric acid	-	-	-	-	-	+	-	-	-	NR	NR
α -Ketovaleic acid	+	-	+	+	+	+	-	+	-	NR	NR
L-Malic acid	+	-	-	-	-	-	-	-	-	NR	NR
Pyruvic acid methyl ester	+	-	+	-	+	+	-	+	-	NR	NR
Succinic acid monomethyl ester	-	+	+	-	-	-	+	+	-	NR	NR
Propionic acid	-	-	-	+	-	-	-	-	-	NR	NR
Pyruvic acid	+	-	+	-	+	+	+	-	-	NR	NR
Succinamic acid	+	-	-	-	-	-	-	+	-	NR	NR

Succinic acid	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
L-Alaninamide	+	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
D-Alanine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
L-Alanine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NR
L-Alanyl – glycine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
L-Arginine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NR
L-Asparagine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
L-Glutamic acid	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NR
L-Serine	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NR
Putrescine	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
2, 3-Butanediol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
Glycerol	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	NR
Adenosine	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
Deoxyadenosine	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NRD	NR
Inosine	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
Thymidine	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
Uridine	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
TMP	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
UMP	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
DL- α -Glycerol phosphate	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	NR	NR
Chemotaxonomic characteristics																			
Menaquinone	MK-6, MK-7, MK-8	MK-8	MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8
Polar lipids	PG, DPG, PE	PG, DPG, PE	PG, DPG, PC	NR	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	NR	PG, DPG, PE	NR	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	NR	PG, DPG, PE
G+C content of DNA (mol%)	51	48	48	39	48	48	48.5	48.3	50.5	47	41.5	40.5	40.5	40.5	40.5	40.5	40.5	40.5	40-43
Source	Marine sponge	Sea water	Algal mat	Cyanobacterial mat	Shrimp and sea food	Marine sediment	Pigeon feces	Deep-sea sediment	Cyanobacterial mat	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods	Fish and frozen foods

*Kaur et al. (2012)

^bWyktyczuk et al. (2012)

^cHao and Komagata (1985); Nakagawa et al. (1996); Engelhardt et al. (2001); Yoon et al. (2003); Shivaji (2009)
 + positive, – negative, v variable, NR not reported, S sensitive, R resistant, MK menaquinone, PG phosphatidylglycerol, DPG diphosphatidylglycerol, PE phosphatidylethanolamine, PC phosphatidylcholine. For antibiotic concentration, check the original papers since for different species different concentrations have been used

Table 25.3

Cellular fatty acid composition (%) of species of the genus *Planococcus* (Data from Kaur et al. (2012); Mykytczuk et al. (2012)). Strains: 1, *P. plakortidis* AS/ASP6 (II)^{T a}; 2, *P. maritimus* TF-9^{T a}; 3, *P. rifetoensis* M8^{T a}; 4, *P. maitriensis* S1^{T a}; 5, *P. citreus* NCIMB 1493^{T a}; 6, *P. salinarum* ISL-16^{T a}; 7, *P. columbae* PgEx11^{T a}; 8, *P. donghaensis* JH1^{T a}; 9, *P. antarcticus* CMS 26or^{T a}; 10, *P. halocryophilus* Or1^{T b}; 11, *P. kocurii* DSM 20747^{T b}

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
Straight chain											
C _{12:0}	NR	NR	NR	2.9	NR	9.6	NR	3.2	NR	NR	NR
C _{14:0}	1.5	NR	0.6	2.4	NR	5.5	NR	2.3	NR	0.5	NR
C _{16:0}	1.9	1.9	6.8	9	4	5.4	NR	6.3	2.3	7.0	1–2.7
C _{18:0}	0.5	NR	5.9	9.2	2	7.4	NR	8.9	NR	2.9	0–2.2
Branched chain											
iso-C _{14:0}	9.6	2.8	2.8	3.4	NR	5	11.1	5.4	1	1.2	6–16
iso-C _{15:0}	13.2	12.1	6.9	5.5	1.9	NR	13.6	2.6	2.5	1.7	6–14
anteiso-C _{15:0}	34.2	40.5	37	35.5	56	35.1	41	32.5	53.1	46.0	40–48
iso-C _{16:0}	11.7	6.1	7.6	8.2	3.6	11.8	11.1	7.7	3.2	2.9	4–11
iso-C _{17:0}	2.9	5.6	4.2	3.1	NR	NR	3	2	3.1	2.3	0–3.0
anteiso-C _{17:0}	5.5	9.3	8.6	7.5	18.8	7.7	5.3	7.4	12.4	18.0	2–14
Unsaturated											
C _{16:1} (ω _{7c}) alcohol	7	0.9	4	5	4.5	10.8	8.4	6.8	3.8	2.1	1–11
Summed feature 4	NR	NR	NR	NR	NR	NR	NR	NR	NR	5.2	NR
Summed feature 5	1.2	4.5	1.9	NR	5.4	NR	2.5	5.1	9.4	NR	NR

^aKaur et al. (2012)

^bMykytczuk et al. (2012)

– negative, NR not reported, summed feature 4; iso-C_{17:1} I and/or anteiso-C_{17:1} B; Summed feature 5: C_{18:2}(ω_{6,9c}) and/or anteiso-C_{18:0}. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

activities and some produce urease. The major fatty acids are iso-C_{15:0} and anteiso-C_{15:0}. MK-8 is the predominant respiratory quinone. Cell wall peptidoglycan type is A4α and contains L-lysine as the diagnostic diamino acid. The major polar lipids are phosphatidylglycerol and diphosphatidylglycerol. The type species *Bhargavaea cecembensis*, DSE10^T, contains the signature nucleotides G, A, C, T, C, A, G, C, and T at positions 182, 444, 480, 492, 563, 931, 1253, 1300, and 1391, respectively, in the 16S rRNA gene sequence. The G+C content of the genomic DNA is 50–54.0 mol%.

Initially, the genus description was based on a single species, and the type strain of the genus, *Bhargavaea cecembensis*, was characterized phenotypically using the HiMedia *Enterobacteriaceae* identification kits KB003 and KB009 A, B, and C. Verma et al. (2012) characterized the three species using the VITEK-2 GP compact system, API ZYM (bioMérieux) and Biolog GP2 MicroPlates along with HiMedia kits besides using the conventional phenotyping methods (Gordon et al. 1973; Kovacs 1956; Simmons 1926).

The type species of the genus is *Bhargavaea cecembensis* (Manorama et al. 2009; emend Verma et al. 2012), and the type strain is DSE10^T = LMG 24411^T = JCM 14375^T.

All the three species of *Bhargavaea* are Gram-positive; nonmotile; nonspore forming; positive for oxidase, catalase, caseinase, gelatinase, nitrate reduction, and β-hemolysis; and negative for indole production, Simmons' citrate, methyl red

and Voges-Proskauer reactions, phenylalanine deaminase, glucose fermentation, and H₂S production. All the three species are positive for esterase (C4), esterase lipase (C8), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase but negative for alkaline phosphatase, lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. None of the strains produced acid from adonitol, arabinose, glucose, dulcitol, inositol, inulin, lactose, mannitol, melibiose, raffinose, sorbitol, sucrose, or xylose; however, acid was produced from trehalose and fructose. Oxidation of different carbon substrates (Biolog GP2) shows that all three species are positive for Tween 40, β-hydroxybutyric acid, α-ketoglutaric acid, α-ketovaleric acid, acetic acid, pyruvic acid methyl ester, succinic acid monomethyl ester, pyruvic acid, succinic acid, L-alaninamide, L-alanyl glycine, L-glutamic acid, β-cyclodextrin, L-serine, and glycerol, while negative for α-cyclodextrin, dextrin, glycogen, inulin, mannan, amygdalin, Tween 80, L-arabinose, D-arabitol, arbutin, cellobiose, D-fructose, L-fucose, D-galactose, D-galacturonic acid, gentiobiose, D-gluconic acid, D-glucose, myo-inositol, L-asparagine, lactose, lactulose, maltose, maltotriose, D-mannitol, D-mannose, melezitose, melibiose, methyl α-D-galactoside, methyl β-D-galactoside, 3-methyl glucose, N-acetyl-L-glutamic acid, methyl α-D-glucoside, methyl β-D-glucoside, methyl α-D-mannoside,

palatinose, D-psicose, raffinose, L-rhamnose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-tagatose, trehalose, turanose, xylitol, D-xylose, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, lactamide, D-lactic acid methyl ester, L-lactic acid, propionic acid, L-pyroglutamic acid, putrescine, 2,3-butanediol, D-fructose 6-phosphate, α -D-glucose 1-phosphate, D-glucose 6-phosphate, and D L-glycerol phosphate. Results from the GP VITEK-2 compact system indicated that all strains are positive for ala-phe-pro arylamidase, Ellman test, L-pyrrolidonyl arylamidase, L-aspartate arylamidase, leucine arylamidase, phenylalanine arylamidase, and tyrosine arylamidase and negative for β -xylosidase, L-lysine arylamidase, L-proline arylamidase, α -galactosidase, β -galactosidase, alanine arylamidase, *N*-Acetyl- β -glucosaminidase, β -mannosidase, cyclodextrin, D-galactose, glycogen, *myo*-inositol, methyl α -D-glucopyranoside acidification, methyl D-xyloside, maltotriose, glycine arylamidase, D-mannitol, melezitose, *N*-acetyl-D-glucosamine, palatinose, L-rhamnose, β -glucosidase, phosphorylcholine, pyruvate, α -glucosidase, D-tagatose, inulin, D-glucose, D-ribose, putrescine assimilation, and aesculin hydrolysis. The differentiating characteristics of species of the genus *Bhargavaea* are listed in [▶ Tables 25.4](#) and [▶ 25.5](#).

Caryophanon (Peshkoff 1939)

Caryophanon (Ca.ry.o'pha.non. Gr. N. *Karyon* nut, kernel, nucleus; Gr. adj. *Phaneros* bright, conspicuous; N. L. neut. N. *Caryophanon* that which has a conspicuous nucleus).

The genus presently contains two species, *Caryophanon latum* and *Caryophanon tenue* (Peshkoff 1939; Trentini 1978; Pringsheim and Robinow 1947). The genus *Caryophanon* is a unique prokaryote because cells are extremely large, exhibit unusual structural complexity, and present in specialized ecological niches. This giant bacterium forms trichomes (multicellular rods) with rounded or tapered ends, and the size of the trichome is 1.0–3.5 μm in diameter and 10–40 μm in length (Pringsheim and Robinow 1947). Several trichomes may form short chains without any branching. Peshkoff first isolated this organism from fresh cow dung in Russia in 1939. Growing the organism was a challenging task. The organism grows poorly on routine nutrient agar but thrives on yeast extract plus meat-extract agar, especially if supplemented with small amounts of sodium acetate. A medium containing 0.5 % Bacto yeast extract, 0.5 % Bacto peptone, and 0.01 % sodium acetate, adjusted to pH 7.4–7.6, proved very favorable for isolation and maintenance (Pringsheim and Robinow 1947). Cow dung agar with lactalbumin hydrolysate (Moran and Witter 1976) or a semisynthetic medium containing (gm per liter) lactalbumin hydrolysate (10.0), sodium acetate (5.0), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (20.0), CaCl_2 (11.0), Cu_2SO_4 (0.4 mg), FeSO_4 (0.152 mg), biotin (0.02 mg), thiamin (0.05 mg), and nitrilotriacetic acid (19.1 mg) (Smith and Trentini 1973; Trentini 1978) supported good growth. Recently Fritze and Claus (2009) could grow both the species of *Caryophanon* using the DSM medium no.34 (per liter: yeast extract [2.0 g], trypticase [2.0 g], soya peptone [2.0 g], sodium

acetate [1.0 g], sodium glutamate [0.1 g], thiamine-HCl \times 2 H_2O [0.2 mg], biotin [0.05 mg], K_2HPO_4 [1.0 g], $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ [0.27 g], Tris-HCl buffer [10 mM] and pH 7.8) for the purpose of fatty acid analyses. In all the cases, biotin is required and thiamine is a stimulator.

Cells of *Caryophanon* can grow from 10 to 40 °C with an optimum of 25–37 °C and can tolerate 4 °C for 4 weeks. In liquid media, it does not multiply, but very small concentrations of agar are sufficient to enable it to grow. After isolating in pure form, the typical cell morphology can be preserved only in liquid media containing cow dung. However, change in morphology of both species during the extended cultivation on agar medium was demonstrated by Peshkov et al. (1978). Cells of *Caryophanon* are Gram-positive, motile rods by means of peritrichous flagella and asporogenous. However, Fritze and Claus (2009) reported that the type strains of the genus *Caryophanon*, *C. latum* (DSM 14151), and *C. tenue* (DSM 14152) lost motility on culture media. Phenotypic characteristics ([▶ Table 25.6](#)) of the genus are based on the study by Fritze and Claus (2009), Pringsheim and Robinow (1947), and Trentini (1978). Cells are strictly aerobic and chemoorganotrophic. Carbohydrates are not utilized as substrates, but acetate and other organic acids are utilized as major carbon sources. Cells are catalase positive and negative for oxidase, urease, nitrate reduction, phenylalanine deaminase, and indole production. Both the species can hydrolyze gelatin but not starch, tyrosine, and hippurate. *C. tenue* can hydrolyze Tween 20, 40, 60, and 80. *C. latum* and *C. tenue* are negative for acid production from L-arabinose, D-glucose, mannitol, and xylose, can utilize citrate and propionate, and were resistant to the antibiotics streptomycin, polymyxin B, and nalidixic acid. The other characteristics are listed in [▶ Tables 25.6](#) and [▶ 25.7](#). According to Adcock et al. (1976), both species of *Caryophanon* had similar antibiotic resistance pattern. The general appearance of the organism and the character of the nuclear structures suggested the existence of a direct relationship to blue-green algae (Peshkoff 1940). However, features such as motility due to the presence of typical, peritrichous flagella, regular binary fission of the composite rods, and the absence of a distinctive slime layer and the 16S rRNA gene sequence placed the genus in Eubacteria. The close phylogenetic relationship between *Caryophanon* and related genera of the family Planococcaceae was described by Stackebrandt et al. (1987). Placement of both *Caryophanon* species within rRNA group two (Ash et al. 1991) was confirmed by Farrow et al. (1994) and Fritze and Claus (2009).

The peptidoglycan of *C. latum* and *C. tenue* was very similar and was found to contain glucosamine, muramic acid, alanine, glutamic acid, and lysine in the molar ration of about 2:2: 1:1 (Becker et al. 1967; Trentini 1978). One of the glutamic acid residues is bound to the ϵ -amino group of lysine and was responsible for the cross-linking of the peptide subunit, and therefore, it is of A4 α type (Stackebrandt et al. 1987). Cell walls of *Caryophanon* species are sensitive to lysozyme. Menaquinones with six isoprene units (MK-6) are the major isoprenologues present in *Caryophanon*

Table 25.4

Diagnostic phenotypic characteristics of species of the genus *Bhargavaea* (Data from Manorama et al. (2009) and Verma et al. (2012))

Characteristic	<i>B. cecembensis</i> LMG 24411 ^{Ta}	<i>B. beijingensis</i> DSM 19037 ^{Ta}	<i>B. ginsengi</i> DSM 19038 ^{Ta}
Morphological characteristics			
Cell shape	Rods	Coccoid rods	Coccoid rods
Cell arrangement	Single, pairs, or short chains	Single or pairs	Single, pairs, or short chains
Cell dimensions (μm)	1.0 × 2.0–8.0	1.0 × 1.2	1.0 × 1.2–2.0
Growth characteristics			
Temp. range (°C)	15–55	7–45	4–45
Optimum growth (°C)	37	30	30
Growth pH range	7–7.5	5.5–11	6–11
Biochemical characteristics			
Urease	+	+	–
Sucrose fermentation	–	+	+
Lysozyme resistance	–	–	+
Acid production from			
Galactose	–	–	+
Salicin	–	+	+
Mannose	+	–	–
Rhamnose	+	–	–
Cellobiose	+	–	–
Maltose	–	–	+
Utilization of substrates (Biolog)			
<i>N</i> -Acetyl-D-glucosamine	–	–	+
<i>N</i> -Acetyl-β-D-mannosamine	–	–	+
α-Hydroxybutyric acid	+	+	–
D-Malic acid	–	+	–
L-Malic acid	+	+	–
Succinic acid	–	+	+
D-Alanine	+	+	–
L-Alanine	+	–	+
Glycyl-L-glutamic acid	–	+	–
Adenosine	–	+	+
2'-Deoxyadenosine	–	+	+
Inosine	–	+	+
Thymidine	–	+	+
Chemotaxonomic characteristics			
Menaquinone	MK-8, MK-9	MK-8	MK-7, MK-8
Polar lipids	PG, DPG, PC, UPL	PG, DPG, UPL	PG, DPG, PC, UPL
Peptidoglycan composition	L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Glu
G+C content of DNA (mol%)	51	48	48
Source	Deep-sea sediment	Tissue of ginseng roots	Tissue of ginseng roots

^aData from Manorama et al. (2009) and Verma et al. (2012)

+ positive, – negative, MK menaquinone, PG phosphatidylglycerol, DPG diphosphatidylglycerol, PC phosphatidylcholine, UPL unidentified phospholipid, L-Lys L-lysine, d-Asp D-aspartic acid, d-Glu D-glutamic acid

(Collins and Jones 1981). The major fatty acids present are iso-C_{15:0} and C_{16:1(ω7c)} alcohol, and composition of other fatty acids of *C. latum* and *C. tenue* is given in Table 25.7 (Fritze and Claus 2009).

The main difference between *C. latum* and *C. tenue* is in their nature of trichome. Cells of *C. latum* in the trichome are larger in width than in length and show cross wall formation. In *C. tenue*, cells within trichomes are slightly larger in length than in

■ **Table 25.5**
Cellular fatty acid composition (%) of species of the genus *Bhargavaea*^a

Fatty acid	<i>B. cecembensis</i> LMG 24411 ^T	<i>B. beijingensis</i> DSM 19037 ^T	<i>B. ginsengi</i> DSM 19038 ^T
Branched chain			
iso-C _{14:0}	8.9	4.2	4.9
iso-C _{15:0}	18.5	37.4	29
iso-C _{16:0}	14	5.9	8.6
anteiso-C _{15:0}	33.4	27	32.6
anteiso-C _{17:0}	9.5	11.6	9.9

^aData from Manorama et al. (2009) and Verma et al. (2012). If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

■ **Table 25.6**
Diagnostic phenotypic characteristics of species of the genus *Caryophanon*^a

Characteristic	<i>C. latum</i> DSM14151 ^T	<i>C. tenue</i> DSM 14152 ^T
Morphological characteristics		
Trichome width (µm)	2.3–3.5	1.0–2.0
Trichome length (µm)	6.0–20.0	4.0–10.0
No. of cells per trichome	4–15	2–3
Biochemical characteristics		
Indole production	+	–
Hydrolysis of		
Tween 80	–	+
Tween 60	–	+
Tween 40	–	+
Tween 20	–	+
Chemotaxonomic characteristics		
G+C content of DNA (mol%)	44–45.6	41.2–41.6
Genome length	1100–1200 × 10 ⁶	900–1000 × 10 ⁶
Source	Cow dung	Cow dung

^aData from Fritze and Claus (2009), Trentini (1978), and Pringsheim and Robinow (1947)

+ positive, – negative

width and lack the multiple septation typical of *C. latum* and show mostly only one cross septum in a trichome (Peshkov and Marek 1972). Further, the size of trichomes of *C. latum* varies between 2.3 × 6.0 and 3.5 × 20 µm, and the cell number ranges from 4 in the shorter to 15 in the longer trichomes. The trichome dimensions of *C. tenue* range between 1.0 × 4.0 and 2.0 × 10.0 µm, and the cell number range from 2 to 3. In addition, *C. latum* has a preference for acetate as a carbon source followed by butyrate (Provost and Doetsch 1962), and in contrast,

■ **Table 25.7**
Cellular fatty acid composition (%) of species of the genus *Caryophanon* (Data from Fritze and Claus (2009))

Fatty acid	<i>C. latum</i> DSM14151 ^{Ta} = NCIMB 9533 ^{Ta}	<i>C. tenue</i> DSM 14152 ^{Ta}
Straight chain		
C _{16:0}	4.0	5.5
Branched chain		
C _{14:0 iso}	4.3	5.4
C _{15:0 iso}	39.4	28.8
C _{16:0 iso}	1.7	10.6
Unsaturated		
C _{16:1(ω7c)} alcohol	8.7	12.5
C _{16:1(ω11c)}	23.3	18.5

^aFritze and Claus (2009)

Note: DSM medium no. 34 was used for growing *Caryophanon*. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

–, absent

C. tenue has a preference for valerate and capronate to acetate (Rowenhagen 1987).

The type species of the genus is *Caryophanon latum* (Peshkoff 1939), and the type strain is ATCC 33407^T = LMG 17312^T = NCIMB 9533^T = VKM B-105^T.

Chryseomicrobium (Arora et al. 2011)

Chryseomicrobium (Chry'se.o.mi.cro'bi.um. Gr. adj. *chruseos* golden; N.L. neut. n. *microbium* microbe from Gr. adj. *mikros* small and Gr. n. *bios* life; N.L. neut. n. *Chryseomicrobium* yellow microbe).

The type strain of *Chryseomicrobium* was isolated from sea water of the Bay of Bengal, India (Arora et al. 2011). The description of the genus is based on a single species, *Chryseomicrobium imtechense*. Cells of *Chryseomicrobium* are Gram-positive, nonmotile, nonspore forming, and rod shaped, and the cells size is 0.3–0.7 µm wide and 1.7–2.9 µm long. Grows from 4 to 45 °C with an optimum of 30 °C, pH range is 6–9, and can tolerate 6 % NaCl. Catalase positive, casein is hydrolyzed but not gelatin, starch, ONPG, Tween 40, Tween 80, and aesculin. Negative for egg yolk reaction, oxidase, urease, H₂S production, indole formation, methyl red and Voges-Proskauer tests, and nitrate is not reduced to nitrite. No growth on MacConkey or Simmons' citrate agar. Acid is produced from glucose, salicin, fructose, maltose, sucrose, inulin, melibiose, and cellobiose.

In the Biolog GP2 system, cells of *Chryseomicrobium* are positive for oxidation of α-ketovaleric acid, glycerol, γ-hydroxybutyric acid, pyruvic acid, L-serine, D-ribose, pyruvic acid methyl ester, L-alanine, 2-deoxyadenosine, and L-arabinose and negative for oxidation of α- and β-cyclodextrin, glycogen,

inulin, mannan, lactose, methyl β -D-glucoside, L-alaninamide, trehalose, D-lactic acid methyl ester, D-alanine, arbutin, L-lactic acid, xylitol, D- and L-malic acid, raffinose, L-asparagine, L-fucose, L-rhamnose, adenosine 5'-monophosphate, melezitose, α -hydroxybutyric acid, glycyl-L-glutamic acid, D-galacturonic acid, melibiose, salicin, propionic acid, L-pyroglutamic acid, uridine 5'-monophosphate, gentiobiose, methyl α -D-galactoside, sedoheptulosan, D-fructose 6-phosphate, N-acetyl-b-glucosamine, D-gluconic acid, methyl β -D-galactoside, D-sorbitol, *p*-hydroxyphenylacetic acid, succinamic acid, putrescine, α -D-glucose 1-phosphate, amygdalin, *myo*-inositol, methyl α -D-glucoside, sucrose, N-acetyl L-glutamic acid, and DL- α -glycerol phosphate.

The cell wall peptidoglycan is of the A4 α type with an interpeptide bridge containing L-Lys-D-Asp, major menaquinones are MK-7 and MK-8, which contribute to 80.7 % and MK-7 (H₂), and MK-6 are present but in lesser amounts. The cellular fatty acid profile includes C_{16:0}, C_{16:1(ω7c)} alcohol, C_{16:1(ω11c)}, iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0}, and iso-C_{17:1(ω10c)} of which iso-C_{15:0} (68.6 %) and C_{16:1(ω7c)} alcohol (10.9 %) are the predominant fatty acids. The polar lipids present are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown phospholipid, an unknown lipid, and an unknown glycolipid. The mol% G+C content of DNA is 53.4.

Cell shape, nonspore-forming nature, presence of high amounts of iso-C_{15:0} and C_{16:1(ω7c)} alcohol, and relatively high DNA G+C content make the genus *Chryseomicrobium* unique. Phylogenetically, the genus is closely related to the clades representing *Planococcus* and *Planomicrobium*.

The type specie of the genus *Chryseomicrobium* is *Chryseomicrobium imtechense* (Arora et al. 2011), and the type strain is MW 10^T = MTCC 10098^T = JCM 16573^T and isolated from a seawater.

***Filibacter* (Maiden and Jones 1984)**

Filibacter (*Fi.li.bac'ter*. L. n. *filum* a thread; N. L. masc. N. *bacter* masculine form of Gr. neut. N. *bactron* a rod; N. L. masc. N. *Filibacter* thread rod).

The type strain of *Filibacter* was isolated from the sediment of a eutrophic lake on a dilute peptone medium by Maiden and Jones (1984). Cells of *Filibacter* are composed of cylindrical filaments that are straight or curved, filaments are neither sheathed nor branched, and cell junctions are clearly marked. Filaments are 1.1 μ m in width and 8–150 μ m in length, and the cell dimensions within the filaments are 1.1 μ m in width and 3–30 μ m in length. Filaments are flexible but do not show any active flexing. Cells of *Filibacter* spread widely over the surface of agar producing whorls of growth and spiral colonies because of the gliding nature of the cell. Cells stained Gram-negative motile by means of gliding, nonspore forming, strictly aerobic, and slimy. They grow from 4 to 26 °C with an optimum at 20 °C and pH optimum of 7–7.4 and had a requirement for vitamins.

No single amino acid is required for growth, but all are required for maximum biomass production, and growth does not occur in absence of vitamins. The unique feature of this organism is its inability to utilize organic compounds in the absence of amino acids. However, histidine is inhibitory due to the possible inhibition of glutamine synthetase. Phenotypic characteristics were described by Maiden and Jones (1984) by testing on API 20B strips, and most of the tests showed negative results. But cells of *Filibacter* are positive for catalase, oxidase, β -galactosidase, lipase, chymotrypsin, phosphoamidase, and urease. Hydrolyzes gelatin but not casein or starch and reduces nitrate to nitrite. Could not utilize glucose, fructose, sucrose, lactose, galactose, mannitol, glucosamine, galacturonic acid, acetate, pyruvate, lactate, citrate, glutamate, butyrate, ethanol, and glycerol as carbon sources (Maiden and Jones 1984). However, acetate, butyrate, and glycerol (at 2 mg L) enhanced the growth in presence of amino acids. Among the amino acids, the combination of the following families supported the growth: the glutamate family (glutamate, glutamine, arginine, and proline) plus aspartate family (aspartate, asparagines, lysine, threonine isoleucine, and methionine) or serine family (serine, glycine, and cysteine) and the aspartate family plus the pyruvate family (alanine, valine, and leucine) and serine or aromatic family (tryptophan, phenylalanine, and tyrosine). The interdependence of combination of amino acids from different families could be due to the coupled oxidation-reduction reactions. No single amino acid or a mixture of amino acids from a single family supported the growth (Maiden and Jones 1984). In addition, none of the substrates of API20 NE and none of carbohydrates (sugars and acids) provided by API 50 CHE panels supported the growth. Peptidoglycan contains lysine and the murein type is L-Lys-D-Glu and peptidoglycan variation is A4 α type and the major menaquinone is MK-7. Membrane fatty acids are dominated by anteiso-C_{15:0} and anteiso-C_{17:0} (Nichols et al. 1986), and the lone β -hydroxy fatty acid found was C_{12:0} β -OH. Cytochromes present are of *c* type, while *b* type also occurs in lesser amount (Maiden and Jones 1984). The mol% G+C content of DNA of strain *Filibacter* is 44.0 %. Morphologically, the genus *Filibacter* is closely related to *Vitreoscilla* with respect to formation of Gram-negative, unpigmented, multicellular, and gliding filaments. However, the genomic riboprinting (Stackebrandt 2009) and chemotaxonomic characteristics supported the association of *Filibacter* with members of *Bacillus* rRNA group two (Ash et al. 1991). Comparison of 16S rRNA oligonucleotide of the genus *Filibacter* with gliding organisms, *Vitreoscilla*, *Cytophaga*, and *Flexibacter* and representative of Gram-positive organisms, further, indicated a close phylogenetic relation with rRNA group two (Clausen et al. 1985).

Fatty acid composition of strain *Filibacter limicola* NCIB11923^T was determined by Nichols et al. (1986) by growing the strain in mineral medium (Maiden and Jones 1985; Jones 1983) containing trypticase soy (27 g L). The composition of the medium was (mg per liter): K₂HPO₄, 28; MgCl₂·6H₂O, 1.27; KNO₃, 4; (NH₄)₂SO₄, 60; MnSO₄·4H₂O, 8; ferric citrate, 6. The pH of the medium was adjusted with 1 M KHCO₃, before

autoclaving, and the final pH was 7.0–7.2. Composition (%) of fatty acids is C_{12:0} (0.3), anteiso-C_{17:0} (21.3), C_{14:0} (1.8), C_{15:0} (0.1), C_{16:0} (9.2), C_{17:0} (tr), C_{18:0} (0.2), C_{14:1} (0.1), C_{14:1(ω5c)} (0.1), C_{16:1(ω5c)} (0.1), C_{16:1(ω7c)} (2.8), C_{16:1(ω7t)} (0.1), C_{16:1(ω9c)} (5.4), br C_{17:1} (0.2), iso-C_{17:1} (6.5), C_{18:1(ω9c)} (0.1), C_{18:1(ω7c)} (0.3), C_{18:1(ω7t)} (0.1), and iso-C_{14:0} (tr) (Nichols et al. 1986).

The type species of the genus is *Filibacter limicola* (Maiden and Jones 1985), and the type strain is 1SS101^T = ATCC 43646^T = NCIMB 11923^T. Bacterium was isolated from lake sediment.

Jeotgalibacillus (Yoon et al. 2001)

Jeotgalibacillus (*Je.ot.ga.li.ba.cil'lus*. Korean n. *jeotgal* jeotgal, traditional Korean food; Gr. n. *baktron* rod; N.L. masc. n. *Jeotgalibacillus* rod from jeotgal).

Genus *Jeotgalibacillus* was described by Yoon et al. (2001a), and the genus presently contains five species, of which two were transferred from *Marinibacillus*. The species present are *Jeotgalibacillus alimentarius* (Yoon et al. 2001a), *Jeotgalibacillus campisalis* (Yoon et al. 2004, 2010a), *Jeotgalibacillus marinus* (Rüger and Richter 1979; Yoon et al. 2010a), *Jeotgalibacillus salarius* (Yoon et al. 2010a), and *Jeotgalibacillus soli* (Cunha et al. 2012). According to the genus description proposed by Yoon et al. (2001a), cells are rod shaped, form round endospores, present in swollen sporangia, and catalase and oxidase positive but urease negative, and nitrate is reduced to nitrite. Majority of the species are negative for lipases, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, α -galactosidase, β -glucuronidase, α -mannosidase, α -fucosidase, and *N*-acetyl- β -glucosaminidase. Cell wall peptidoglycan contains L-lysine at position three of the peptide subunit. Predominant menaquinones are MK-7 and MK-8. Major fatty acid is iso-C_{15:0}. DNA GC content is 44 mol%.

Data is available for all the species with respect to their growth, morphology, enzymes, and acid production characteristics. Species of *Jeotgalibacillus* exhibit white to yellowish orange pigmentation and are strictly aerobic. *J. alimentarius* is facultatively anaerobic, Gram-variable, and spore forming, and the spores are round or ellipsoidal, located terminally or subterminally, and in case of *J. soli*, spores can also be present in the center. The growth temperature ranges from 4 °C to 55 °C, most of them are mesophilic, whereas *J. marinus* is psychrophilic. All of them are halotolerant, and *J. alimentarius* can tolerate up to 20 % of NaCl. Cells are motile by means of peritrichous or single polar or subpolar flagella. Characteristics such as catalase, oxidase, urease, and nitrate reduction are variable among the species. However, in majority of cases, the above properties matched with the genus description of Yoon et al. (2001a). Besides these, all five species shared the following common characteristics: negative for alkaline phosphatase and positive for production of acid from D-fructose, D-glucose, maltose, sucrose, and trehalose but not from L-arabinose, inositol,

rhamnose, and sorbitol. The peptidoglycan variation is A1 α where the linkage between the peptide chains is direct, major menaquinone is MK-7, and the major lipids present were aminophospholipids APL1 to 4. Most of the strains are characterized with respect to their acid production, enzymes, and antibiotic susceptibility. The differentiating characteristics and fatty acid profiles of all the type species are given in [Tables 25.8](#) and [25.9](#).

Phylogenetically, *Jeotgalibacillus* is closely related to *Lysinibacillus*, *Solibacillus*, and *Bacillus*. The genus is unique compared to the members of the family Planococcaceae in containing the A1 α type of peptidoglycan variant and presence of aminophospholipids.

Type species is *Jeotgalibacillus alimentarius* (Yoon et al. 2001a), and the type strain is YKJ-13^T = JCM 10872^T = KCCM 80002^T.

Kurthia (Trevisan 1885)

Kurthia (*Kurth'i.a*. N.L. fem. Gen. N. *Kurthia* named for H. Kurth, the German bacteriologist who described the type species).

Genus *Kurthia* was described by Trevisan (1885) to accommodate species that are characterized by bird's feather like morphology on gelatin nutrient agar. Cells are aerobic, Gram-positive rods, motile by means of peritrichous flagella, nonspore forming, containing L-Lys-D-Asp type of peptidoglycan, MK-7 as the major menaquinone, iso-C_{15:0} and anteiso-C_{15:0} as major fatty acids, and DNA mol % G+C range of 36–38. Presence of PG, DPG, and PE as predominant polar lipids was characterized in *K. zopfii* and *K. gibsonii*. Besides the above characteristics, 16S rRNA gene sequence analysis is another unambiguous marker in assigning the strains to this genus. The rods are approximately 0.6–1.2 μ m in diameter and the length varies from 2 to 5 μ m in young cultures and 5–10 μ m in old cultures that form filaments. The genus contains three species: *K. zopfii* (Kurth 1883; Trevisan 1885), *K. gibsonii* (Shaw and Keddie 1983), and *K. sibirica* (Belikova et al. 1986). Optimum temperature for growth is 20–30 °C, and *K. gibsonii* can grow up to 45 °C.

Phenotypic characterization of *K. zopfii* and *K. gibsonii* was done by Shaw and Keddie (1983) and Keddie and Shaw (1986), respectively, and that of *K. sibirica* was by Belikova et al. (1986). Pukall and Stackebrandt (2009) characterized all the three species of *Kurthia* using the Biolog GP2 microtiter plates for substrate utilization, and the results are given in [Table 25.5](#). The phenotypic characteristics that differentiate the three species of *Kurthia* as determined by Biolog GP2 plates (Pukall and Stackebrandt 2009) and fatty acids composition (Pukall and Stackebrandt 2009; Goodfellow et al. 1980; Collins et al. 1979) of all the three species are listed in [Tables 25.10](#) and [25.11](#), respectively. Polar lipid analysis was done in case of *K. zopfii* and *Kurthia gibsonii*, and they are phosphatidyl glycerol, diphosphatidyl glycerol, and phosphatidylethanolamine.

■ Table 25.8

Diagnostic phenotypic characteristics of species of the genus *Jeothgalibacillus* (Data from Yoon et al. (2001a); Yoon et al. (2004); Ruger and Richter (1979); Yoon et al. (2010a); Cunha et al. (2012))

Characteristic	<i>J. alimentarius</i> JCM 10872 ^{Ta}	<i>J. campisalis</i> JCM 11810 ^{Tb,d} ;	<i>J. marinus</i> DSM 1297 ^{Tc,d}	<i>J. salaries</i> DSM 23492 ^{Td}	<i>J. soli</i> DSM 23228 ^{Te}
Morphological characteristics					
Colony color	Orange-yellow	Light orange-yellow	White	Light yellow	White
Gram-stain	Variable	Variable	+	Variable	+
Spore shape	Round	Round or ellipsoidal	Round	Ellipsoidal	NR
Spore position	ST or T	ST or T	ST or T	ST	C or PC
Swollen sporangia	+	Slightly	- or slightly	Slightly	NR
Flagella type	Peritrichous	Single polar	Peritrichous	Peritrichous	Single polar or subpolar
Growth characteristics					
Anaerobic growth	+	–	–	–	–
Optimum pH	7–8	7–8	7	7	8–8.5
Temperature range (°C)	10–55	5–39	5–30	5–40	15–40
Optimum temperature (°C)	30–35	30	12–23	30	30–37
NaCl tolerance (%)	20	15	7	18	9
Biochemical characteristics					
Nitrate reduction	+	+	–	–	–
Catalase	+	–	–	+	+
Oxidase	+	–	+	+	+
APIZYM					
Esterase (C4)	NR	–	–	+	W
Esterase (C8)	NR	W	–	+	W
Leucine arylamidase	NR	–	–	–	+
Alpha-Chymotrypsin	NR	–	–	–	+
Naphthol-AS-BI-phosphohydrolase	–	W	+	–	–
β-Galactosidase	+	+	–	–	–
α-Glucosidase	+	–	–	–	–
β-Glucosidase	NR	–	–	–	+
Hydrolysis of					
Gelatin	+	v	+	–	–
Starch	–	+	–	–	+
Tween 80	+	–	–	+	–
Acid production from sugars					
Cellobiose	–	+	–	W	–
Galactose	+	–	–	+	–
Lactose	–	–	+	–	–
Mannitol	+	+	–	+	+
D-Mannose	–	–	+	–	–
Melibiose	+	+	–	–	–
Melezitose	+	W	NR	+	–
Raffinose	+	NR	–	–	+
Ribose	+	–	NR	+	+
Xylose	–	–	+	–	–

■ Table 25.8 (continued)

Characteristic	<i>J. alimentarius</i> JCM 10872 ^{Ta}	<i>J. campisalis</i> JCM 11810 ^{Tb,d,}	<i>J. marinus</i> DSM 1297 ^{Tc,d}	<i>J. salaries</i> DSM 23492 ^{Td}	<i>J. soli</i> DSM 23228 ^{Te}
Chemotaxonomic characteristics					
Menaquinone	MK-7, MK-8	MK-7, MK-8	MK-7	MK-7, MK-8	MK-7
Polar lipids	NR	APL-1, APL-2, APL-3, APL-4	APL-1, APL-2, APL-3, APL-4	NR	APL-1, APL-2, APL-3, APL-4
G+C content of DNA (mol%)	44	41.8	37–42	42.9	39.4
Source	Jeotgal, sea food	Marine solar saltern	Deep-sea water	Marine sediment	Soil

^aYoon et al. (2001a)^bYoon et al. (2004)^cRüger and Richter (1979)^dYoon et al. (2010a)^eCunha et al. (2012)+positive, – negative, *W* weakly positive, *v* variable, *NR* not reported, *ST* subterminal, *T* terminal, *C* central, *PC* paracentral, *S* sensitive, *R* resistant, *MK* menaquinone, *APL* aminophospholipid

■ Table 25.9

Cellular fatty acid composition (%) of species of the genus *Jeotgalibacillus* (Data from Yoon et al. (2010a); Cunha et al. (2012))

Fatty acid	<i>J. alimentarius</i> JCM 10872 ^{Ta}	<i>J. campisalis</i> JCM 11810 ^{Ta}	<i>J. marinus</i> DSM 1297 ^{Ta}	<i>J. salaries</i> DSM 23492 ^{Ta}	<i>J. soli</i> DSM 23228 ^{Tb}
Branched chain					
iso-C _{14:0}	4.2	14.1	5.3	10.8	1.6
iso-C _{15:0}	46.3	2.5	22.2	9.4	22.0
anteiso-C _{15:0}	15.8	49.8	47.7	35.9	45.5
iso-C _{16:0}	6.3	5.2	5.1	12	2.5
iso-C _{17:0}	7.6	–	2.6	1.7	5.8
anteiso-C _{17:0}	5.8	7.4	10.7	12.2	11.3
Unsaturated					
C _{16:1} (ω 7c) alcohol	6.7	12.3	2.6	12.6	1.8

^aYoon et al. (2010a)^bCunha et al. (2012)

– absent. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

The type species of the genus is *K. zopfii* (Trevisan 1885), and the type strain is ATCC 33403^T = CCUG 38890^T = CIP 103249^T = DSM 20580^T = JCM 6101^T = LMG 17318^T = NBRC 101529^T = NCIMB 9878^T = NCTC 10597^T = VKM B-1568^T.

Paenisporosarcina (Krishnamurthi et al. 2009)

Paenisporosarcina (*Pae'ni.spo'ro.sar.ci'na*. L. adv. *paeni* almost; N.L. fem. n. *Sporosarcina* a bacterial genus name; N.L. fem. n. *Paenisporosarcina* almost a *Sporosarcina*, because it is closely related to this genus but is phylogenetically distinct).

Genus *Paenisporosarcina* was described by Krishnamurthi et al. (2009b) and emended by Reddy et al. (2013). Originally

the genus was carved from *Sporosarcina* based on the presence of L-Lys-D-Asp in the peptidoglycan, iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, and C_{16:1}(ω 7c) alcohol as major fatty acids. During the description of *Paenisporosarcina indica*, Reddy et al. (2013) found that *Sporosarcina antarctica* (Yu et al. 2008) and *Paenisporosarcina indica* were phylogenetically closely related to the members of *Paenisporosarcina* but contained L-Lys-D-Glu in their peptidoglycan. In order to accommodate the above two species, Reddy et al. (2013) emended the genus *Paenisporosarcina*, described *Paenisporosarcina indica*, and transferred *Sporosarcina antarctica* (Yu et al. 2008) to the genus *Paenisporosarcina* as *Paenisporosarcina antarctica*. The emended description of the genus *Paenisporosarcina* is as follows: cells of *Paenisporosarcina* are Gram-positive rods and/or cocci, strictly aerobic, nonmotile, form round

■ Table 25.10

Diagnostic phenotypic characteristics of species of the genus *Kurthia* (Data from Shaw and Keddie (1983); Belikova et al. (1986); Pukall and Stackebrandt (2009))

Characteristic	<i>K. zopfii</i> DSM 20580 ^{Ta}	<i>K. gibsonii</i> DSM 20636 ^{Ta}	<i>K. sibirica</i> DSM 4747 ^{Tb}
Morphological characteristics			
Cell shape	Rods or chains	Rods or chains	Rods
Growth characteristics			
Temperature range (°C)	5–35	5–45	5–37
Optimum temperature (°C)	25	25	20–25
NaCl tolerance (%)	5	7.5	6.5
Biochemical characteristics			
Phosphatase	–	+	+
DNAase	–	+	NR
RNAase	+	–	NR
Acid production			
Glycerol	–	+	+
Ethanol	+	–	–
Substrate utilization^{c#}			
<i>N</i> -acetyl-β-D-glucosamine	++	–	–
D-Fructose	–	–	++
D-Mannose	–	–	+
D-Psicose	–	–	++
Sedoheptulosan	–	–	W
γ-Hydroxybutyric acid	+	–	–
Pyruvatic acid methyl ester	+	++	–
Succinic acid monomethyl ester	++	++	–
L-Alanine	+	W	–
L-Glutamic acid	W	–	+
Glycerol	W	+	–
Inosine	+	+	–
Uridine	+	+	–
Adenosine-5'-monophosphate	W	+	–
Thymidine-5'-monophosphate	–	+	–
Uridine-5'-monophosphate	+	++	–
Mol % G+C content of DNA	36–38	36–38	37
Source	Intestinal contents	Hen and cow dung	Intestinal tract

^aShaw and Keddie (1983)

^bBelikova et al. (1986)

^cPukall and Stackebrandt (2009)

+ positive, ++ strongly positive, – negative, NR not reported, W weakly positive

endospores in a terminal or subterminal position, and catalase positive. The major fatty acids are iso-C_{14:0}, anteiso-C_{15:0}, and C_{16:1ω7c} alcohol. MK-7 and/or MK-8 are the major menaquinones, the cell wall peptidoglycan is of the A4α type with L-Lys-D-Asp or L-Lys-D-Glu, and the polar lipids present are diphosphatidylglycerol, phosphatidylethanolamine, and an aminophospholipid (APL1). The G+C content of the genus ranges from 38.0 to 46.0 mol%. The type species is *Paenisporosarcina quisquiliarum*.

The other characteristics of the genus are as follows: all the described species were negative for nitrate reduction, lysine decarboxylase, ornithine decarboxylase, Voges-Proskauer test, indole production, H₂S production, and production of acid from L-arabinose, D-lactose, D-mannose, D-mannitol, and L-rhamnose. Positive for utilization of D-fructose, D-maltose, pyruvate, D-xylose, and D-glucose, but not trehalose, D-lactose, L-rhamnose, melezitose, melibiose, *N*-acetyl-B-glucosamine, D-sorbitol, myo-inositol, and sucrose. Three species

■ Table 25.11

Cellular fatty acid composition (%) of species of the genus *Kurthia* (Data from Pukall and Stackebrandt (2009))

Fatty acid	<i>K. zopfii</i> DSM 20580 ^{Ta}	<i>K. gibsonii</i> DSM 20636 ^{Ta}	<i>K. sibirica</i> DSM 4747 ^{Ta}
Straight chain			
C _{16:0}	4.6	6.0	2.4
Branched chain			
iso-C _{14:0}	6.8	8.9	2.0
iso-C _{15:0}	42.9	26.8	65.4
anteiso-C _{15:0}	39.3	31.1	12.2
iso-C _{16:0}	—	8.8	1.1
Unsaturated			
iso-C _{17:1(ω 10c)}	—	—	6.5

^aPukall and Stackebrandt (2009)

—, absent. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

Paenisporosarcina quisquiliarum, *Paenisporosarcina macmurdoensis*, and *Paenisporosarcina indica* were positive for hydrolysis of starch but negative for hydrolysis of tyrosine, aesculin, Tween 80; production of acid from D-xylose; and utilization of xylitol, salicin, and methyl α-D-glucoside.

The two species of the Genus *Paenisporosarcina*, viz., *Paenisporosarcina quisquiliarum* and *Paenisporosarcina macmurdoensis*, were characterized using the Biolog GP2 system (Krishnamurthi et al. 2009b), and it was found that both species were positive for oxidation of adenosine 2'-deoxyadenosine, L-alanine, dextrin, D-fructose, D-mannose, maltotriose, D-ribose, turanose, thymidine, uridine, and D-xylose but not methyl β-D-glucoside; L-alaninamide; α-cyclodextrin; D-lactic acid methyl ester; D-alanine; β-cyclodextrin; arbutin; L-lactic acid; xylitol; D- and L-malic acid; glycogen; raffinose; L-asparagine; inulin; L-fucose; adenosine 5'-monophosphate; mannan; melezitose; α-hydroxybutyric acid; glycyl-L-glutamic acid; Tweens 20, 40, and 60; D-galacturonic acid; melibiose; salicin; propionic acid; L-pyrogutamic acid; uridine 5'-monophosphate; gentiobiose; methyl D-galactoside; sedoheptulosan; γ-hydroxybutyric acid; L-serine; D-fructose 6-phosphate; D-gluconic acid; methyl β-D-galactoside; D-sorbitol; p-hydroxyphenylacetic acid; succinamic acid; putrescine; α-D-glucose 1-phosphate; amygdalin; myo-inositol; methyl α-D-glucoside; sucrose; N-acetyl-L-glutamic acid; and DL-glycerol phosphate. *Paenisporosarcina antarctica* was characterized by API strips, and besides sharing some common characteristic with above three species, it exhibited the following characteristics: positive for naphthol-AS-BI-phosphohydrolase and negative for leucine arylamidase, valine arylamidase, cystine arylamidase, lecithinase, acid phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, and α-chymotrypsin; hydrolysis of trypsin; production of acid from glycerol, maltose, melibiose, melezitose, raffinose, and

D-sorbitol; and utilization of malate, succinate, capric acid, adipic acid, and phenyl acetic acid. The diagnostic characteristics of four species of the genus are listed in ● Tables 25.12 and ● 25.13.

Planomicrobium (Yoon et. 2001)

Planomicrobium (*Pla.no.mi.cro'bi.um*. Gr. n. *planos* wanderer; Gr. adj. *micro* small; Gr. n. *bios* life; M.L. n. *Planomicrobium* motile microbe).

Genus *Planomicrobium* was created by Yoon et al. (2001b) to accommodate strains that are coccoid or short rods in the early growth period but later on change to rods. The genus includes strains that are Gram-positive to Gram-variable, aerobic, nonspore forming, motile by means of a single polar flagellum or peritrichous flagella, catalase positive, and urease negative and contain L-Lys-D-Asp or L-Lys-D-Glu as the peptidoglycan type, preponderance of MK-8 followed by MK-7 or by the predominance of MK-8 followed by MK-7 and MK-6, and iso-C_{14:0}, anteiso-C_{15:0}, C_{16:1(ω7c)} alcohol, and iso-C_{16:0} as the dominant cellular fatty acids. The G+C content of the genomic DNA is 35–47 mol%. At the time of description of the genus, there were only three species (Yoon et al. 2001b), but currently, the genus contains nine species, *Planomicrobium alkanoclasticum* (Engelhardt et al. 2001; Dai et al. 2005), *Planomicrobium chinense* (Dai et al. 2005), *Planomicrobium flavidum* (Jung et al. 2009), *Planomicrobium glaciei* (Zhang et al. 2009), *Planomicrobium koreense* (Yoon et al. 2001b), *Planomicrobium mcmeekini* (Junge et al. 1998; Yoon et al. 2001b), *Planomicrobium okeanoikoites* (Nakagawa et al. 1996; Yoon et al. 2001b), *Planomicrobium psychrophilum* (Reddy et al. 2002; Dai et al. 2005), and *Planomicrobium stackebrandtii* (Mayilraj et al. 2005; Jung et al. 2009). All the nine species form a monophyletic clade clearly separating out from the parent genus, the *Planococcus* (Jung et al. 2009), from which it was carved. In addition, the genus *Planomicrobium* contains the signature nucleotides C and G at positions 183 and 190, respectively, which unambiguously distinguishes it from the phylogenetically closest genus, the *Planococcus* (Dai et al. 2005). The phenotypic characteristics that were specific for the genus at the time of description, such as oxidase, nitrate reduction, and hydrolysis of aesculin, casein, gelatin, starch, and Tween 80, are variable among the nine species and thus cannot be used as characteristics of the genus (● Table 25.10). However, the morphology, chemotaxonomic markers, 16S rRNA gene signature nucleotides can easily distinguish the genus *Planomicrobium* from other genera of the family.

The type species is *Planomicrobium koreense* (Yoon et al. 2001b). The type strain is JG07^T = CIP 107134^T = JCM 10704^T = KCTC 3684^T.

The growth characteristics of species belonging to the genus *Planomicrobium* are listed in ● Table 25.10. Phenotypic characterization, such as acid production, carbon utilization, and qualitative enzymes tests, for type strains, were carried out, in majority of the cases, using noncommercial methods for *P. chinense* (Dai et al. 2005), *P. flavidum* (Jung et al. 2009),

Table 25.12

Diagnostic phenotypic characteristics of species of the genus *Paenisporosarcina* (Data from Reddy et al. (2003) and Krishnamurthi et al. (2009b))

Characteristic	<i>Paenisporosarcina quisquiliarum</i>	<i>Paenisporosarcina macmurdoensis</i>	<i>Paenisporosarcina indica</i>	<i>Paenisporosarcina antarctica</i>
Morphological characteristics				
Cell shape	Rods	Rods/cocci	Rods	Rods
Colony color	Cream	Creamish white	Red/white	Light yellow
Spore position	T	ST	ST	NA
Growth characteristics				
Growth temperature range (°C)	15–37	0–30	0–25	0–23
Optimum temperature (°C)	25–30	20	20	17–18
pH Range	6–9	6–9	6–8	5–10
pH Optimum	7–8	7	7	6–8
Biochemical characteristics				
Oxidase	+	–	–	+
Arginine dihydrolase	+	–	–	–
Hydrolysis of				
Gelatin	–	+	+	–
Acid production from				
D-Fructose	+	–	–	–
D-Galactose	+	–	–	–
D-Glucose	+	–	–	–
Sucrose	+	–	–	–
Utilization of carbon compounds				
D-Galactose	–	+	–	–
D-Ribose	+	–	–	NR
Succinate	+	–	–	–
L-Alanine	+	–	–	NR
Oxidation of carbon compounds (Biolog GP2)				
Maltose	+	–	NR	–
Succinic acid monomethyl ester	+	–	NR	NR
Pyruvic acid	+	–	NR	NR
Methyl D-glucose	+	–	NR	NR
α-Ketovaleric acid	+	–	NR	NR
D-Tagatose	–	+	NR	NR
D-Arabitol	–	+	NR	NR
Lactulose	–	+	NR	NR
Methyl α-D-mannoside	–	+	–	NR
Cellobiose	–	+	–	–
D-Mannitol	–	+	–	–
Chemotaxonomic characteristics				
Menaquinone (major)	MK-7, MK-8	MK-7	MK-7	MK-7
Menaquinone (minor)	MK-9, MK-10, MK-11	–	–	–
Polar lipids	DPG, PG, PE, APL, UL	DPG, PG, PE, APL, UL	DPG, PG, APL, UL1-UL4, PL	NR
G+C content of DNA (mol %)	46.0	44	NR	39.2
Source	Landfill	Cyanobacterial mats	Soil	Soil

+ Positive, – negative, NR not reported, MK menaquinone, DPG diphosphatidylglycerol, PG phosphatidylglycerol, PE phosphatidylethanolamine, PL phospholipid, APL unknown phospholipid, UL unknown lipid

Table 25.13

Cellular fatty acid composition (%) of species of the genus *Paenisporosarcina* (Data are from Krishnamurthi et al. (2009b); Reddy et al. (2013); Yu et al. (2008))

Fatty acid	<i>P. quisquiliarum</i> SK55 ^{Ta}	<i>P. macmurdoensis</i> CMS21w ^{Ta}	<i>P. indica</i> PN2 ^{Tb}	<i>P. antarctica</i> N-05 ^{Tc}
Branched chain				
C _{14:0} iso	4.7	11.6	7.4	7.6
C _{15:0} iso	39.6	15.1	12.0	1.4
C _{15:0} ante	19.3	22.2	50.2	39.8
C _{16:0} iso	4.0	9.3	5.5	7.0
Unsaturated				
C _{16:1(ω7c)} alcohol	9.6	23.8	5.6	18.9
Summed feature 4	5.5	6.5	2.5	11.9

^aKrishnamurthi et al. (2009b)

^bReddy et al. (2013)

^cYu et al. (2008)

Summed feature 4: anteiso-C_{17:1} B and/or iso I; If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

P. koreense (Yoon et al. 2001b), *P. psychrophilum* (Reddy et al. 2002), *P. mcmeekinii* (Junge et al. 1998), *P. okeanokoites* (Nakagawa et al. 1996), and *P. staeckebbrandtii* (Mayilraj et al. 2005). Biolog GP MicroPlate system was used for *P. alkanoclasticum* (Engelhardt et al. 2001) and API ZYM, API 20E and API 20NE for *P. glaciei* (Zhang et al. 2009). Since phenotypic characterization was performed by various methods, most of the species are lacking the uniform characteristics that can be used to draw the similarities or differences. However, certain tests, such as methyl red, Voges-Proskauer, indole production, phenylalanine deaminase, were either negative or not reported for majority of species. In acid production, most of the type strains were negative for L-arabinose, D-mannose, xylose, cellobiose, lactose, melibiose, raffinose, rhamnose, and sucrose. Substrate utilization and antibiotic susceptibility were done only in few strains. The diagnostic and differential characteristics are listed in ▶ Tables 25.14 and ▶ 25.15.

Rummeliibacillus (Vaishampayan et al. 2009)

Rummeliibacillus (*Rum.me'li.i.ba.cil'lus*. N.L. n. *Rummelius* Rummel; L. masc. n. *bacillus* a rod, and also a bacterial genus name; N.L. masc. n. *Rummeliibacillus* a bacterium close to the genus *Bacillus* and named in honor of former NASA Planetary Protection Officer Dr. John Rummel, an astrobiologist responsible for bringing planetary protection into the public domain).

Rummeliibacillus is strictly aerobic, Gram-positive, and spore forming. The spores are round and terminally located in swollen sporangia. Strains are motile by means of peritrichous flagella. Besides these, the most straightforward placement of an unidentified strain into the genus *Rummeliibacillus* is a combination of 16S rRNA gene sequence analysis and presence of the peptidoglycan type, L-Lys-D-Glu or L-Lys-D-Asp

(variation A4α), MK-7 as the menaquinone type and phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine as major polar lipid with moderate amounts of an unknown aminophospholipid (APL1), minor amounts of two unknown phospholipids (PL1, PL2), and an unknown aminolipid (AL). Fatty acid profiles consist largely of anteiso-C_{15:0} (approximately 50 %) and iso-C_{15:0} (approximately 25 %) acids. The main characteristic that differentiates *Rummeliibacillus* from *Viridibacillus* (genus that is closely related to *Rummeliibacillus*) is the absence of green pigment. The G+C content of the genomic DNA of the genus is approximately 35.0 mol%.

The genus presently contains two species, *Rummeliibacillus stabekisii* and *Rummeliibacillus pycnus* (Vaishampayan et al. 2009). *Rummeliibacillus pycnus* was originally described as *Bacillus pycnus* (Nakamura et al. 2002), but due to its 16S rRNA gene sequence-based phylogenetic closeness, the species was transferred to the genus *Rummeliibacillus*. Both the species were characterized using API 20NE and API 50 CH strips, and both the species are positive for catalase and negative for nitrate reduction and Voges-Proskauer test. *R. stabekisii* is negative for oxidase, can hydrolyze gelatin, and produces acid from adonitol, L-arabinose, cellobiose, D-galactose, D-lactose, D-mannose, D-mannitol, D-ribose, D-xylose, and citrate. *R. pycnus* produces acid from citrate and pyruvate, does not produce indole from tryptophan, and does not decompose casein; tyrosine; urea; Tweens 40 and 80; and lecithin. Oxidizes pyruvate, β-hydroxybutyrate but not deoxyadenosine, inosine, AMP, and UMP. The other prominent characteristics are listed in ▶ Tables 25.16 and ▶ 25.17. The genus is phylogenetically closely related to the genera *Kurthia* and *Viridibacillus*.

The type species of the genus is *Rummeliibacillus stabekisii* (Vaishampayan et al. 2009), and the type strain is KSC-SF6g^T = NBRC 104870^T = NRRL B-51320^T.

■ Table 25.14

Diagnostic phenotypic characteristics of species of the genus *Planomicrobium* (Data from Engelhardt et al. (2001); Dai et al. (2005); Jung et al. (2009); Zhang et al. (2009); Yoon et al. (2001b); Junge et al. (1998); Reddy et al. (2002); Mayilraj et al. (2005)). 1. *P. alkanoclasticum* MAE2^{T a,b}; 2. *P. chinense* JCM 12466^{T b,c}; 3. *P. flavidum* KCTC 13261^{T c}; 4. *P. glaciei* JCM 15088^{T c,d}; 5. *P. koreense* JCM 10704^{T c,e}; 6. *P. mcmeekinii* ATCC 700539^{T c,e,f}; 7. *P. okeanoikoites* ATCC 33414^{T c,e}; 8. *P. psychrophilum* CMS 53or^{T b,c,g}; 9. *P. stackebrandtii* DSM 16419^{T c,h}

Characteristic	1	2	3	4	5	6	7	8	9
Morphological characteristics									
Colony color	Orange	Yellow-orange	Light yellow	Yellow-orange	Yellow-orange	Pale orange	Yellow-orange	Orange	Orange
Cell shape	Rods	Cocci/short rods	Cocci/short rods	Cocci/short rods	Cocci/short rods	Cocci/short rods	Rods	Rods	Cocci
Cells size (µm)	0.4–0.8 × 1.7–2.6	0.8 × 1.0	0.4–0.8 × 0.4–1.6	NR	NR	0.8–1.2 × 0.8–1.0	0.4–0.8 × 1.0–2.0	NR	NR
Gram-stain	v	+	v	+	v	+	v	+	+
Growth characteristics									
pH Range	NR	5–10	6–8	5–10	6–9	NR	NR	6–12	5.6–11
Temperature range (°C)	15–41	10–45	4–37	4–28	4–38	0–37	20–37	2–30	15–30
NaCl tolerance (%)	15	10	13	11	7	7	6	12	7
Biochemical characteristics									
Oxidase	–	NR	+	–	–	–	+	+	–
Hydrolysis of									
Starch	+	–	–	–	–	–	–	–	–
Casein	NR	–	W	+	+	+	+	+	–
Gelatin	+	+	–	+	+	+	+	+	+
Aesculin	NR	–	–	NR	+	–	–	+	NR
Tween 80	–	–	+	–	–	–	–	–	NR
Nitrate reduction	–	+	NR	+	–	+	–	–	–
Acid production from sugars: Jung et al. (2009)									
Cellobiose	+	–	–	NR	+	–	–	–	NR
D-Fructose	+	NR	+	–	–	+	+	+	+
D-Glucose	–	+	–	–	+	+	–	–	–
Lactose	–	–	–	–	+	–	–	–	+
Maltose	–	NR	–	NR	+	W	–	–	NR
Mannitol	+	–	–	NR	–	–	–	–	–
Melibiose	–	–	–	NR	+	–	–	–	NR
Raffinose	–	–	–	NR	–	–	–	–	+
Ribose	–	NR	–	+	–	–	+	–	NR
L-Rhamnose	–	–	–	NR	–	–	–	–	+
Sucrose	–	–	–	NR	–	–	–	–	+
D-Xylose	–	–	–	NR	–	–	+	–	–
Chemotaxonomic characteristics									
Menaquinone	MK-7, MK-8	MK-8	MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8	MK-7, MK-8
Peptidoglycan composition	NR	L-Lys-D-Glu	NR	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Asp	Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Glu

■ Table 25.14 (continued)

Characteristic	1	2	3	4	5	6	7	8	9
G+C content of DNA (mol%)	45.3	34.8	45.9	49	47	35	46	44.5	40
Source	Intertidal beach sediment	Coastal sediment	Marine solar saltern	Glacier water	Sea food	Sea ice	Ocean bed	Cyanobacterial mat sample	Cold desert of Himalayas

^aEngelhardt et al. (2001)^bDai et al. (2005)^cJung et al. (2009)^dZhang et al. (2009)^eYoon et al. (2001b)^fJunge et al. (1998)^gReddy et al. (2002)^hMayilraj et al. (2005)

+ positive, – negative, v variable, W weakly positive, NR not reported, MK menaquinone, l-Lys L-lysine, d-Glu D-Glutamate, d-Asp D-Aspartate

■ Table 25.15

Cellular fatty acid composition (%) of species of the genus *Planomicrobium* (Data from Engelhardt et al. (2001); Zhang et al. (2009); Jung et al. (2009); Mayilraj et al. (2005)). 1. *P. alkanoclasticum* MAE2^{T a}; 2. *P. chinense* CGMCC 1.3454^{T b}; 3. *P. flavidum* ISL-41^{T c}; 4. *P. glaciei* 0423^{T b}; 5. *P. koreense* DSM 15895^{T b}; 6. *P. mcmeekinii* DSM 13963^{T b}; 7. *P. okeanoikoites* NBRC 12536^{T b}; 8. *P. psychrophilum* CMS 53or^{T d}; 9. *P. stackebrandtii* DSM 16419^{T d}

Fatty acid	1	2	3	4	5	6	7	8	9
Straight chain									
C _{15:0}	NR	NR	NR	NR	NR	NR	NR	NR	5.5
Branched chain									
iso-C _{14:0}	6.0	22.9	8.0	15.2	16.2	19.4	42.9	5.3	4.7
iso-C _{15:0}	7.5	4.9	1.8	1.6	6.3	4.9	Tr	5.8	2.9
anteiso-C _{15:0}	45.5	40.6	39.0	37.0	43.6	36.5	5.1	53.5	49.8
iso-C _{16:0}	17.1	6.8	11.5	5.8	11.5	12.9	27.2	7.1	5.7
iso-C _{17:0}	7.4	Tr	2.8	NR	Tr	1.2	NR	-	2.8
anteiso-C _{17:0}	10.2	1.8	11.3	3.0	2.9	2.7	Tr	4.0	4.6
Unsaturated									
C _{16:1(ω7c)} alcohol	NR	12.0	11.0	4.6	9.6	9.4	15.1	10.1	8.5
C _{16:1(ω11c)}	6.4	5.5	1.0	3.2	4.0	2.5	3.6	NR	1.7
Summed feature 4	9.3	NR	8.4	NR	NR	NR	NR	8.6	8.6

^aEngelhardt et al. (2001)^bZhang et al. (2009)^cJung et al. (2009)^dMayilraj et al. (2005)– absent, NR not reported, Tr traces; summed feature 4: iso-C_{17:1} I and/or anteiso-C_{17:1} B. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

Solibacillus (Krishnamurthi et al. 2011)

Solibacillus (*So.li.ba.cil'lus*. L. n. solum soil; L. n. Bacillus a bacterial genus; N.L. n. *Solibacillus* a Bacillus-like organism isolated from soil).

The type strain of *Solibacillus silvestris* was isolated from the top soil layer of a beech forest soil near Braunschweig, Germany. It was originally described as *Bacillus silvestris* by Rheims et al.

(1999). But *Bacillus silvestris* differed from other members of the genus *Bacillus*, in that it contained A4 α type of peptidoglycan instead of A4 γ type of peptidoglycan present in *Bacillus* strains (Schleifer and Kandler 1972; Kämpfer et al. 2006). In addition, *Bacillus silvestris* also differs in that it contains iso-C_{15:0} and iso-C_{16:1} as predominant fatty acids in contrast to the type species of *Bacillus* and other members which contains iso- and anteiso-C_{15:0}. Based on these differences, Krishnamurthy et al.

■ Table 25.16

Diagnostic phenotypic characteristics of species of the genus *Rummeliibacillus* (Data from Vaishampayan et al. (2009); Nakamura et al. (2002))

Characteristic	<i>R. stabekisii</i> KSC-SF6g ^{Ta}	<i>R. pycnus</i> JCM 11075 ^{Ta,b}
Morphological characteristics		
Cell size (µm)	1.07–1.14 × 2.64–3.32	1.0 × 3.0–5.0
Growth characteristics		
Temperature range (°C)	28–55	10–45
Optimum temperature (°C)	28–32	28
Tolerance to NaCl (%)	7	5
Biochemical characteristics		
Indole production	–	+
Hydrolysis of		
Gelatin	+	–
Starch	+	–
Acid production		
D-Glucose	–	+
Chemotaxonomic characteristics		
Peptidoglycan composition	L-Lys-D-Asp	L-Lys-D-Glu
G+C content of DNA (mol%)	34.3	35
Source	Payload Hazardous Servicing Facility	Soil

^aVaishampayan et al. (2009)

^bNakamura et al. (2002)

+ positive, – negative, S sensitive, L-Lys L-lysine, d-Asp D-Aspartate, d-Glu D-Glutamate

(2009a) created the genus *Solibacillus* to accommodate the species *Bacillus silvestris* and reclassified it as *Solibacillus silvestris*.

Cells of *Solibacillus silvestris* are Gram-positive, strictly aerobic, rod shaped and the rods measure 0.5–0.7 µm in width by 0.9–2.0 µm in length, motile by means of peritrichous flagella, endospore forming, and the spores are round and lie terminally in a swollen sporangium. Cells can grow from 10 to 40 °C with an optimum temperature of 20–30 °C, pH range is 6–7, and can tolerate a NaCl concentration of 5 %. *Solibacillus silvestris* is catalase positive and negative for oxidase, Voges-Proskauer test, indole production, arginine dihydrolase, phenylalanine deaminase, and nitrate-to-nitrite reduction test and could not hydrolyze casein, gelatin, starch, tyrosine, aesculin, and Tween 80.

Oxidation of substrates provided in the API 50CH panel indicates that glycerol and ribose are utilized as sole carbon sources, but no acid is produced. Negative for utilization of D-glucose, erythritol, D- and L-arabinose, D- and L-xylose, adonitol, methyl β D-xyloside, galactose, mannose, D-mannitol, D-fructose, L-sorbose, rhamnose, dulcitol, inositol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose,

■ Table 25.17

Cellular fatty acid composition (%) of species of the genus *Rummeliibacillus* (Data from Vaishampayan et al. (2009))

Fatty acid	<i>R. stabekisii</i> KSC-SF6g ^{Ta}	<i>R. pycnus</i> JCM 11075 ^{Ta}
Branched chain		
iso-C _{14:0}	6.7	4.7
iso-C _{15:0}	26	74.2
anteiso-C _{15:0}	49.9	9.9
anteiso-C _{17:0}	7.0	1.2

^aVaishampayan et al. (2009)

Strains were grown in TSB at 25–32 °C for 30–48 h. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

melibiose, sucrose, trehalose, inulin, melezitose, D-raffinose, starch, glycogen, xylitol, p-gentiobiose, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate, citrate, and propionate. The peptidoglycan contains L-Lys-D-Glu and represents peptidoglycan type A4α. Polar lipids comprise phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, minor amounts of phosphatidylserine, and one unknown phospholipid. The predominant isoprenoid quinone is of the MK-7 type. The fatty acid composition is characterized by the presence of iso-C_{15:0} and iso-C_{16:1}, contributing to 61.0 %, and the other fatty acids are C_{17:0}, C_{16:1} (ω7c) alcohol, iso-C_{17:1} (ω 10c) anteiso-C_{17:1}, iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0}, and anteiso-C_{17:0}. The G+C content of the DNA is 39.3 mol % and phylogenetically a member of the *Bacillus* RNA group 2 (Ash et al. 1991).

The type species of the genus is *Solibacillus silvestris* (Rheims et al. 1999; Krishnamurthi et al. 2009) and the type strain is HR3-23^T = DSM 12223^T = ATCC BAA-269^T = CIP 106059^T.

Sporosarcina (Kluyver and van Niel 1936 Emend. Yoon et al. 2001)

Sporosarcina. (*Spo.ro.sar.ci'na*. M.L. n. *spora* a spore; M.L. fem. n. *Sarcina* generic name; M.L. fem. n. *Sporosarcina* spore-forming *Sarcina*).

The genus *Sporosarcina* was described by Kluyver and van Niel in 1936 and emended by Yoon et al. (2001c). The genus presently contains 12 validly described species (<http://www.bacterio.cict.fr/s/sporosarcina.html>), namely, *S. aquimarina* (Yoon et al. 2001c), *S. contaminans* (Kämpfer et al. 2010), *S. globispora* (Larkin and Stokes 1967; Yoon et al. 2001c), *S. koreensis* (Kwon et al. 2007), *S. luteola* (Tominaga et al. 2009), *S. newyorkensis* (Wolfgang et al. 2012), *S. pasteurii* (Miquel 1889; Yoon et al. 2001c), *S. psychrophila* (Nakamura 1984; Yoon et al. 2001c), *S. saromensis* (An et al. 2007), *S. soli* (Kwon et al. 2007), *S. thermotolerans* (Kämpfer et al. 2010), and *S. ureae* (Beijerinck 1901). *S. macmurdoensis* (Reddy et al. 2003) and *S. antarctica*

(Yu et al. 2008) were transferred to the genus *Paenisporosarcina* as *Paenisporosarcina macmurdoensis* (Krishnamurthi et al. 2009b) and *Paenisporosarcina antarctica* (Reddy et al. 2013).

Members of the genus *Sporosarcina* are characterized by the presence of spherical or rod-shaped cells that are Gram-positive to Gram-variable, motile or nonmotile, endospore forming, spores are spherical or round, and location is central or terminal or subterminal. Facultatively anaerobic or strictly aerobic, catalase and oxidase positive (not determined for *S. pasteurii* but positive for the type strain of *S. pasteurii*). The diagnostic amino acid at position three of the peptide subunit of the peptidoglycan is L-lysine, and peptidoglycan composition in nine out of thirteen species is L-Lys-D-Glu. In other species, it is L-Lys-L-Ala-L-Asp (*S. aquimarina*) or L-Lys-Gly-D-Glu (*S. newyorkensis* and *S. ureae*) or L-Lys-D-Asp (*S. pasteurii*). However, the peptidoglycan variation in all the species is A4 α . The predominant menaquinone in all the species is MK-7, and the major fatty acid is anteiso-C_{15:0}. The genus-specific phenotypic characteristics that were described by Yoon et al. (2001c) have deviated with the addition of new species wherein the hydrolysis of gelatin, urea, and starch are variable. The G+C content of the genomic DNA ranges from 38 to 47 mol%.

The type species is *Sporosarcina ureae* (Beijerinck 1901; Kluyver and van Niel 1936). The type strain is ATCC 6473^T = DSM 2281^T = IFO (now NBRC) 12699^T = JCM 2577^T = LMG 17366^T = VKM B-595^T.

Phylogenetically, based on 16S rRNA gene sequence, the genus is closely related to the clade represented by *Filibacter*, *Bacillus*, *Paenibacillus*, *Lysinbacillus*, and *Viridibacillus* (Wolfgang et al. 2012). The genus *Sporosarcina* can be differentiated from other members of the family based on its morphology, chemotaxonomic markers, and 16S rRNA genes sequence.

Phenotypic characterization of type strains were carried out in majority of the cases, and the diagnostic characteristics are listed in ► Tables 25.18 and ► 25.19. Species of the genus are psychrophilic (Yu et al. 2008; Nakamura 1984) to moderately thermotolerant (Kämpfer et al. 2010) and halotolerant. Majority of the species are negative (exceptions are listed in brackets), for hydrolysis of casein (*S. pasteurii*), aesculin, and Tween 80 (*S. luteola* and *S. ureae*), acetate (*S. soli*) and citrate utilization, Voges-Proskauer test, indole production, H₂S production, arginine dihydrolase (*S. koreensis*), lysine decarboxylase, ornithine decarboxylase, and phenylalanine deaminase (*S. soli* and *S. ureae*). Acid is not produced from L-arabinose, lactose (*S. globispora*; *S. ureae*), mannitol (*S. psychrophila*), maltose (*S. luteola*) and sucrose (*S. ureae*).

Ureibacillus (Fortina et al. 2001)

Ureibacillus (Ur.e.i.ba.cil'lus. L. n. *urea* urea; L. dim. n. *bacillus* from *Bacillus*, a genus of aerobic endospore-forming bacteria; *Ureibacillus* a ureolytic aerobic bacillus).

Genus *Ureibacillus* was created by Fortina et al. (2001) to accommodate a number of moderately thermophilic sporeformers that are phylogenetically distinct and from

a separate clade in the genus *Bacillus*. Cells are Gram-negative rods, motile by means of peritrichous flagella, and bear spherical endospores which lie in terminal or subterminal positions in swollen sporangia. Members of the genus are strictly aerobic and moderately thermophilic. The cross-linkage of peptidoglycan type is of the L-Lys-D-Asp (variation A4 α). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phospholipids, and glycolipids of unknown composition. The major cellular fatty acid is iso-C_{16:0} that contributes more than 60.0 % in so far described species. The G+C content of DNA ranges from 35 to 42 mol%.

The type species is *Ureibacillus thermosphaericus* (Andersson et al. 1995; Fortina et al. 2001), and the type strain is P-11^T = CIP 104857^T = DSM 10633^T = HAMBI 1900^T = LMG 17959^T.

Following strains were described till today under this genus: *U. composti* (Weon et al. 2007), *U. suwonensis* (Kim et al. 2006), *U. terrenus* (Fortina et al. 2001), *U. thermophilus* (Weon et al. 2007), and *U. thermosphaericus* (Andersson et al. 1995; Fortina et al. 2001). Among all these, *U. thermosphaericus* was originally described as *Bacillus thermosphaericus*.

Phenotypic characterization of all the isolates was done traditionally, without using any commercial kits. All the five isolates exhibited similar characteristics with respect to Gram-staining, spore formation, motility, and anaerobic growth. In addition, the following characteristics were shared by all the isolates: positive for catalase and oxidase; negative for Voges-Proskauer test; indole production; nitrate reduction; hydrolysis of casein, gelatin, and starch; production of acid from L-arabinose, D-glucose, mannitol, and xylose. Diagnostic characteristics that differentiate the species are listed in ► Tables 25.20 and ► 25.21. Genus *Ureibacillus* can be distinguished from the other members of the family *Planococcaceae* based on the growth temperature, Gram-staining, fatty acid methyl esters, and polar lipids.

Viridibacillus (Albert et al. 2001)

Viridibacillus (*Vi.ri.di.ba.cil'lus*. L. adj. *viridis* green; L. masc. n. *bacillus* rod; N.L. masc. n. *Viridibacillus* the green bacillus/rod).

Genus *Viridibacillus* was established by Albert et al. (2007) to accommodate strains that belong to *Bacillus* rRNA group 2 (Ash et al. 1991) and produce green pigment on R2A medium after 48 h growth. Cells of the genus are Gram-positive, spore forming, motile rods. Endospores are round and are located terminally in swollen or slightly swollen sporangia. Growth occurs in the presence of 2 % NaCl but not in 7 % NaCl (w/w). Sporulation and release of endospores (free spores) are abundant on R2A agar after 24 and 48 h at 25 °C growth temperature. The quinone system consists of MK-8 (69–81 %) and moderate amounts of MK-7 (19–30.5 %). In the fatty acid profile, the major fatty acids are iso-C_{15:0} are anteiso-C_{15:0}, and both of them contribute close to 70.0 %. The polar lipids present are phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine and moderate amounts of an unknown

Table 25.18

Diagnostic phenotypic characteristics of species of the genus *Sporosarcina* (Data from Yoon et al. (2011c); Kämpfer et al. (2010); Kwon et al. 2007; Tominaga et al. (2009); Wolfgang et al. (2012); An et al. (2007); Kwon et al. (2010); Kämpfer et al. (2012)). 1. *S. aquimarina* JCM 10887^{T a}; 2. *S. contaminans* DSM 22204^{T b}; 3. *S. globispora* ATCC 23301^{T a}; 4. *S. koreensis* JCM 16400^{T c}; 5. *S. luteola* JCM 15791^{T d}; 6. *S. newyorkensis* DSM 23544^{T e}; 7. *S. pasteurii* DSM 33^{T a}; 8. *S. psychrophila* JCM 9075^{T a}; 9. *S. saromensis* JCM 23205^{T f}; 10. *S. soli* DSM 16920^{T c}; 11. *S. thermotolerans* DSM 22203^{T b}; 12. *S. ureae* DSM 2281^{T f}

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12
Morphological characteristics												
Colony color	Light orange	Beige	White	Light orange	Yellow	Gray	White	White	Beige	Light orange	Beige	White
Cell shape	R	R	R	R	R	R	Sp	R	R	R	R	Sp
Gram-stain	v	+	v	+	v	+	+	+	+	+	+	+
Spore shape	Round	Spherical	Round	Oval	NR	Round to oval	NR	Round	Spherical	Round	Spherical	NR
Spore position	T	T	ST	T	T	ST	T	ST	T	C	T	NR
Cell arrangement	NR	NR	Single or pairs	Single or short chains	NR	Single or palisades	NR	Single or pairs	NR	Single or pairs	NR	NR
Motility	+	+	+	+	+	+	+	+	+	-	-	+
Growth characteristics												
Anaerobic growth	+	-	+	-	+	+	+	+	-	-	-	-
Optimum pH	6.5-7	7	7	7	7	7.2-9.5	9	7	6.5	8	7	7
Temperature range (°C)	4-37	15-50	5-30	15-40	10-40	10-42	5-37	0-30	5-40	15-37	15-50	-37
Optimum temperature (°C)	25	30-37	25	30	25-30	22-28	30	20	27	30	30-37	25
NaCl tolerance (%)	13	10	5	7	7.5	13	10	5	9	5	3	3
Biochemical characteristics												
Nitrate reduction	+	-	+	-	+	-	+	+	-	+	-	+
Urease	+	-	+	+	-	+	+	+	+	+	-	+
DNase	+	+	NR	+	-	+	NR	NR	NR	+	+	-
Hydrolysis of												
Gelatin	+	-	+	+	+	-	+	+	+	-	+	-
Starch	-	-	-	+	-	-	-	-	+	-	+	-

Tyrosine	–	+	NR	+	–	NR	–	NR	+	–	–	
Acid production from sugars												
D-Fructose	+	NR	+	–	+	–	–	–	+	–	–	
D-Glucose	–	–	+	–	+	–	NR	–	+	–	+	
Galactose	–	NR	+	–	+	–	NR	–	+	NR	–	
Glycerol	+	NR	+	–	+	–	NR	NR	NR	NR	NR	
Lactose	–	NR	+	–	–	–	NR	–	–	–	+	
Xylose	–	–	+	–	+	–	NR	–	+	–	–	
Chemotaxonomic characteristics												
Polar lipids	NR	PG, DPG, PE	NR	PG, DPG, PE	PG, DPG, PE	PG, DPG, PE	NR	NR	NR	PG, DPG, PE	PG, DPG, PE	
Peptidoglycan composition	L-Lys-L-Ala-L-Asp	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-L-Gly-D-Glu	L-Lys-D-Asp	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-D-Glu	L-Lys-L-Gly-D-Glu	
G+C content of DNA (mol%)	40	NR	40	46.5	43.6	42.4	39	44	46	44.5	40–42	
Source	Sea water	Industrial clean room	Soil, river water	Soil	Surface of soya sauce instrument	Patient's blood	Soil, water, sewage	Soil and river water	Sediment	Soil	Human blood	Contaminant from clean-room floor

^aYoon et al. (2011c)

^bKämpfer et al. (2010)

^cKwon et al. 2007

^dTominaga et al. (2007)

^eWolfgang et al. (2012)

^fAn et al. (2007)

+ positive, – negative, v variable, NR not reported, R rod, Sp spherical, T terminal, ST subterminal, C central, UPL unidentified phospholipid, PG phosphatidylglycerol, DPG diphosphatidylglycerol, PE phosphatidylethanolamine, L-Lys L-lysine, L-Ala L-Alanine, d-Glu D-Glutamate, d-Asp D-Aspartate, L-Asp L-Aspartate, L-Gly L-Glycine

■ Table 25.19

Cellular fatty acid composition (%) of species of the genus *Sporosarcina* (Data from Wolfgang et al. (2012); Yoon et al. (2001c); An et al. (2007)). Taxa: 1. *S. newyorkensis* 6062^{T a}; 2. *S. ureae* DSM 2281^{T a}; 3. *S. aquimarina* DSM 14554^{T a}; 4. *S. soli* DSM 16920^{T a}; 5. *S. koreensis* DSM 16921^{T a}; 6. *S. thermotolerans* CCUG 53480^{T a}; 7. *S. contaminans* CCUG 53915^{T a}; 8. *S. luteola* DSM 23150^{T a}; 9. *S. globispora* DSM 4^{T b}; 10. *S. psychrophila* KCTC 3446^{T b}; 11. *S. pasteurii* KCTC 3558^{T b}; 12. *S. saromensis* HG645^{T c}. Fatty acid methyl esters for which values were less than 0.1 % have been omitted from the table

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
Straight chain												
C _{14:0}	0.6	0.3	2.4	2.5	0.5	9	0.4	1.2	0.6	0.9	1.1	-
C _{16:0}	0.8	0.6	3	2.2	0.2	10.6	0.5	0.5	0.9	1.5	4.7	1.4
Branched chain												
iso-C _{14:0}	26.1	1.8	4.3	5.5	20.9	7.5	4.7	11	3.4	4.1	15.4	5.6
iso-C _{15:0}	32.3	7.6	4.3	45.5	34.6	27.7	55.8	37.6	4	6.4	6.9	49.5
anteiso-C _{15:0}	15.2	62.7	76.8	37.7	30.8	39.6	23.5	41.1	61.8	68.4	48.6	33.3
iso-C _{16:0}	10	0.6	1.6	1.1	4.4	—	3.6	2.6	1.5	2.1	7.5	4.2
anteiso-C _{17:0}	1.4	11.3	3.2	1.1	1	—	2.8	1.6	6.9	6.6	4.0	2.4
Unsaturated												
C _{16:1(ω7c)} alcohol	6.6	2.7	1	0.5	3.9	—	3.6	2.2	5.5	2.4	2.8	—
C _{16:1(ω11c)}	3.4	3.5	2.4	1.5	1.2	5.6	1.4	1.1	3.1	1.8	3.2	—
Summed feature 4	0.8	6.7	0.7	—	1	—	1.1	0.4	10.6	3.9	0.7	—

^aWolfgang et al. (2012)

^bYoon et al. (2001c)

^cAn et al. (2007)

—, absent; summed feature 4 contains iso-C_{17:1} I and/or anteiso-C_{17:1} B. If a given fatty acid is present above 5.0 % in at least one species the composition in the other species is given

aminophospholipid (APL1), two unknown phospholipids (PL1, PL2), and three unknown polar lipids. Cell wall peptidoglycan variation is A4a, and it is either L-Lys-D-Glu or L-Lys-D-Asp as the peptidoglycan type. The G+C content of genomic DNA of species of the genus ranges from 35 to 40.4 mol%.

The type species of the genus is *Viridibacillus arvi* (Heyrman et al. 2005), and the type strain is DSM 16319^T = LMG 22166^T.

The genus currently contains three species, *V. neidei* (Nakamura et al. 2002; Albert et al. 2007), *V. arenosi* (Heyrman et al. 2005; Albert et al. 2007), and *V. arvi* (Heyrman et al. 2005; Albert et al. 2007). Species *V. arenosi* (Heyrman et al. 2005; Albert et al. 2007) and *V. arvi* were characterized using API Biotype 100 kit, and substrate utilization were carried out using API 50CHB kits. Strain *V. neidei* was studied based on Biolog GP system. All three species are positive for catalase and gelatin hydrolysis; negative for hydrolysis of aesculin, casein, starch, and Tween 80; for tests of Voges-Proskauer, indole production, methyl red; for H₂S production, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization; and for nitrate reduction. Both *V. arvi* and *V. arenosi* (Heyrman et al. 2005; Albert et al. 2007) produced acid only from D-fructose in the API 50CHB strips, and no substrate was used as a sole carbon source in the API Biotype 100 kit. Species *V. neidei* (Nakamura et al. 2002; Albert et al. 2007) is negative for phenylalanine deaminase and requires biotin, thiamin, and cystine for growth. It oxidizes pyruvate and β-hydroxybutyrate

but not citrate, propionate, L-alanine, glycyl L-glutamate, 2'-deoxyadenosine, inosine, AMP, and UMP in the Biolog GP system. Species *V. neidei* has been partially characterized for antibiotic susceptibility, and it is sensitive to chloramphenicol, tobramycin, streptomycin, erythromycin, and tetracycline. Three species are characterized by the presence of MK-8 as the major menaquinone contributing 70–80 % followed by MK-7 (20–39 %) and trace amounts of MK-6. Polar lipids are nearly identical, and the peptidoglycan is L-Lys-D-Asp in case of *V. arvi* and *V. arenosi*, whereas *V. neidei* contains L-Lys-D-Asp type. The characteristics that diagnose the three species are listed in [Tables 25.22](#) and [25.23](#).

Isolation, Enrichment, and Maintenance Procedures

Species of the genus *Planococcus* have been isolated using various kinds of media such as antarctic bacterial medium (0.5 % peptone, 0.2 % yeast extract, and 1.5 % agar, pH 6.4) with 1.5 % NaCl or in the absence of salt, nutrient agar containing 1–5 % NaCl, sea water agar (1.0 % beef extract, 1.0 % peptone, 2.0 % agar, tap water 250 mL, and sea water 750 mL; pH 7.2), Zobell marine agar 2216 (Difco Laboratories, Detroit, USA) with 6 % NaCl, 100-fold-diluted marine agar (MA; Difco) containing 1 % starch, trypticase soy agar (TSA) or broth (TSB; Difco) with or

■ Table 25.20

Diagnostic phenotypic characteristics of species of the genus *Ureibacillus* (Data from Weon et al. (2007); Kim et al. (2006); Fortina et al. (2001); Weon et al. (2007); Andersson et al. (1995))

Characteristic	<i>U. composti</i> DSM 17951 ^{Ta}	<i>U. suwonensis</i> DSM 16752 ^{Tb}	<i>U. terrenus</i> DSM 12654 ^{Tc}	<i>U. thermophilus</i> DSM 17952 ^{Td}	<i>U. thermosphaericus</i> DSM 10633 ^{Tc, e}
Morphological characteristics					
Cell shape	Rods	Rods	Rods	Rods	Cocci/short rods
Cell size	0.7–0.9 × 2.5–4.0	0.5–0.7 × 1.5–2.0	NR	0.8–1.2 × 2.5–3.5	NR
Spore shape	Spherical	Spherical or oval	Round or oval	Round	Round or oval
Spore position	ST or T	ST or T	ST or T	ST or T	T
Cell arrangement	NR	Single or chains	Single or chains	NR	Single or chains
Growth characteristics					
Temperature range (°C)	37–60	35–60	42–65	30–65	32–64
NaCl tolerance (%)	5	5	Variable	5	5
Biochemical characteristics					
Phenylalanine deaminase	+	W	–	+	–
Urease	–	–	+	–	+
Hydrolysis of					
Aesculin	+	–	+	+	+
Tyrosine	–	–	+	+	–
Chemotaxonomic characteristics					
Menaquinone	MK-7, MK-8, MK-9	MK-7, MK-8, MK-9, MK-10, MK-6	MK-7, MK-8, MK-9, MK-10, MK-11	MK-7, MK-8, MK-9	MK-7
Mol% G+C content of DNA	42.4	41.5	39.6–41.5	38.5	35.7–39.2
Source	Compost	Compost	Soil	Compost	Air

^aWeon et al. (2007)

^bKim et al. (2006)

^cFortina et al. (2001)

^dWeon et al. (2007)

^eAndersson et al. (1995)

+ positive, – negative, W weakly positive, NR not reported, ST subterminal, T terminal, MK menaquinone

without salt (5 % NaCl), and R2A solid media (Difco, USA) supplemented with 0.4 g L⁻¹ sodium acetate.

Species of the genus *Bhargavaea* were enriched and isolated on Zobell marine agar and Luria-Bertani (LB) agar plates that had been seeded with a tissue suspension of ginseng roots using the standard dilution plating technique (Manorama et al. 2009; Verma et al. 2012).

Caryophanon strains have been isolated and grown on different agar media adjusted to pH 7.5–8.5. Clarified manure extract agar (Peshkov 1967), cow dung agar (Smith and Trentini 1972), cow dung agar with lactalbumin hydrolysate (Moran and Witter 1976), and peptone-yeast extract-acetate agar (Pringsheim and Robinow 1947). A semisynthetic medium (Smith and Trentini 1973; Trentini 1978) was also found to support good growth of most strains of *C. latum* and *C. tenue*. Biotin has also been found to be essential for growth, whereas

thiamine appears to stimulate the growth of both species. Kele and McCoy (1971) also developed a defined liquid medium for *C. latum*.

Chryseomicrobium imtechense was isolated on TSA by dilution plating method (Arora et al. 2011).

Filibacter limicola was isolated using MYP medium [mg L⁻¹: yeast extract (Difco), 10; peptone (Oxoid L37), 100; K₂HPO₄, 28; MgCl₂·6H₂O, 127; KNO₃, 4; (NH₄)₂SO₄, 60; MnSO₄·4H₂O, 8; Ferric citrate, 6] with the final pH adjusted to 7.0–7.2 with 1 M KHCO₃ before autoclaving (Stackebrandt 2009). Strains of *Filibacter limicola* also grow well, though less abundant, in different media such as tryptic soy broth (Oxoid CM129), CASO agar (g L: casein peptone, 15.0; soy peptone, 5.0; sodium chloride, 5.0; agar, 15.0, and pH 7.3 at 20 °C) supplemented with inosine (Stackebrandt 2009), casamino acids, or complete amino acid mixture in the presence of vitamins (Maiden and

Table 25.21

Fatty acid composition (%) of species of the genus *Ureibacillus* (Data from Weon et al. (2007))

Fatty acids	<i>U. composti</i> DSM 17951 ^{Ta}	<i>U. suwonensis</i> DSM 16752 ^{Ta}	<i>U. terrenus</i> DSM 12654 ^{Ta}	<i>U. thermophilus</i> DSM 17952 ^{Ta}	<i>U. thermosphaericus</i> DSM 10633 ^{Ta}
Straight chain					
C _{16:0}	5.4	6.4	7.9	3.7	8.1
Branched chain					
iso-C _{15:0}	2.9	–	1.4	4.0	10.2
iso-C _{16:0}	72.2	77.3	67.5	60	68.4
iso-C _{17:0}	2.3	1.2	5.5	5.9	2.5
anteiso-C _{17:0}	2.7	–	3.4	6.8	–
Unsaturated					
C _{16:1(ω7c)} alcohol	7.2	8.5	4.3	8.4	3.0

^aWeon et al. (2007)

For the fatty acid analyses, cells for all strains were harvested after growth on R2A agar at 50 °C for 2 days. If a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

Table 25.22

Diagnostic phenotypic characteristics of species of the genus *Viridibacillus* (ata from Heyrman et al. (2005); Albert et al. (2007); Nakamura et al. (2002))

Characteristic	<i>V. arvi</i> DSM 16317 ^{Ta, b}	<i>V. arenosi</i> DSM 16319 ^{Ta, b}	<i>V. neidei</i> DSM 15031 ^{Tb, c}
Morphological characteristics			
Colony color	Cream	Cream	White
Gram-stain	Variable	Variable	+
Spore shape	Spherical	Spherical	Round
Swollen sporangia	Slightly	v	+
Growth characteristics			
Anaerobic growth	W	–	–
Temperature range (°C)	5–40	5–37	5–45
Optimum temperature (°C)	30	30	28–33
pH range	6–9	6–9	5–10
Biochemical characteristics			
Nitrate reduction	–	+	–
Urease	+	–	–
Acid production from sugars			
D-Fructose	+	–	+

^aHeyrman et al. (2005)

^bAlbert et al. (2007)

^cNakamura et al. (2002)

+ positive, – negative, v variable, W weakly positive

Jones 1984), anaerobe agar (BBL, Becton-Dickinson), and in all the above cases, no growth occurred in absence of vitamins (Stackebrandt 2009).

Strains of the genus *Jeotgalibacillus* were isolated by a dilution plating/serial dilution using marine agar (Difco) or marine agar supplemented with up to 10 % (w/v) NaCl. Strains also grow on sea water medium (De Clerck and De Vos 2009; Cunha et al. 2012).

Kurthia strains were enriched on nutrient gelatin plates [per liter of distilled water: meat extract (Lab Lemco powder, Oxoid), 4 g; peptone (Difco), 5 g; yeast extract (Difco), 2.5 g; NaCl, 5 g; gelatin (BDH Chemical Co., Poole, UK), 100 g; pH 7.0] (YNG; Keddie 1981) and incubated at 20 °C with the lids upright and observed daily to check for filamentous out-growths beyond the zone of gelatin liquefaction around the streak. To get pure cultures, a small piece of nutrient gelatin containing the outgrowth is then streaked on a nutrient agar (NA) medium.

Paenisporsarcina quisquiliarum was isolated by dilution plating of a surface soil sample onto TSBA plates (tryptic soy broth solidified with 1.5 % agar), whereas *Paenisporsarcina macmurdoensis* was isolated using ABM agar (0.5 % peptone, 0.2 % yeast extract, and 1.5 % agar, pH 7.2; Shivaji et al. 1988; Reddy et al. 2003). *Planomicrobium* strains were isolated using media containing NaCl (varying concentrations), marine agar (Difco), or artificial sea water basal medium with 1 % peptone and 0.5 % yeast extract (Eguchi et al. 1996). *Planomicrobium alkanoclasticum* was isolated on Bushnell-Haas medium (g L: Magnesium sulfate, 0.2; calcium chloride, 0.02; monopotassium phosphate, 1.0; dipotassium phosphate, 1.0; ammonium nitrate, 1.0; ferric chloride, 0.050; agar 20.0; pH 7.0) (Engelhardt et al. 2001).

■ Table 25.23

Fatty acid composition (%) of species of the genus *Viridibacillus* (Data from Albert et al. (2007))

Fatty acid	<i>V. arvi</i> DSM 16317 ^T	<i>V. arenosi</i> DSM 16319 ^T	<i>V. neidei</i> NRRL BD-87 ^T			
Time (h)	21	30	21	30	21	30
Branched chain						
iso-C _{15:0}	44.7	46.2	55.2	55.5	52.4	54.7
anteiso-C _{15:0}	21.6	21.7	11.3	11.2	15.4	15.3
anteiso-C _{17:0}	5.3	4.8	1.8	1.8	2.8	2.4
Unsaturated						
C _{16:1(ω11c)}	3.8	3.9	8.2	7.0	4.1	3.8
iso-C _{17:1(ω10c)}	5.4	5.2	7.0	7.1	7.0	6.8
Summed feature 4	7.2	6.8	5.1	5.1	6.3	5.8

Albert et al. (2007). Summed feature 4: iso-C_{17:1} l/anteiso-C_{17:1} B

Strains were grown on TSA at 23 °C for 21 or 30 h as indicated; if a given fatty acid is present above 5.0 % in at least one species, the composition in the other species is given

Strains of the genus *Rummeliibacillus* were isolated by streaking serially diluted samples on TSA (Vaishampayan et al. 2009; Nakamura et al. 2002).

Solibacillus silvestris (Rheims et al. 1999; Krishnamurthi et al. 2009a) was isolated by a dilution plating/serial dilution using TSA (Vaishampayan et al. 2009).

Strains of the genus *Sporosarcina* were isolated by streaking serially diluted samples on different media. NA plates supplemented with 30–100 g urea/L, tryptic soy yeast extract agar (Difco), which was adjusted to pH 8.5 with NaOH before sterilization and filter-sterilized urea solution (1 %), trypticase soy agar supplemented with artificial sea water (pH 7.5) (Yoon et al. 2001c), TSA (Kwon et al. 2007), ABM agar (Reddy et al. 2003), JCM57 medium (10 g of glucose, 1.0 g of asparagine, 0.5 g of K₂HPO₄, 2.0 g of yeast extract, 15 g of agar per liter of distilled water, pH adjusted to 7.3), 1/10 diluted MA 2216 (An et al. 2007), PYG medium (0.5 % Bacto peptone, 0.02 % yeast extract, 0.5 % glucose, 0.3 % beef extract, 0.05 % NaCl, and 0.15 % MgSO₄ · 7H₂O, w/v, pH adjusted to 7.0) (Zhang et al. 2007), blood agar (Kämpfer et al. 2010), brain-heart infusion (BHI) (Oxoid, Hampshire, United Kingdom) agar (Tominaga et al. 2009), and TSA plates supplemented with 5 % sheep blood (Wolfgang et al. 2012).

Strains of *Ureibacillus* were isolated by using TSA (Weon et al. 2007; Kim et al. 2006) and CESP agar (casitone, 15 g; yeast extract, 5 g; soytone, 3 g; peptone, 2 g; MgSO₄, 0.015 g; FeCl₃, 0.007 g; MnCl₂, 0.002 g; made up to 1 L with distilled water, pH 7.2) (Fortina et al. 2001).

Strains of *Viridibacillus* were isolated on TSA plates (Heyrman et al. 2005; Nakamura et al. 2002).

Some members of family Planococcaceae cannot be isolated without prior enrichment. Species of the genus *Caryophanon* have been isolated only after an enrichment step. Samples of fresh cow dung are collected, placed in closed bottles, and kept at room temperature (Pringsheim and Robinow 1947; Weeks and

Kelley 1958); after 1–2 days, when *Caryophanon* had multiplied in the sample, a loopful of dung is suspended in a few drops of tap water, and samples that are microscopically rich in *C. latum* are streaked on cow dung agar or peptone-yeast extract-acetate agar (Fritze and Claus 2009). For enrichment of *Planococcus* strains, sea water agar or NA containing 5–7 % NaCl can be used (Shivaji 2009). *P. halocryophilus* was enriched in R2A medium (Difco, USA) supplemented with 0.4 g L⁻¹ sodium acetate incubated at 5 °C. Following 11 months of incubation, 100 µL of culture was transferred to R2A (Difco, USA) solid media at 5 °C, resulting in the isolation (Myktyczuk et al. 2012). *Planomicrobium alkanoclasticum* was enriched in Bushnell-Haas medium (Brown and Braddock 1990) containing crude oil (0.5 g), at 15 °C for 14 days (Dai et al. 2005). *S. antarctica* was enriched prior isolation in PYG medium (0.5 % Bacto peptone, 0.02 % yeast extract, 0.5 % glucose, 0.3 % beef extract, 0.05 % NaCl, and 0.15 % MgSO₄ · 7H₂O, w/v, pH adjusted to 7.0) (Zhang et al. 2007) supplemented with cycloheximide, nystatin, and nalidixic acid (all at 25 mg mL⁻¹) and shaken at 10 °C for 4 days at 150 r.p.m. The culture was further diluted (1:10) and spread onto PYG agar plates and incubated at 4 °C for 2 weeks for isolation (Yu et al. 2008).

The members of the family are preserved using routine techniques like cryopreservation. For short-term preservation, the log-phase cultures on agar plates can be preserved at 4 °C for 4–6 weeks. *Planococci* cultures can be stored at 4 °C in screw-capped tubes or on plates containing semisolid medium after inoculating and overnight growth at optimum temperature. Species of the genus *Kurthia* can be maintained on yeast extract nutrient agar (YNA) medium at 25 °C for at least 6 months at room temperature (20 °C). *Caryophanon* cultures can be maintained for 4 weeks at 4 °C on clarified manure extract agar or cow dung agar after incubation at about 25 °C for 48 h. The psychrophilic or psychrotolerant cultures, grown at 20 °C, can be maintained at 10 °C for some weeks. Vegetative

cultures of *S. ureae* grown on nutrient agar are viable for up to a year when stored at 4–10 °C in the dark. Endospores survive several years in screw-capped tubes under the same conditions (Claus and Fahmy 1986). Strains of *S. ureae* form spores in nutrient agar supplemented with urea (final concentration 0.2 %) if incubated at 25 °C. Sporulation of *Sporosarcina* cultures can be enhanced by adding 50 mg of MnSO₄ · H₂O per liter to the medium of MacDonald and MacDonald (1962) (Claus and Fahmy 1986). For long-term preservation, they can be freeze-dried using cryoprotectants such as skim milk (10–20 %, w/v), serum containing 5 % meso-inositol, glycerol (10–25 %), and DMSO (5 %) prepared in the appropriate medium. The cultures could also be stored in liquid nitrogen at –196 °C or at –20 or –70 or –80 °C in freezers.

Ecology

Habitat

Species of the genus *Planococcus* have been isolated from various aquatic and terrestrial habitats such as sea water (Yoon et al. 2003; Zobell and Upham 1944), Antarctic sea (Wang et al. 2011b), Antarctic sea ice (Bowman et al. 1997), Antarctic soil (Shivaji et al. 1988), Antarctic cyanobacterial mats (Alam et al. 2003; Reddy et al. 2002), sub-Antarctic sediments of Isla de Los Estados, Argentina (Olivera et al. 2007), high Arctic permafrost (Mykytczuk et al. 2012), high Arctic hypersaline spring channels (Lay et al. 2012), northeast Siberian sea coast permafrost sample (Hinsa-Leasure et al. 2010), coastal soil (Siddikee et al. 2010), mangrove soil (Kannan et al. 2006), glacial soil (Mayilraj et al. 2005), Palk Bay sediment (Nithya et al. 2011), marine sediment (Choi et al. 2007), marine solar saltern (Yoon et al. 2010b), salt water of Lake Red (Sovata, Romania) (Borsodi et al. 2010), saline and hyperalkaline Lonar Lake (Surakasi et al. 2010), sulfur spring (Romano et al. 2003), spacecraft assembly facility (Venkateswaran et al. 2001), and fish-brining tanks (Georgala 1957). Species of *Planococcus* have also been isolated from various life-forms including marine clams and fish (Hao and Komagata 1985; Novitsky and Kushner 1976), pigeon feces (Suresh et al. 2007), a shrimp (Alvarez 1982), a marine sponge *Plakortis simplex* (Schulze) (Kaur et al. 2012), from *Agaricus bisporus* composting phase II (He et al. 2009), a cuttlefish (*Sepia pharaonis*) fillets (Jeyasekaran et al. 2012), a sea urchin *Hemicentrotus pulcherrimus* (Huang et al. 2009), a sea anemone (Xiao et al. 2009), a snail (*Nassarius semiplicatus*) (Wang et al. 2008), reed (*Phragmites australis*), periphyton communities (two Hungarian soda ponds) (Rusznayk et al. 2008), fish (Radwan et al. 2007), brown and red algae (Beleneva and Zhukova 2006), and corals in (Beleneva et al. 2005). *Planococcus* sp. are pathogenic (10 %) to *Hylesia metabus* larvae, at doses of 3–4 × 10⁷ cells.

Bhargavaea cecembensis was isolated from a deep-sea sediment sample (depth of 5904 m) from the Chagos-Laccadive ridge system in the Indian Ocean (Manorama et al. 2009).

Bacillus beijingensis and *Bacillus ginsengi* were isolated from the internal tissue of ginseng roots (Qiu et al. 2009).

C. latum and *C. tenue* were both isolated from fresh cattle dung. In most successive studies, *C. latum* was found only in cattle manure or cattle manure-contaminated materials like bedding straw, barn dust, or barnyard soil. *C. latum* has not been found as part of the natural flora of the intestinal tract of cattle (Trentini 1978) and seems to be a natural, specific, and temporary coprophilic resident of cattle dung. It seems to be dispersed to new droppings by contaminated air and by flying insects (Trentini 1978; Trentini and Machen 1973). Recently *Caryophanon* strains have been reported from *Agaricus bisporus* composting phase II (He et al. 2009) and gingival scrapings from dogs (Saphir and Carter 1976).

Chryseomicrobium imtechense was isolated from a marine sample from Bay of Bengal, India (Arora et al. 2011).

Filibacter limicola has been isolated from sediments of Blelham Tarn, a eutrophic freshwater lake (Maiden 1983).

Jeotgalibacillus alimentarius was isolated from jeotgal, traditional Korean fermented seafood (Yoon et al. 2001a). The other species of the genus have been isolated from various habitats such as from forest soil sample (*J. soli*; Chen et al. 2010b), from a marine solar saltern (*J. salaries* and *J. campisalis*; Yoon et al. 2010a), and from sediments of the Iberian deep sea, the tropical Atlantic, and the Arctic and Antarctic Oceans (*J. marinus*, Yoon et al. 2010a). *Jeotgalibacillus* strains have also been reported from non-saline soil samples collected from Xiaoxi National Natural Reserve (Chen et al. 2010a), decomposing reed rhizomes in a Hungarian soda lake (Borsodi et al. 2005), and hull of a ship in the form of biofilms (Inbakandan et al. 2010).

Strains of *Kurthia* have been isolated from the feces of patients suffering from diarrhea, but there is no evidence of pathogenicity in members of the genus (Keddie 1981). *K. zopffii* and *K. gibsonii* were isolated from meat (Gardner 1969) and meat products (Keddie 1981) and from feces of farm animals (chickens and pigs) (Keddie 1981). Authentic *Kurthia* species have also been isolated sporadically from milk, soil, and surface waters, presumably as a result of contamination with animal dung (Keddie 1981), from bottled drinking water (Jeena et al. 2006), from the nasal cavity of sea lions (Hernández-Castro et al. 2005), from the stomach and intestinal contents of the Suisun mammoth (Belikova et al. 1980), from the housefly (Wei et al. 2012), from Maari, a Baobab seed fermented product (Parkouda et al. 2010), from cigarettes (Rooney et al. 2005), from activated sludge (Kahru et al. 1998), from refrigerated meat (Pin and Baranyi 1998), in dental plaque from the beagle dog (Wunder et al. 1976), and from sloughing spoilage of California ripe olives (Patel and Vaughn 1973).

Paenisporosarcina macmurdoensis was isolated from cyanobacterial mat samples collected from ponds of Wright Valley, McMurdo Region, Antarctica (Matsumoto 1993), and *Paenisporosarcina quisquiliarum* from surface soil at a landfill site in Chandigarh, India (Krishnamurthi et al. 2009b).

Species of *Planomicrobium* have been isolated from diverse habitats including fresh water, marine habitats, cold regions, and

mesophilic climates. *Planomicrobium koreense* was isolated from the Korean traditional fermented seafood jeotgal (Yoon et al. 2001b). A few of them have been isolated from marine habitats like *Planomicrobium alkanoclasticum*, which was isolated from intertidal beach sediment (Engelhardt et al. 2001), *Planomicrobium chinense* from coastal sediment from the Eastern China Sea (Dai et al. 2005), *Planomicrobium mcmeekinii* from Antarctic sea ice (Junge et al. 1998), *Planomicrobium okeanokoites* from marine mud (Zobell and Upham 1944), and *Planomicrobium flavidum* from a marine solar saltern of the Yellow Sea (Jung et al. 2009). A few of the species were also isolated from cold habitats like *Planomicrobium glaciei* from frozen soil collected from the China no. 1 glacier (Zhang et al. 2009), *Planomicrobium stackebrandtii* from a cold desert of the Himalayas (Mayilraj et al. 2005), and *Planomicrobium psychrophilum* from a cyanobacterial mat sample from McMurdo Dry Valleys, Antarctica (Reddy et al. 2002). *Planomicrobium* strains were also isolated from biofilms of full-scale drinking water distribution systems (Liu et al. 2012) and ancient permafrost sediments from the Kolyma lowland of Northeast Eurasia (Vishnivetskaya et al. 2006).

Rummeliibacillus stabekisii was isolated from the Payload hazardous servicing facility at the Kennedy Space Center, FL, USA (Vaishampayan et al. 2009), and *Rummeliibacillus pycnus* was isolated from soil (Nakamura et al. 2002).

Solibacillus silvestris (Krishnamurthi et al. 2009a) was isolated from the top soil layer of a beech forest soil near Braunschweig, Lower Saxony, Germany (Rheims et al. 1999).

Sporosarcina species have been mostly isolated from soils and water from different habitats. *S. antarctica* was isolated from soil samples collected off King George Island, West Antarctica (Yu et al. 2008); *S. globispora* and *S. psychrophila* from soil and river water (Larkin and Stokes 1967; Nakamura 1984); *S. koreensis* and *S. soli* from upland soil in Suwon, Korea (Kwon et al. 2007); *S. pasteurii* from soil, sewage, and incrustations on urinals (Miquel 1889); *S. saromensis* isolated from surface water in Lake Saroma (An et al. 2007); *S. aquimarina* from sea water in Korea (Yoon et al. 2001c); *S. contaminans* and *S. thermotolerans* from an industrial clean-room floor and from a human blood sample, respectively (Kämpfer et al. 2010); *S. luteola* from soy sauce (Tominaga et al. 2009); and *S. newyorkensis* from clinical specimens and raw cow's milk (Wolfgang et al. 2012). *Sporosarcina* strains were also isolated from chicken-waste-impacted farm soil (You et al. 2012), ancient algal mats from the McMurdo Dry Valleys, Antarctica (Antibus et al. 2012), ice and brine (Bakermans and Skidmore 2011), surimi seafood products (Coton et al. 2011), petroleum hydrocarbon-contaminated soil (Wan et al. 2011), arctic terrestrial and marine environments (Kim et al. 2010), sea urchin *Hemicentrotus pulcherrimus* (Huang et al. 2009), from bovine slurry waste (Murayama et al. 2010), sea anemone *Stichodactyla haddoni* (Williams et al. 2007), farm-packaged product (Huck et al. 2008), and deep-sea sediment of the South China Sea (Liu and Shao 2007).

Species of *Ureibacillus* have also been isolated from a diverse range of habitats including air, soil, compost, and human waste. Most species have been isolated from various different types of compost like *U. composti* and *U. thermophilus* from livestock-manure composts (Weon et al. 2007) and *U. suwonensis* from cotton waste composts (Kim et al. 2006). *U. terrenus* was isolated from soil (Fortina et al. 2001) and *U. thermosphaericus* from air (Fortina et al. 2001). *Ureibacillus* strains were also isolated from the Ramsar hot springs in Iran (Abbasalizadeh et al. 2012), oyster mushroom (*Pleurotus* sp.) (Vajna et al. 2012), terrestrial systems (Portillo et al. 2012), dried human feces (Hoyles et al. 2012), rice straw (Wang et al. 2011a), autothermal thermophilic aerobic digestion of sewage sludge (Liu et al. 2010), personal-use composting reactor (Watanabe et al. 2010), pig manure composting (Xie et al. 2009), anaerobic sludge (Nakasaki et al. 2009), conventional dairy farms (Coorevits et al. 2008), hot aerobic poultry and cattle manure composts (Wang et al. 2007), and aerobic digestion of swine waste (Gagné et al. 2001).

Viridibacillus species have been isolated from soil samples (Heyrman et al. 2005; Nakamura et al. 2002).

Pathogenicity, Clinical Relevance

The members of the family Planococcaceae are reported to be nonpathogenic to humans, although a few reports indicate their presence in diseased individuals. A few species and strains of *Planococcus* have been demonstrated to be pathogenic to other animals. For instance, a strain of *Planococcus* sp. is pathogenic (10 %) to *Hylesia metabus* larvae (Osborn et al. 2002), and *Planococcus* W29 has strong inhibitory activity against *Serratia liquefaciens* which is a fish pathogen. Abdel Gabbar et al. (1995) implicated *P. halophilus* in an outbreak of necrotic hepatitis in chickens, whereas Oeding (1971) reported that *P. citreus* has no antigenic relationship to *Staphylococci* and *Micrococci*. The principle components responsible for the pathogenic activity have been identified as aromatic rings, phenolic groups, and a covalently bonded glycopeptide which is water soluble with a molecular weight of about 2800 (Austin and Billaud 1990). *Caryophanon latum* and *C. tenue* are not known to be pathogenic. The *Caryophanon* sp. 52E5 and *Planomicrobium* sp. 34D8 inhibited swarming in *Serratia marcescens* MG1 (Alagely et al. 2011). There is no evidence of pathogenicity in authentic members of the genus *Kurthia*, although a number of strains of *Kurthia* have been isolated from various clinical sources (Faoagali 1974; Severi 1946; Yang et al. 1985) and most frequently from the feces of patients suffering from diarrhea (Elston 1961; Jarumilinta et al. 1976). The genus *Planomicrobium* is essentially a marine genus with no previously reported pathogenicity (Skerratt et al. 2002). Chomarar et al. (1990) reported the isolation of *S. ureae* from a bronchial biopsy in a child with cystic fibrosis, but the strain is not pathogenic.

Antibiotic susceptibility has been studied in few species of the genus *Planococcus*. *P. citreus*, *P. maritimus*, *P. maitriensis*, and *P. antarcticus* are susceptible to chloramphenicol and tetracycline.

P. maritimus, *P. maitriensis*, and *P. antarcticus* were sensitive to bacitracin, lincomycin, and streptomycin but varied in their susceptibility to ampicillin, erythromycin, gentamicin, penicillin-G, and rifampin (Alam et al. 2003; Jeffries 1969; Kocur et al. 1970; Novitsky and Kushner 1976; Reddy et al. 2002; Yoon et al. 2003). *P. columbae*, *P. halocryophilus*, *P. halophilus*, *P. plakortidis*, and *P. salinarum* are sensitive to a number of antibiotics (Suresh et al. 2007; Mykytczuk et al. 2012; Novitsky and Kushner 1976; Kaur et al. 2012; Yoon et al. 2010b).

J. campisalis, *J. marinus*, and *J. salarius* are susceptible to carbenicillin, cephalothin, chloramphenicol, gentamicin, neomycin, oleandomycin, and penicillin-G; resistant to polymyxin B; and are variable to ampicillin, kanamycin, lincomycin, novobiocin, streptomycin, and tetracycline (Yoon et al. 2010a).

Planomicrobium flavidum is susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, lincomycin, neomycin, novobiocin, oleandomycin, penicillin-G, and streptomycin, but not to polymyxin B or tetracycline.

Bacillus pycnus (*Rummeliibacillus pycnus*; Vaishampayan et al. 2009) has been partially characterized for the antibiotic susceptibility, and it is susceptible to chloramphenicol, tobramycin, streptomycin, erythromycin, and tetracycline.

Application

Bioremediation

Planococcus sp. strain S5 isolated from activated sludge, which is able to grow on salicylate or benzoate as sole carbon source has been used for sodium salicylate degradation. This strain harbor a plasmid pLS5 which has genes coding for catechol 1, 2-dioxygenase and catechol 2,3-dioxygenase involved in degradation of salicylate and benzoate (Labuzek et al. 2003). Strain S5 is also able to utilize phenol as the sole carbon and energy source and can grow on 1 or 2 mM phenol, and this was attributed to both catechol 1,2- and catechol 2,3-dioxygenase. Catechol 2, 3-dioxygenase was optimally active at 60 °C and pH 8.0 and showed meta-cleavage activities for various catechols like catechol (100 %), 3-methylcatechol (13.67 %), 4-methylcatechol (106.33 %), and 4-chlorocatechol (203.80 %). The high reactivity of this enzyme towards 4-chlorocatechol is different from that observed for other catechol 2,3-dioxygenases (Hupert-Kocurek et al. 2012). Strain S5 can thus be used to bioremediate sites contaminated with the above aromatic hydrocarbons and phenolic compounds. *P. citreus* has also been used for the bioremediation of Cr (VI) (Cheng et al. 2010).

S. ginsengisoli CR5 tolerates high concentrations (50 mM) of As (III), a highly toxic metalloid. Microbially induced calcite precipitation (MICP)-based bioremediation by *S. ginsengisoli* is a viable, environmental friendly technology for remediation of arsenic-contaminated sites (Achal et al. 2012).

Mercuric reductase plays a significant role in biogeochemical cycling and detoxification of Hg and is useful in clean up of Hg-

contaminated effluents. *Sporosarcina* sp. strain G3 tolerates up to 40, 525, 210, 2900, and 370 μM of Cd, Co, Zn, Cr, and Hg, respectively, and reduces and detoxifies redox-active metals like Cr and Hg. The chromate reductase and MerA activities in the crude cell extract of the strain G3 were 1.5 and 0.044 units/mg protein, respectively (Bafana 2011).

Wang et al. (2011a) established a microbial consortium, designated WCS-6, which is capable of degrading lignocellulose. The consortium included nine bacterial isolates related to *Bacillus thermoamylovorans* BTa, *Paenibacillus barengoltzii* SAFN-016, *Proteobacterium* S072, *Pseudoxanthomonas taiwanensis* CB-226, *Rhizobiaceae* M100, *Bacillus* sp. E53-10, betaproteobacterium HMD444, *Petrobacter succinimandens* 4BON, and *Tepidiphilus margaritifera* N2-214 and five DNA sequences related to *U. thermosphaericus*, uncultured bacterium clone GC3, uncultured *Clostridium* sp. clone A1-3, *Clostridium thermobutyricum*, and *Clostridium thermosuccinogenes*.

Vargas-García et al. (2007) studied the effect of inoculation of microbial isolates, *Bacillus shackletoni*, *Streptomyces thermovulgaris*, and *U. thermosphaericus* in composting processes to improve lignocellulose degradation and reported that *U. thermosphaericus* decreased the final lignin content in a range between 17.2 % and 24.3 % with high efficiency. The biological detoxification of lignocellulosic hydrolysate by *U. thermosphaericus* is gentle, more precise in their action, and the efficiency is quite comparable to other physical and chemical methods (Okuda et al. 2008; Parawira and Tekere 2011). *U. thermosphaericus* was also resistant to Hg²⁺ salts with minimum inhibitory concentration (MIC) value of 30 μg mL⁻¹. The resistance to mercury in *U. thermosphaericus* was attributed to mercuric reductase activity which removed Hg²⁺ from the medium by the formation of a black precipitate, identified as HgS. According to Glendinning et al. (2005), this is a new mechanism of Hg tolerance, based on the production of nonvolatile thiol species.

An unidentified species of *Sporosarcina* sp. along with two unknown species of *Bacillus* sp. and *Pseudomonas* sp. were demonstrated to be very effective in the bioleaching of aluminum, arsenic, copper, manganese, iron, and zinc in Pb/Zn smelting at 65 °C and pH 1.5 (Cheng et al. 2009).

P. maitriensis produces extracellular polymeric substance (EPS) with oil spreading potential comparable to that of Triton X100 and Tween 80 and could emulsify xylene, hexane, and oils from jatropha, paraffin, and silicone and reduce the surface tension (from 72 to 46.07 mN m⁻¹) and interfacial tension. EPS could thus be used for bioremediation, enhanced oil recovery, and in cosmetics due to its emulsifying and tensiometric properties (Kumar et al. 2007).

Formation of Added-Value Products

Kurthia produces two enzymes, namely, carbamoylase and hydantoinase, which are key enzymes for the production of optically pure amino acids from dl-5-substituted hydantoins

(SSH) (Mei et al. 2009). *Kurthia* sp. has also been used to produce L-proline from glutamic acid or aspartic acid with the aid of detergents (Kato et al. 1972a, b).

Eleven biotin biosynthetic genes have been identified in *Kurthia* sp. strain 538-KA26 (Kiyasu et al. 2001) with potential use in biotech industry.

P. maritimus produces a number of carotenoids such as the red-pigmented glyco-carotenoic acid ester and methyl glucosyl-3,4-dehydro-apo-8'-lycopenoate with antioxidative activity (Shindo et al. 2008). These antioxidants could be used as drugs for stroke and neurodegenerative diseases, as dietary supplements and also as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline. Recently, Krishnaveni and Jayachandran (2009) demonstrated that crude extracts of both *P. maritimus* KP8 and *Staphylococcus arlettae* KP2 downregulated the synthesis of inflammatory mediators such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and cyclooxygenase-2 (COX-2), besides markedly inhibiting p38 mitogen-activated protein (MAP) kinase suggesting that the crude ethyl acetate extracts from these isolates have the potential of inhibiting inflammation in mitogen-induced peripheral blood mononuclear cells (Krishnaveni and Jayachandran 2009).

Endophytic *S. aquimarina* Sjam16103 are known to have plant growth-promoting activity due to their ability to produce indole acetic acid and siderophore which help to solubilize phosphate molecules and fix atmospheric nitrogen (Janarthine and Eganathan 2012). In fact more and more agriculturists are now resorting to the use of endophytic bacteria to promote plant growth due to their abilities to fix nitrogen, to produce phytohormones, to solubilize phosphate, and to control disease (Janarthine and Eganathan 2012). Zhang et al. (2010) demonstrated that *Sporosarcina* sp. strain N52 produces intracellular glucan from L-arabinose, and additionally strain N52 could be used for waste management and bioconversion of organic materials to the valuable alpha-glucan which can be used as a food additive.

Enzymes

Cold-active enzymes have a high catalytic efficiency at low temperature and thus are suitable for processes that need to be accomplished at low temperature. For instance, the catalytic efficiency of β -galactosidase of *Planococcus* sp. L4 at 5 and 20 °C is 14 and 47 times more than that of *Escherichia coli* β -galactosidase at 20 °C, respectively. Hence, cold-active β -galactosidase from the strain L4 can be used for removal of lactose from milk and dairy products at cold conditions or as a reporter enzyme for psychrophilic genetic systems (Hu et al. 2007). *Sporosarcina* sp. RRLJ1 produces a protease with potential for commercial use as it has high activity at pH 6.5 (Boruah and Bezbaruah 2000). *S. ureae* produces an alkaline stable (pH 7.75–12.5) urease which could be used for several applications at alkaline pH (McCoy et al. 1992).

Other Applications

P. rifietoensis and several other strains can control gray moulds (which cause disease in strawberry fruits) and eventually increase the yield of fruits and can be a key substitute to synthetic fungicides. These strains can also be used under storage and greenhouse conditions (Essghaier et al. 2009). *Planococcus* strains from Antarctica have the ability to produce cold-active antimicrobial compounds with potential use in chilled food preservation. Thus, Antarctic soils could represent an untapped reservoir of novel, cold-active antimicrobial-producers (O'Brien et al. 2004).

Geologically stored carbon dioxide (CO₂) needs to be trapped to avoid increase in CO₂ in the atmosphere. Microbes also have a role to play in this important aspect. For instance, microbially induced calcium carbonate precipitation has the potential in remediation of a broad array of structural damages including sealing of concrete cracks with CaCO₃, a property which has been extensively studied in *S. pasteurii* (Achal et al. 2009a). Thus, microbially induced mineral precipitation (MICCP) technologies may effectively seal and strengthen fractures to alleviate CO₂-leakage potential (Phillips et al. 2012). Martin et al. (2012) suggested the use of an anaerobic strain instead of *Sporosarcina pasteurii* to precipitate CaCO₃ in the anoxic subsurface via ureolysis since *S. pasteurii* cannot grow anaerobically. Bergdale et al. (2012) used genetically engineered microorganisms with dual abilities of producing extracellular polymeric substances (EPSs) as well as inducing MICCP to remediate the structural damages. They transformed *Pseudomonas aeruginosa* strain 8821 capable of producing EPS with the entire *Sporosarcina pasteurii* urease gene cluster including *ureA*, *ureB*, *ureC*, *ureD*, *ureE*, *ureF*, and *ureG* and used the recombinant strain to induce calcite precipitation in the presence of EPS to provide a stronger matrix than MICCP alone as a biosealant.

Another strategy developed by Okwadha and Li (2011) used a biosealant which was obtained by using PCB, urea, Ca²⁺, and bacteria (*S. pasteurii* strain ATCC 11859). It was envisaged that the biosealant would have reduced water permeability and high resistance to carbonation and would be thermally stable up to 840 °C for environmentally sustainable development (Okwadha and Li 2011).

Achal et al. (2011) developed a mutant of *S. pasteurii* strain Bp M-3 which could utilize corn steep liquor or lactose mother liquor (low-cost industrial waste) from starch/dairy industry as a nutrient source for the growth and production of calcite (Achal et al. 2009a, b, 2011).

A novel carotenoid from *S. aquimarina* strain SF238 acetyl-4,4'-diapolycopene-4,4'-dioate has excellent antioxidative properties which can protect the cells from photosensitized peroxidation reactions like other related 4,4'-diapolycopene-4,4'-dioate derivatives (Steiger et al. 2012).

Ranganathan et al. (2006) used bacteriotherapy to alleviate uremic intoxication by ingestion of live *S. pasteurii* (nonpathogenic alkalophilic urease positive) which are able to catabolize

uremic solutes in the gut. The bacterium was able to remove urea from 21 ± 4.7 mg to 228 ± 6.7 mg per hour using 10 cfu (colony forming units) in in vitro study. *S. pasteurii* does not disturb the microbial community in the human intestine, thus implying that *S. pasteurii* reduced blood urea-nitrogen levels and significantly prolonged the lifespan of uremic animals (Ranganathan et al. 2006).

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