2.3 Environmental Distribution and Taxonomic Diversity of Alkaliphiles

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Background

Two categories exist for discrimination of bacteria that are able to grow at a high pH (pH 9). The first group of bacteria is categorized as alkali-tolerant bacteria. The bacteria belonging to this group can grow at pH 9, but their optimum growth pH is around 7. In addition, they cannot grow at a pH higher than 10. For example, several *Virgibacillus* spp. (e.g., *Virgibacillus chiguensis* [Wang et al. 2008]) are able to grow at pH 8–9 but not at pH 10. Another example is alkali-tolerant *Anoxybacillus* spp. (e.g., *Anoxybacillus flavithermus* [Pikuta et al. 2000]). They are able to grow at pH 9 but not at pH 10, and their optimum growth pH is around 7. The second group of bacteria is categorized as alkaliphilic bacteria. This category of bacteria is the main issue in this review. Alkaliphilic bacteria can be defined as the bacteria that can grow at above or at pH 10 and/or grow equally well or better in terms of growth intensity or velocity above or at pH 9 compared with those grown at a pH lower than 9. Alkaliphilic bacteria can be further divided into facultative alkaliphiles, which can grow well below or at pH 8, and obligate alkaliphiles, which cannot grow well below or at pH 8. Some genera of bacteria include neutralophilic, alkali-tolerant, and alkaliphilic (e.g., *Bacillus*). Even among the same species of bacteria, there are those that are both alkali-tolerant and alkaliphilic bacteria (e.g., *Bacillus horikoshii* [Nielsen et al. 1995]). Even among the same species of bacteria, there are those that are both obligate and facultative alkaliphiles (e.g., *Bacillus pseudofirmus* (Nielsen et al 2005) [Nielsen et al. 1995]). It is considered that the above-described differences in categorized bacteria are due to the differences in the physiological function for adaptation at high pH and/or neutral pH.

The first reported alkaliphilic bacterial species is *Sporosarcina pasteurii* (formerly *Bacillus pasteurii*) described by Chester (1889). The bacterium has the ability to convert urea to ammonium carbonate and requires ammonia in addition to an alkaline environment. Strains belonging to S. pasteurii can be isolated from soil, water, sewage, and incrustation on urinals. In 1934, Vedder reported *Bacillus alcaliphilus* as the second example of isolation of alkaliphiles. The bacterium lacks urease activity and does not require ammonia in addition to an alkaline enveronment. This species was isolated from various materials using preliminary enrichment in broth at pH 10. In 1960, Takahara and Tanabe isolated an indigo-reducing alkaliphile, which can grow at pH 12.1 from indigo fermentation liquor, and proposed it as a strain belonging to the genus *Bacillus*. They proposed a new species for the strain as *Bacillus alkaliphiles* (Takahara and Tanabe 1962). However, the species name has not been approved and the strain is not available presently.

During the 1970s–1980s, numerous alkaliphiles belonging to the genus *Bacillus* were isolated for industrial applications of their enzymes and for physiological studies on environmental adaptation mechanisms. However, most of these bacterial strains have not been appropriately identified up to the species level. Gordon and Hyde (1982) firstly classified numerous alkaliphilic *Bacillus* strains. They grouped alkaliphilic *Bacillus* spp. into *Bacillus firmus*, *Bacillus lentus* groups I–III, and the *B. firmus*–*B. lentus* complex on the basis of their physiological and biochemical characteristics. A reclassification of these strains, together with additional strains, was carried out by Fritzie et al. (1990). Their reclassification was based on several phenotypic properties that correlated with distinct DNA G+C mol% values. Among the strains used in the study of Spanka and Fritzie (1993), a new species, *B. cohnii*, was proposed; it is distinct from the other strains by the absence of diaminopimelic acid (DAP) in its cell wall. The phenotypic and genotypic heterogeneities of alkaliphilic *Bacillus* strains within the same group in the study by Fritz et al. were revealed by phylogenetic analysis based on 16S rRNA gene sequence analysis (Nielsen et al. 1994). In addition to the reported alkaliphilic bacteria described above, nine new species were proposed by Nielsen et al. (1995) on the basis of results of numerical taxonomy and DNA–DNA hybridization. Strains used for physiological studies on environmental adaptation mechanisms (Goto et al. 2005; Krulwich and Guffanti 1989; Krulwich et al. 2007; Yumoto 2002, 2003) belong to the species defined by Nielsen et al. (1995). Since the study by Nielsen et al. (1995), more than 30 new species alkaliphilic *Bacillus* have been proposed. In addition, numerous strains belonging to genera other than the genus *Bacillus* and included in the phylum Firmicutes have been proposed (Yumoto 2007). Furthermore, since the finding of alkaliphiles in soda lakes in Africa, the taxonomic distribution of alkaliphiles has become wider (Duckworth et al. 1996; Jones et al. 1998). In addition to the phylum Firmicutes, alkaliphilic microorganisms so far isolated are presently taxonomically distributed to the following phyla: Cyanobacteria, Actinobacteria, Proteobacteria (including alpha, gamma, and delta subdivision), Bacteroidetes, Thermotogae, Spirochaetas, Archaea (Euryarchaeota), and yeast (Jones et al. 1998). In the 2000s, numerous alkaliphiles are continuously being isolated and the number of approved species of alkaliphiles is continuously increasing. In this review, we focus on the environmental distribution and taxonomic diversity of alkaliphiles belonging to the phylum Firmicutes.

Aims and Significance of Taxonomy of Alkaliphilic Fermicutes

Bacteria have gone through generations of changes over a long period on earth. They exhibit tremendous genetic diversities and can adapt to a wide range of environments compared with other organisms. In addition, bacteria contribute to global material circulation. Therefore, it can be said that the maintenance of environmental conditions on earth depends on the activity of bacteria. It is expected that each existing bacterial cell in the environment has a certain role in a community of bacteria. On the basis of the above facts, it is important to know the environmental and taxonomic distributions of bacteria to understand their functions and communities in the environment. In addition, understanding the relationship between taxonomic diversity and geographical distribution, the variation of strategy for adaptation in an environment, and the concomitant evolutional process of bacteria is also important. If such knowledge is available, it will contribute to the understanding of the contribution of microorganisms to environmental sustainability on earth. However, it is not easy to understand the genetic diversity of bacteria even in a limited environment and a limited category of bacteria in a short term. Furthermore, it is not easy to consider the ecological function of such numerous species of bacteria. However, if we consider only alkaliphilic bacteria, it would be much easier to think about the above problems because bacteria can be isolated selectively from an environmental sample. In addition, alkaliphilic bacteria are more widely distributed in the environment than other extremophiles (Horikoshi and Grant 1998). Therefore, we are able to consider a wide range of environments for a limited category of bacteria compared with neutralophilic bacteria. This approch will simplify the difficult problem in microbial ecology and will bring us a simpler model of bacterial environmental distribution and taxonomic diversity compared with an exhaustive approach.

It is considered that four environmental categories exist for bacterial habitats considering the size and naturally existing or artificial environments (➲ *Fig. 2.3.1*). These are large natural and artificial environments, and small natural and artificial environments. If we apply these categories to the habitats of alkaliphiles, we can categorize the bacterial habitats as follows:

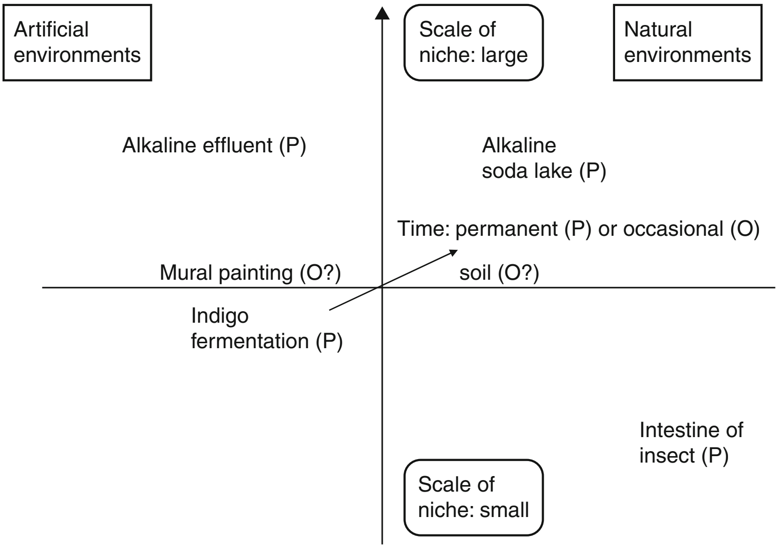

Fig. 2.3.1

Classification of habitats of alkaliphiles. The horizontal axis indicates natural or artificial environments and the vertical axis indicates the scale of the habitat. The third axis indicates time of exposure to alkaline conditions. P: permanent alkaline condition, O: occasionally alkaline condition. It is difficult to define soil and soil-like solid environments because it is difficult to monitor pH in small niches

large natural environments such as alkaline soda lakes, large artificial environments such as alkaline containing waste water treatment systems, small natural environments such as the gut of termites, and small artificial environments such as alkali-fermented food “Hongeohoe” in Korea and laboratory enrichment culture for alkaliphiles. To a greater or lesser extent, many microorganisms can be classified into these four environmental categories. It will be important to know the differences in actual status and dynamics of bacterial flora between natural and artificial environments, and global material circulation and symbiotic systems for application of microbial ecosystems to benefit the human society. However, it is obviously difficult to understand these differences by exhaustively considering all bacteria. In this case also, the study of a limited category of alkaliphilic bacteria will simplify the analysis. If we present a more concrete proposal of a study of the above-mentioned problem on the four categories of bacterial habitats, clarification of the differences in physiological function between the same species of bacterial strains isolated from different habitats may also clarify the relationship between ecological niches and their physiological function. It is also interesting to considerer why alkaliphilic bacteria distribute not only in alkaline environments but also in conventional garden soil. To solve such a problem, it is important to accumulate data on the characteristics of corresponding species from different habitats. Targeting alkaliphilic bacteria will be useful from the microbial ecological and physiological points of view.

Considering the different characteristics in terms of cytochrome content, cell wall composition and fatty acid composition of alkaliphiles, as well as alkaline adaptation mechanisms of alkaliphiles may differ among bacterial species or strains (Aono and Horikoshi 1983; Clejan et al. 1986; Yumoto et al. 1997). In addition, it is considered that a certain diversity of alkaline adaptation mechanisms may exist among various bacterial species or strains. Whole-genome analysis was completed in several strains of alkaliphiles (Takami et al. 2000, 2002). It is expected that diversities and origins of specific physiological functions of alkaliphiles will be clarified by selection of strains based on phylogenetic position and estimation of gene expressions of physiologically important proteins and biological materials.

Environmental Distributions

Hyperthermophiles and psychrophiles are adapted to specific environments and cannot grow under conventional laboratory conditions used for the growth of common microorganisms such as *Escherichia coli* (i.e., atmospheric pressure of 37°C, moderate ionic strength and neutral pH). These extremophiles are distributed in their specific environments in nature. In general, although there are several exceptions, major populations of extremophiles are adapted to their specific environments and they are distributed in their specific environments. Compared with such extremophiles, alkaliphiles are unique because typical alkaliphiles (e.g., alkaliphilic *Bacillus* spp.) usually exist in conventional environments such as gardens and agricultural soil and manure (Horihoshi 2006; Nielsen et al. 1995). Alkaliphilic microorganisms are also present in high pH environments such as naturally occurring alkaline environments, including soda lakes (Borsodi et al. 2005; Jan-Roblero et al. 2004; Jones et al. 1998; Joshi et al. 2008; Rees et al. 2004), underground alkaline water (Roadcap et al. 2006; Takai et al. 2001; Tiago et al. 2004), relatively small alkaline niches such as intestines of insects (Broderick et al. 2004; Thongaram et al. 2003), and artificial alkaline environments such as indigo fermentation liquor (Nakajima et al. 2005; Takahara and Tanabe 1960; Yumoto et al. 2004b, 2008) and alkaline wastes formed as by-products of food-processing industries (Collins et al. 1983; Nakamura et al. 2004; Ntougias and Russel 2000, 2001; Yumoto et al. 2004a).

Soil Samples

Numerous strains of alkaliphilc *Bacillus* spp. have been isolated from soil samples (Horikoshi 2006; Yumoto 2007). In addition to the isolation of *Bacillus* spp., several strains of alkaliphiles, *Alkalibacillus* spp. (Fritze 1996; Jeon et al. 2003) and *Paenibacilus* spp. have also been isolated (Lee et al. 2002; Yoon et al. 1998). The genus *Paenibacillus* belongs to a family different from that of the genus *Bacillus* (*Paenibacillaceae*). Indeed, alkaliphiic *Bacillus* spp. can easily be isolated from ordinary garden soil using a conventional medium containing 1% Na_2CO_3 as reported by Horikoshi (2006). Alkaliphilic *Bacillus* spp. have been isolated not only from alkaline soils but also from soils of neutral pH. The reason why alkaliphiles exist in soils other than alkaline soils has not been clarified yet, although there are several possibilities. During nitrogen circulation, the decay of proteins and the hydrolysis of urea by microorganisms may lead to localized alkalization in the soil. Another possible explanation for the localized alkalization in the soil is the production of alkaline substances by alkaliphiles and/or other microorganisms. For example, Horikoshi (2006) reported that some alkaliphiles produce extracellular alkaline substances that provide a favorable pH to their ambient environment. Another reason for the universality of alkaliphilc *Bacillus* spp. in soil may be their ability to form spores. Alkaline adapted *Bacillus* spp. living in a vast or small alkaline environment may be scattered in the soil, for example, by dust storms. Indeed, alkaline soils are very common around the world. The pH of alkaline soils is around 10 or even higher. In addition, some of alkaliphiles can adapt to neutral pH also; thus, facultative alkaliphilic *Bacillus* spp. are distributed in a wide range of soil types compared with obligate alkaliphiles. Generally, most of the alkaliphilic *Bacillus* spp. are facultative alkaliphiles, and the population of obligate alkaliphiles is small among isolates from an ordinary soil sample.

Felske et al. (1999) estimated the predominant bacteria in Dutch grassland soils by 16S rRNA gene targeting PCR amplification using directly extracted DNA from the soils as the template, and compared them with the most abundant culturable bacteria. Although the pH of the media used for isolation of strains was 7 or 4, a strain exhibiting the highest similarity (94.8%) in the 16S rRNA gene with the alkaliphile *B. cohnii* was isolated. In this study, comparison of the culture-dependant approach and culture-independent approach by 16S rRNA gene sequence analysis of clone library on the basis of extracted DNA from the same soil samples showed no correlation between the collection of cultured strains and the 16S rRNA gene clone library. Therefore, it is not clear whether a bacterial strain related to *B. cohnii* is the predominant population in soils.

Garbeva et al. (2003) estimated predominant *Bacillus* spp. in Dutch agricultural soil under different culture management regimes using genus *Bacillus*-targeting 16S rRNA gene clone libraries. In total, 128 clones (42–43 clones per treatment) were analyzed. Although the soil sample was slightly acidic (pHs 5.5–6.5), 3 clones that exhibited 99% similarity with alkaliphilic *Bacillus halodurans* were detected. It is considered that only major groups of *Bacillus* spp. were detected in this experiment. Therefore, it is suggested that *B. halodurans* is the major population among genus Bacillus in the soils used in this study.

To isolate halophilic bacteria from soil, samples taken from 360 places were collected and spread on agar plates containing 20% NaCl with pH adjusted to 5.0, 7.0, and 9.0 (Echigo et al. 2005). The samples were from ordinary garden soil, yards, and roadways in areas surrounding Tokyo, Japan. Twenty-seven alkaliphilic strains were isolated out of 176 isolates. These isolates were tentatively identified according to their similarity in the 16S rRNA gene sequence as follows: *Alkalibacillus haloalkaliphilus* (Jeon et al. 2005) (formerly *Bacillus haloalkaliphilus* [Fritze 1996]) *Filobacillus milosensis*, *Gracillibacillus halotolerans*, *Halobacillus trueperi*, ‘*Bacillus nitriophilus*,’ and ‘*Planococcus psychrotoleratus*.’ However, their similarities are not sufficiently high (87.3–97.2%) to classify them into their corresponding species except *A. haloalkaliphilus*. Therefore, most of the isolated alkaliphiles are unknown species. Among the isolated alkaliphiles, four strains of an isolate exhibit 100% similarity and six strains exhibit 98.0–99.8% similarity with *A. haloalkaliphilus* (formerly *B. haloalkaliphilus*). *A. haloalkaliphilus* is a spore-forming obligate alkaliphile and is strictly an aerobe. The genus *Alkalibacillus* belongs to the same family as the genus *Bacillus* (family: *Bacillaceae*).

There are soils containing a substantial concentration of sodium carbonate among the dominant soluble salts in their upper profile such as soda or sodic, soda solonchaks. It is considered that such places are unique extreme habitats for microorganisms characterized by high alkalinity. Such soils are distributed in dry steppe and semidesert areas such as southwestern Siberia, northeastern Mongolia, north China in Central Asia, Egypt in Africa, India, Hungary in Europe, and North American steppes. A bacterial strain was isolated by enrichment with isobutyronitrile as the sole carbon, energy, and nitrogen source at pH 10 from soda solonchak soils obtained from southwestern Siberia (Sorokin et al. 2008a). The isolate was an obligate alkaliphile (pH range of growth between 7 and 10.2 [optimum, 9]) and a moderately salt-tolerant bacterium, and was proposed as a new species called *Bacillus alkalinitrilicus*. On the other hand, another alkaliphilic bacterium was isolated from northeastern Mongolia using a nitrogen-free alkaline medium at pH 10 (Sorokin et al. 2008b). The isolate was able to fix dinitrogen gas and grew on a nitrogen-free alkaline medium, and was proposed as the species named *Bacillus alkalidiazotrophicus*. The isolate was also an obligate alkaliphile with a pH range for growth between 7.8 and 10.6 (optimum, 9.5).

On the basis of the studies of the isolation and characterization of alkaliphilic bacteria, it seems that most of the alkaliphiles living in soils belong to the genus *Bacillus*. However, many as yet undetermined genera or species still exist in the environment, as shown by the above-mentioned study on halophilic alkaliphiles.

Soda Lakes

Microbial diversities of soda lakes in Africa, Eurasia, and North America have been investigated by DNA clone analysis by amplification of DNA and isolation of microorganisms from environments (Borsodi et al. 2005; Duckworth et al. 1996; Humayoun et al. 2003; Jan-Roblero et al. 2004; Jones et al. 1998; Joshi et al. 2008; López-García et al. 2005; Mesbah et al. 2007; Rees et al. 2004). Results of those studies indicate that soda lakes are habitats of diverse microorganisms belonging to the following: Cyanobacteria, Actinobacteria, Firmicutes, Verrucomicrobiales, Proteobacteria (including alpha, beta, gamma, and delta subdivisions), Bacteroides, Verrucomicrobia, Spirochaetes, Chloroflexi, Thermotogae, and candidate divisions of bacteria.

It is considered that East African soda lakes are the most stable and productive naturally occurring environments, with pH generally higher than 10 and occasionally reaching 12 (Jones et al. 1998). This is due to the almost unlimited supply of CO_2 combined with high ambient temperature and strong daily light. The primary production in soda lakes are mainly supported by a dense population of Cyanobacteria. The density of bacterial members is remarkably constant at about 10^5 cfu ml^{-1}. The environmental conditions of soda lakes are due to a combination of geological, geographical, and climatic factors. Soda lakes are characterized by the presence of large amounts of Na^+, Cl^-, and $HCO^{3-}/CO_3{}^{2-}$ as the major ions in solution. Eleven strains associated with the genus *Bacillus* were isolated in the study of phylogenetic diversity in east African soda lakes. Three strains belonged to rRNA group 6 (Nielsen et al. 1995; Yumoto 2007) of the genus *Bacillus* and are close but not identical to obligately alkaliphilic *Bacillus pseudofirmus* that was isolated from soil and manure (➲ *Fig. 2.3.2*). The remaining eight strains belonged to rRNA group 7 (Nielsen et al. 1995; Yumoto 2007), among which, two strains in a distinct subgroup are related to facultatively alkaliphilic *Bacillus saliphilus* (Romano et al. 2005). The remaining 6 strains are less related to known species. Isolates in rRNA group 7 are diversified also in phenotypic characteristics. Strains related to *B. pseudofirmus* seem to predominate in shoreline mud and dry foreshore soda soil that are subjected to fluctuating conditions of alkalinity and salinity with seasonal changes in water level. On the other hand, the strain in rRNA group 7 exhibiting a high Na^+ requirement for growth appear to be more prevalent in lake water and sediments where conditions are less changeable. No alkaliphilic *Bacillus* strains that were the same species from soil samples were isolated from soda lakes. This finding suggests that the origins of alkaliphiles are different. From the analysis of anaerobic environments of soda lakes, ten strains were isolated (Jones et al. 1998). Among them, seven strains were related to *Clostridium* "cluster XI." They formed a group distinct from that of previously reported obligatory anaerobic alkaliphiles. They do not require NaCl for their growth. Other isolates are haloalkaliphiles requiring 12–16% (w/v) NaCl for their growth. Although they are closely related to *Clostridium* "cluster VI" among the

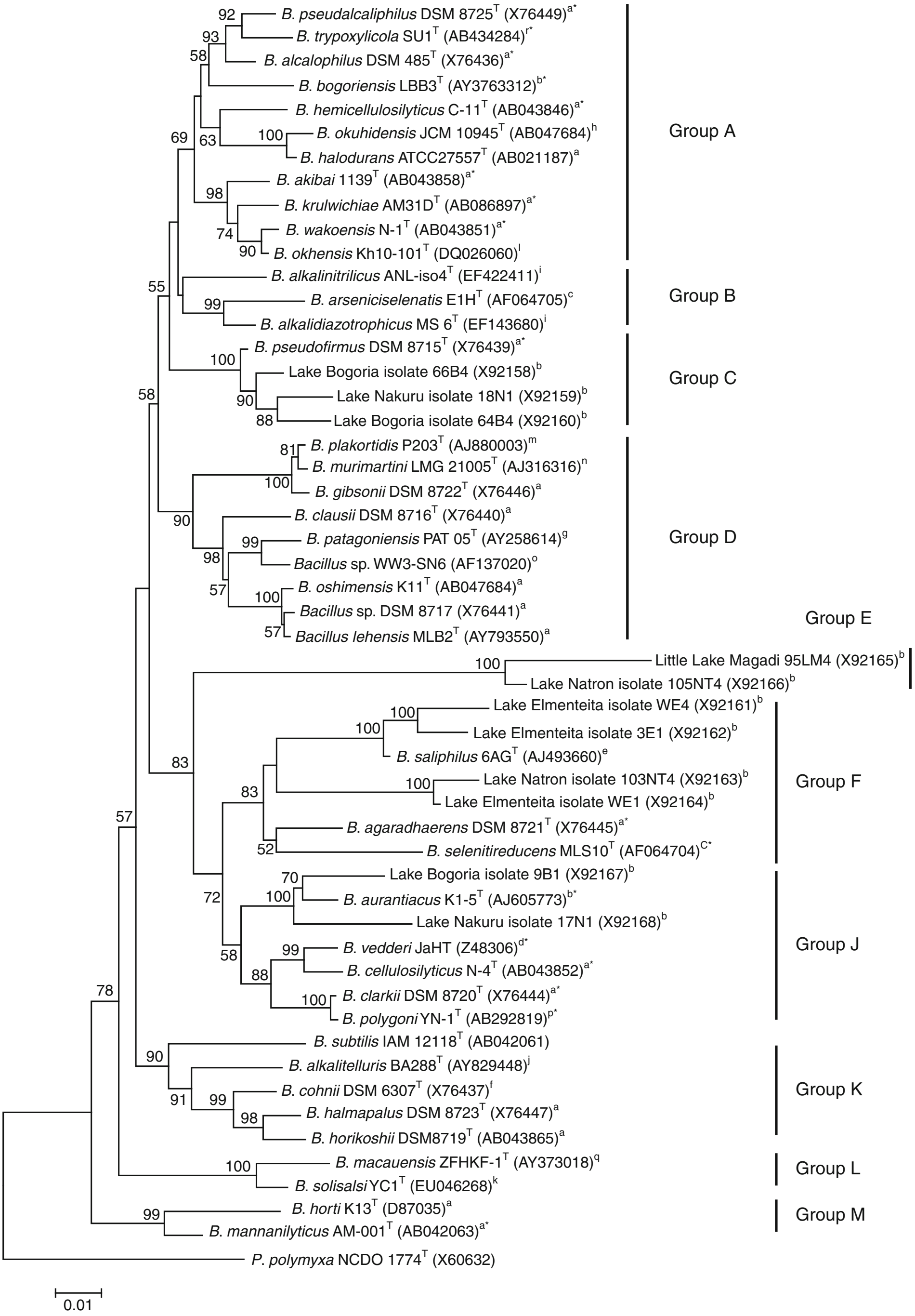

Fig. 2.3.2 (Continued)

reported species, they formed a separate position in the phylogenetic tree constructed on the basis of the 16S rRNA gene. Therefore, they represent a new genus of obligately anaerobic haloalkaliphiles. DNA was extracted from water and sediment samples taken from soda lakes of the Kenyan-Tanzanian Rift Valley and subjected to denaturing gradient gel electrophoresis (DGGE) on the basis of the 16S rRNA gene (Rees et al. 2004). Eight genes related to the phylum Firmicutes were detected. Three genes were related to *B. pseudofirmus* and four genes were related to *Clostridium* spp. One gene exhibited a low similarity with a reported sequence (81.4% identity to *B. halodurans*). These results suggest that most of the genes were reflected in the results of culture-dependent detection, but some of the strains corresponding to genes exhibiting a low similarity with reported sequences have not been isolated.

Mono Lake is an alkaline (pH 9.8), hypersaline (84–94 g liter^{-1}) soda lake located east of the Sierra Nevada Mountains, California, USA (Humayoun et al. 2003). The lake has strong geochemical gradients, a simple food web, and a simple microbial community. Therefore, it is suitable for studying how bacterial populations function to facilitate biochemical processes. Anoxic bottom water at a high pH with prolonged meromixis has high levels of toxic inorganic compounds such as sulfide and ammonia. These conditions may influence microbial activity and flora. In addition, the environment has high concentrations of dissolved organic carbon (DOC), dissolved inorganic phosphorus and standing corps of bacteria. These findings suggest that understanding the reasons for the situation of soda lakes will help clarify the consequence of the microbial loop in the ecology. This study revealed the depth-dependent distribution of microbial diversity in the lake. Bacterial sequences belonging to the phylum Firmicutes were detected in 19% of 212 detected gene sequences. None of the obtained sequences were closely related to any of the 16S rRNA gene sequences in the gene database. Apart from the data on soda lakes in east Africa, no sequence similar to those of *Bacillus* spp. was detected. Twenty-seven sequences formed an independent separate lineage. The remaining sequences exhibited following similarities: two sequences were distantly related (86%) to the obligate anaerobe *Anaeobranca bogoriae*; two sequences were distantly related (85%) to the obligate anaerobe *Natronoanaerobium salstagnum* isolated from Lake Magadi, Kenya; and two strains were distantly related (83%) to facultatively anaerobic *Enterococcus* spp. Only the last two sequences were obtained from the sample collected from the shallowest part.

The microbial diversities of the Indian soda lake Lonar Crater Lake have been studied by molecular analyses and culture-dependent isolation (Wani et al. 2006; Joshi et al. 2008). It is postulated that the lake originated as a meteorite impact crater around 50,000 years ago on the

Fig. 2.3.2

Phylogenetic tree derived from 16S rRNA gene sequences of alkaliphilic *Bacillus* spp., constructed using the neighbor-joining method. *Bacillus subtilis* IAM 12118^T and *Paenibacillus polymixa* NCDO 1774 are used as the representative strain of rRNA group 1 (Ash et al. 1991) and the outgroup of the phylogenetic tree, respectively. The two strains are not alkaliphiles. Origin of alkaliphiles: a, soil; b, soda lake; c, bottom sediment of soda lake; d, bauxite-processing red mud tailing pond; e, algal mat from mineral pool; f, soil and horse meadow; g, rhizosphere of shrub; h, hot spa; i, soda soil; j, sandy soil; k, saline soil; l, saltpan; m, sea; n, mural paint; o, alkaline effluent olive plant; p, indigo fermentation; q, influent of a water treatment plant; r, larval guts of beetle. The asterisks exhibit obligate alkaliphiles. GenBank/EMBL/DDBJ accession numbers are given in parentheses. Numbers indicate bootstrap values greater than 500. Bar: 0.01 K_{nuc}

basis of geological studies. It is the third largest crater in the world. In addition, it is the only known crater formed by a meteoric impact in basaltic rock. Water entered into the lake through rain, ground water seepage, and springs suited in the cliffs at the edge of the lake. The water in the lake is alkaline (pHs 10–10.5) and this high pH is due to the high concentration of sodium carbonate. The lake does not contain any industrial seepage. Therefore, it is intriguing to understand the microbial diversity in this soda lake. DNA was extracted from environmental samples, the 16S rRNA gene was amplified by PCR, and the 16S rRNA gene clone library was constructed (Wani et al. 2006). Five hundred clones were randomly selected for the microbial diversity analyses. Forty-four of unique phylotypes were obtained after restriction fragment length polymorphism (RFLP) analysis and subsequent sequencing. Among them, 34% (15 clones) of the clones were classified as Firmicutes as the major constituent in this analysis. The 15 clones were classified into 5 groups as follows: 2 clones were related to the genus *Alkalibacterium*; 5 clones were not affiliated with any known taxa and formed a cluster with 5 other uncultured bacteria from hypersaline and hydrothermal vent environments; 2 clones were distantly related to *Alkaliphilus crotonoxidans*, whereas 1 clone exhibited a maximum similarity with *Natronoincola histidinovorans*; 1 clone was related to *Anoxynatronum sibiricum*; and 2 clones were related to *B. pseudofirmus*. These findings indicate that this soda lake environment contains aerobic bacteria, facultative anaerobes, obligate anaerobes, and an uncultured cluster. Culture-dependent investigation for clarification of bacterial diversity of Lonar Crater Lake was also carried out. One hundred and ninety-six strains were isolated using different enrichment media (Joshi et al. 2008). Out of the 196 strains, 64 were selected at first selection considering the pH and salt tolerances of the isolates. Then, 31 strains were finally selected on the basis of their enzyme profile and were further subjected to phylogenetic analysis. The result indicated that the classification of the selected bacterial strains could be done as follows: 21 strains belonging to Firmicutes, 3 strains to Actinobacteria, 1 strain of α-Proteobacteria, 1 strain to β-Proteobacteria, and 5 strains to γ-Proteobacteria. The phylum Firmicutes contains the following genera: *Bacillus*, *Paenibacillus*, *Alkalibacillus*, *Exiguobacterium*, *Planococcus*, *Enterococcus*, and *Vagococcus*. Although several isolates were related to reported alkaliphilic *Bacillus* spp. such as *B. cohnii* (96% similarity) and *B. horikoshii* (99%), some of the strains were highly related to species reported to be not alkaliphiles such as *Bacillus cereus* (1 strain, 99% similarity; 1 strain, 100% similarity), *Bacillus benzoevorans* (99%), *B. firmus* (99%), *Enterococcus casseliflavus* (100%), and *Bacillus fusiformis* (100%). Apart from the strains from soda lakes in east Africa, only two strains were isolated and identified as obligate alkaliphiles and they do not belong to the phylum Firmicutes.

Gut of Insects

Termites harbor abundant microorganisms in their gut. The first proctodeal segment of the gut in higher termites has a high pH (i.e., pHs 10–12) and is rich in K^+. Thongaram et al. (2003) screened alkaliphilic bacteria from the first proctodeal region of higher termites by isolation of alkaliphilic bacteria and analysis of alkaliphilic *Bacillus*-targeting 16S rRNA gene clone libraries. The samples used in the study were soil-feeding, soil/wood-interface, wood-feeding, and unknown-feeding higher termites. Twenty-one alkaliphilic bacterial strains were isolated. Among the isolates, 20 strains were found to be affiliated with the genus *Bacillus* by phylogenetic analysis based on 16S rRNA gene sequences. The remaining one strain was affiliated with the genus *Paenibacillus* and the closest relative was *Paenibacillus lautus* among the approved species. The isolates affiliated with the genus *Bacillus* was classified into 6 groups clustered with reported 16S rRNA gene sequences as follows: eight strains with *Bacillus* sp. DSM 8717 (*Bacillus oshimensis* among approved species), six strains with *Bacillus gibsonii* or *B. horikoshii*, two strains with *Bacillus clausii*, two strains with *Bacillus horti*, one strain with *Bacillus halmapalus*, and one strain with *Bacillus* sp. N−1 (*Bacillus alcalophilus* among approved species). Although the bacterial flora in the termite gut has been studied extensively by clone analysis, very few *Bacillius* clones have been detected. Therefore, a new primer was developed for detecting particularly *B. clausii*, *B. gibsonii*, *B. horikoshii*, *Bacillus patagoniensis* (formerly *Bacillus* sp. DSM 8714), and two alkaliphilic isolates from the termite gut. From the results, 31 DNA clones were found to be related to the above-mentioned targeted species, *Bacillus* sp. DSM 8717, and two isolates in this study. Although the clone analysis showed several clones not belonging to the genus *Bacillus*, the obtained sequences were related to the 16S rRNA gene sequence of the isolates from the same origin. Many isolates grew better in an alkaline medium containing K_2CO_3 than in that containing Na_2CO_3. Although the pH of the gut of higher termites is high, most of the isolates are facultative alkaliphiles.

The bacterial community in the gypsy moth larval midgut has been investigated by culturing, PCR amplification, and terminal restriction fragment length polymorphism (T-RFLP) (Broderick et al. 2004). The midgut of the gypsy moth is typically pHs 8–10, but can reach pH 12. The identification of PCR-amplified genes revealed that most of the obtained genes were identified as belonging to γ-Proteobacteria and Fermicutes and that the cultured isolates represent more than one-half of the phylotypes identified. The sequences indentified as Firmicutes include mainly the genera *Enterococcus* and *Staphylococcus*. In addition, the genera *Bacillus* and *Paenibacillus* were also included. Although several new bacterial phylotypes are identified in the gypsy moth midgut, their overall diversity is lower than that in the termite gut.

The above results indicate that life style and food strongly affect the bacterial community of the insect gut.

Groundwater

Analysis of the diversity of microbial communities in extremely alkaline ($pH > 12$) groundtemporary water owing to reaction with the fill from the Lake Calumet area of Chicago, Illinois, where historic dumping of steel slag has occurred in a wetland, was performed by 16S rRNA gene targeting PCR amplification by directly extracting DNA from samples (Roadcap et al. 2006). Many of the sequences are related to those from the soda lakes or cement-contaminated groundwater of high pH in gold mines. These are related to *Alkaliphilus*, *Natronoincola*, *Bacillus*, and *Anaerobranca*. However, these exhibit certain phylogentetic distances from known alkaliphiles. Therefore, there are possibilities of isolation of many new species from this environment. However, isolation of bacteria has not been attempted. Hence, characteristics of bacteria living in the groundwater are as yet unknown.

Sea and Sea-Related Samples

Generally, most of the isolates from seawater are Gram-negative bacteria. However, it has been reported that Gram-positive bacteria exist in mud or sediment at the bottom of the sea. Although it seems that soil samples do not exhibit a high pH, Ivanova et al. (1999) reported that strains belonging to Gram-positive alkaliphilic *Bacillus* were isolated from samples of marine origin. They isolated 20 strains of aerobic endospore-forming bacilli from samples of marine origin obtained from different areas of the Pacific Ocean. A group of four alkaliphiles were tentatively identified as a facultative alkaliphile, *B. horti*, from soil in the sea. Takami et al. reported that a halotolerant and facultative alkaliphile, *Oceanobacillus iheyensis*, has been isolated from deep-sea sediment collected at a depth of 1,050 m on the Iheya Ridge of the Nansei Islands in Japan (27°44.18′N, 126°54.15′E) (Lu et al. 2001). The isolate is a strictly aerobic and spore-forming bacterium.

Marine non spore-forming lactic acid bacteria, *Marinilactibacillus psychrotolerans* and *Halolactibacillus halophilus*, have been isolated from living and decomposing marine organisms (Ishikawa et al. 2003, 2005). They are facultative anaerobes. *Marinilactibacillus psychrotolerans* and *Halolactibacillus* spp. are facultatively alkaliphilic bacteria. Halophilic and alkaliphilic lactic acid bacteria have been isolated from various cheeses produced in Europe (Ishikawa et al. 2007). These alkaliphiles are possibly of marine origin because sea salt is added to cheeses. The identification based on the 16S rRNA gene sequence of the isolates revealed that they belong to the genus *Marinilactibacillus* or *Alkalibacterium*. These microorganisms may contribute to the ripening of cheeses. Recently, ten new species of bacteria belonging to the genus *Alkalibacterium* were isolated from decaying marine algae, decaying seagrass, raw fish, salted fish, and salted and fermented shrimp paste (Ishikawa et al. 2009). Although previously reported *Alkalibacterium* spp. are all obligate alkaliphiles, these sea-related isolates are all facultative alkaliphiles. These finding indicate that alkaliphilic lactic acid bacteria are widely distributed in the sea and sea-related environments.

The above reports indicated that most of the alkaliphiles that are distributed in the sea and sea-related samples are facultative alkaliphiles.

Indigo Fermentation Liquor

The above-described habitats of alkaliphiles are natural environments. Indigo fermentation liquor is an artificial environment in which alkaliphilic microorganisms thrive. Indigo fermentation liquor has been produced from a Japanese indigo plant (*Polygonum tinctorium*) by two steps of processing by microorganisms. Harvested indigo leaves are first air-dried and appropriately wetted for the subsequent processing by microorganisms. In this process, the temperature of a pail of *Polygonum* leaves reaches about 55°C. It is very difficult to control the appropriate water content by adding of water and stirring to maintain the appropriate activity of microorganisms. In this process, the noncolored substance (indican) turns into blue indigo. This fermented *Polygonum* agglomerats into an indigo ball for convenience of transfer. The produced indigo ball is further processed by microbial reduction under alkaline conditions (pH above 10), at which insoluble indigo is converted into the soluble form “lueko indigo,” by the action of microorganisms. This microbial reduction process can be substituted by chemical reduction using dithionite. The fermentation procedure for reducing indigo declined around 1960 in Japan because it takes a much longer time than chemical reduction and has difficulties in maintenance. Recently, traditional methods have been re-evaluated because they yield better products in terms of color than do chemical reduction.

First an indigo-reducing bacterium has been isolated by Takahara and Tanabe (1960) and proposed as a new species called *B. alkaliphiles* (1962). Although the strain is not available presently, it exhibited optimum growth at pHs 10–11.5 and requires a peptide consisting of seven amino acids, which is contained in the fermentation liquor, for its propagation. The fermentation liquor inoculated with the isolate is reduced state, whereas the control, i.e., not inoculated with the isolate, took 168 h to be reduced. Since it is considered that a reducing agent is necessary for chemical reduction, Padden et al. (1999) isolated a novel thermophilic indigo-reducing bacterium, *Clostridium isatidis*, from a woad vat as an alternative. However, the bacterium is alkali tolerant according to the definition mentioned above.

Indigo-reducing obligately alkaliphilic bacteria, *Alkalibacterium psychrotolerans*, *Alkalibacterium iburiense*, and *Alkalibacterium indicireducens* have been isolated from indigo fermentation liquor (Nakajima et al. 2005; Yumoto et al. 2004, 2008). They are facultative anaerobes and produce lactic acid. They share several common characteristics, whereas they exhibit different substrate utilization and growth characteristics. It is considered that there are several types of indo reducing bacterium and probably more than three types of species and they support the reduction of indigo by interacting with each other and with non-indigo-reducing bacteria. Spontaneously occurring microorganisms reduce indigo in the actual fermentation process. Indigo-reducing microorganisms probably come from the indigo ball. Actually, the pH becomes higher in the first *Polygonum* fermentation process owing to ammonia production during this process. However, determination of further upstream origin of indigo-reducing microorganisms is difficult because they are obligate alkaliphiles. Alklaliphilic bacteria other than indio-reducing microorganisms exist in the vat for indigo fermentation. The microflora involved in indigo fermentation, and the relationship between indigo-reducing bacteria and other factors are not known. This interaction may be very important for the maintenance of the fermentation fluid.

Other Environments

Alkaliphiles are distributed not only in indigo fermentation liquor but also in other artificial environments such as effluents from an agricultural or fishery processing plants. The environments of effluents are different from those of indigo fermentation in size and existence of continuous inflow of water from the upstream of facility. The first example of an alkaliphile isolated from an effluent from agricultural plant is *Exiguobacterium aurantiacum* (Collins et al. 1983). The strain was isolated from potato-processing plant effluent. On the other hand, *Exiguobacterium oxidotolerans* T-2-2^T was isolated from fishery processing plant effluent (Yumoto et al. 2004a). The latter isolate was not isolated from alkaline effluent. Although there are not so many examples, it is suggested that strains belonging to the genus *Exiguobacterium* are distributed in effluents from agricultural or fishery processing plants. Facultatively alkaliphilic *Bacillus* sp. WW3-SN6 was isolated from alkaline effluent derived from the preparation of edible olives (Ntougias and Russel 2000). An alkaline solution containing NaOH was used to precipitate a bitter compound of the fruit. The isolate is related to alkaliphilic *B. patagoniensis* that was isolated from the rhizosphere of the perennial shrub *Atiplex lampa* (Oliver et al. 2005). In addition, *Alkalibacterium olivoapovliticus* was also isolated from alkaline effluent derived from the preparation of edible olives (Ntougias and Russel 2001). Other species belonging to the genus *Alkalibacterium* are isolated from indigo fermentation liquor as described above. These findings suggest that strains belonging to the genus *Alkalibacterium* are related to plant environments. In addition, the facultative alkaliphile *Amphibacillus xylanus* was isolated from compost of grass and rice straw (Niimura et al. 1990). These findings suggest that certain alkaliphilic bacteria are related to substances from plants. Alkaliphilic Firmicutes have been isolated from animal manure. Strains of obligately alkaliphilic *B. pseudofirmus* have been isolated from deer and ostrich manure, and strains of facultatively alkaliphilic *B. halodurans* have been isolated from chicken, tiger, pigeon, and elephant manure (Nielsen et al. 1995). Strains of facultatively alkaliphilic *B. cohnii* were isolated from old horse feces. These findings suggest that certain alkaliphilic *Bacillus* spp. are related to animal manure or feces.

In addition to the reports described above, *Oceanobacillus oncorhynchi* was isolated from the skin of rainbow trout (*Oncoehynchus mykiss*), suggesting that certain alkaliphiles exist in the skin of fishes. The above examples suggest that alkaliphilic Firmicutes species are distributed in effluents of agricultural or fishery processing plants, rhizospheres of plants, manure of animals, and feces and skin of fish (Yumoto et al. 2005b). However, this wide distribution of alkaliphilic Firmicutes microorganisms cannot be accounted to only their spore-forming ability because some of the microorganisms described above do not have this ability.

Taxonomy of Isolated Alkaliphilic Firmicutes

The taxonomic distribution of alkaliphilic Firmicutes based on the approved species is listed in ➲ *Table 2.3.1*. The taxonomic distribution becomes wider than that of 10 years ago and it is expected that it will become wider in the next decade. This means that alkaliphiles distribute in a wide range of taxonomic positions. In this review, we mainly focus on the genus *Bacillus* and consider the relationship between characteristics and phylogenetic position, because until now, a lot of bacterial strains belonging to the genus *Bacillus*, which exhibit various phylogenetic positions determined on the basis of the 16S rRNA gene sequence, have been isolated from various of environments. These strains include both facultative and obligate alkaliphiles.

Genus Bacillus

Over 40 alkaliphilic *Bacillus* species had been approved by the end of 2009. The characteristics of 41 approved species belonging to alkaliphilic *Bacillus* are listed in ➲ *Table 2.3.2*. They are listed in order of serial number of G+C content of DNA. Alkaliphilic *Bacillus* share G+C contents of 33.8–42% and 48.4–53.8%. The most frequent source of isolation is soil. They distribute in not only common places such as soil, sea, and feces but also specific environments such as hot spa, sea anemone, and insect gut. Most grow in the mesophilic temperature range of 10–40°C. *Bacillus okuhidaensis* strains grow in the themophilic temperature range of 30–60°C. Most of them grow in the pH range of 7–10, 8–10 or 11. However, some of them grow in a particular pH range such as *Bacilllus solisalsi* (growth pH range of 5–13). The summarized characteristics of alkaliphilic *Bacillus* spp. indicate that they distribute not only in alkaline environments but also in conventional environments. The results suggest that the diversity of alkaliphilic *Bacillus* spp. is not defined only by bacterial habitats and various as yet undiscovered species distribute in various environments. On the other hand, this is quite surprising that one kind of extremophile exhibits this wide range of distribution. These characteristics are only found in alkaliphilic *Bacillus* spp. among the various genera of extremophiles. This means that alkaliphilic *Bacillus* spp. are quite common microorganisms rather than rare microorganisms in various environments on earth.

Table 2.3.1

Taxonomic distribution of alkaliphiles in phylum Firmicutes

Phyrum	Class	Order	Family	Genus
Firmicutes				
	Bacilli			
		Bacillales		
			Bacillaceae	
				Alkalibacillus
				Amphibacillus
				Anoxybacillus
				Bacillus
				Caldalkalibacillus
				Cerasibacillus
				Halolactobacillus
				Natonobacillus
				Oceanobacillus
				Salsuginibacillus
				Terrabacillus
			Paenibacillaceae	
				Paenibacillus
			Planococcaceae	
				Sporosarcina
				Planococcus
			Staphylococcaceae	
				Salinicoccus
			Bacillus Family XII Incertae Sedis	
				Exiguobacterium
			Unclassified Bacillales	
				Alkalilactobacillus
			Unclassified Bacilli	
				Desulfurispirillum
		Lactobacillales		
			Carnobacteriaceae	
				Alkalibacterium
				Marinilactibacillus
			Enterococcaceae	
				Enterococcus
				Vagococcus

Table 2.3.1 **(Continued)**

Phyrum	Class	Order	Family	Genus
	Clostridia			
		Clostridiales		
			Clostridiaceae	
				Alkaliphilus *Anoxynatronum* *Natronincola* *Tindallia*
		Natranaerobiales		
			Natranaerobiaceae	
				Natranaerobius
			Clostridialses Family XIV Incertae Sedis	
				Anaerobranca
			Eubacteriaceae	
				Alkalibacter

A phylogenetic tree constructed on the basis of the 16S rRNA gene sequence of 39 approved species of alkaliphilic *Bacillus* spp. and isolates from soda lakes is shown in *Fig. 2.3.2*. Most of the alkaliphilic *Bacillus* spp. are approved species and isolates from soda lakes belong to a similar phylogenetic position and form several groups. They are classified into rRNA group 1, 6 and 7 (Ash et al. 1991; Nielsen et al. 1994; Yumoto 2007). In this review, we would like 516 to propose a new classification of alkaliphilic Bacillus spp., as described in *Fig. 2.3.2*. Group A contains at least 8 species belonging to obligate alkaliphiles out of 11 species. Most of them were isolated from soil. It is considered that although most of them prefer high-pH environments, they are widely distributed in soil. This is attributed to their localization in alkaline environments and the limited scale of alkaline niches may exist widely in soil environments, and/or soil environments may share localized common characteristics with localized alkaline environments such as the intestine of insects. Actually, *Bacillus trypoxylicola* belonging to this group has been isolated from larval guts of the Japanese beetle. Group B consists of only facultative alkaliphiles isolated from soda soil or bottom sediments of soda lakes. The presented results suggest that bacteria belonging to this group are distributed only in limited environments. Group C contains only one approved species, obligate alkaliphilic *B. pseudofirmus*. This species is distributed in common environments such as soil, animal manure, and fresh lake water. It is expected that this group consists of obligate alkaliphiles, because the other three strains were isolated from vast and permanently alkaline environments, namely, African soda lakes. Group D contains 9 species of facultative alkaliphiles. The origins of six species are soil or soil-related environments. *Bacillus plakortidis* has been isolated from the Norwegian Sea. *Bacillus murimartini* and *Bacillus* sp. WW3-SN6 have been isolated from mural paintings and alkaline effluent, respectively. These findings suggest that this group of bacteria is distributed not only in vast natural environments but also in artificial environments. The above-described groups A–D belong to rRNA group 6 (Nielsen et al. 1994; Yumoto 2007). Obligate alkaliphiles belong to only groups A–C and all the species in group D are facultative alkaliphiles.

Table 2.3.2

Characteristics of alkaliphilic *Bacillus* spp.

Species	Isolated location	pH range (opt.[a]) (pH)	Temp. range (opt.) (°C)	Aerobe or anaerobe	Other characteristics	G+C content of DNA (%)	References
Bacillus chonii	Garden soil, indigo ball, horse faces, horse meadow, feces, contamination in agar plate	7–10 (9)	10–47	Aerobe	Grows at 7% NaCl	33.8–35.0	(Nielsen et al. 1995; Ohta et al. 1975; Spanka and Fritze 1993; Yumoto et al. 2000)
Bacillus alkalinitrilicus	Soda solonchak soils	7–10.2 (9)	15–41 (32)	Aerobe	Utilizes aliphatic nitriles, grows at 0.1–1.5 M NaCl	35.1	(Sorokin et al. 2008a)
Bacillus akibai	Soil	8–10 (9–10)	20–45 (37)	Aerobe	Grows at 7% NaCl	34.4	(Nogi et al. 2005)
Bacillus alcalophilus	Soil, feces, soda lake	8–10 (9–10)	10–40 (ND)	Aerobe	Grows at 8% NaCL	36.2–38.4	(Nielsen et al. 1995; Nogi et al. 2005; Vedder 1934)
Bacillus agaradhaerens	Soil	8–11 (10 or above)	10–45 (ND)	Aerobe	Grows at 16% NaCl	36.5–36.8	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus hemicellulosilyticus	Soil	8–11 (10)	10–40 (37)	Aerobe	Grows at 12% NaCl	36.8	(Nogi et al. 2005)
Bacillus alklidiazotrophicus	Soda soil	7.5–10.6 (9.0–9.5)	15–43 (33–35)	Aerotolerant anerobe	0.1–1.2 M Na^+ (optimum 0.2–0.4 M)	37.1	(Sorokin et al. 2008b)
Bacillus mannanilyticus	Soil	8–10 (9)	20–45 (37)	Aerobe	Grows at 3% NaCl	37.4	(Nogi et al. 2005)
Bacillus bongoriensis	Soda lake	8–11 (10)	10–40 (37)	Aerobe	Nonmotile	37.5	(Vargas et al. 2005)
Bacillus trypoxylicola	Leval guts of the Japanease horned beetle	8–10 (9)	ND (30)	Aerobic	Nonmotile	37.4–37.7	(Aizawa et al. 2010)

Table 2.3.2 (Continued)

Species	Isolated location	pH range (opt.[a]) (pH)	Temp. range (opt.) (°C)	Aerobe or anaerobe	Other characteristics	G+C content of DNA (%)	References
Bacillus alklitelluris	Sandy soil	7.0–11.0 (9–9.5)	15–40 (30)	Facultatively anaerobe	0–4% NaCl	37.9	(Lee et al. 2008)
Bacillus wakoensis	Soil	8–11 (9–10)	10–40 (37)	Aerobe	Grows at 10% NaCl	38.1	(Nogi et al. 2005)
Bacillus pseudoalcalophilus	Soil	8–10 (about 10)	10–40 (ND)	Aerobe	Grows at 10% NaCl	38.2–39.0	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus vedderi	Bauxite-processing red mud tailing pond	Opt. temp. about 10	maximum temp. for growth 40–50	Aerobe	Grows at 7.5% NaCl	38.3	(Agnew et al. 1995)
Bacillus pseudofirmus	Soil, animal manure, Fresh lake water	8–10 (about 9)	10–45 (ND)	Aerobe	Grows at 16% NaCl	38.7–40.9	(Nielsen et al. 1995)
Bacillus halmapalus	Soil	7–10 (about 8)	10–40 (ND)	Aerobe	No growth at 5% NaCl	38.6	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus oshimensis	Soil	7–10 (ND)	13–41 (28–32)	Aerobe	Grows at 0–20% NaCl, nonmotile	39.1–40.8	(Nogi et al. 2005; Yumoto et al. 2005a)
Bacillus cellulosilyticus	Soil	8–10 (9–10)	20–40 (37)	Aerobe	Grows at 12% NaCl	39.6	(Nogi et al. 2005)
Bacillus murimartini	Mural painting from a church	7–10 (8.5)	10–30 (15–20)	Aerobe	Grows at 0–4% NaCl	39.6	(Borchert et al. 2007)
Bacillus patagoniensis	Rhizosphere of shrub	7–10 (8)	5–40 (ND)	Aerobe	Grows at 15% NaCl	39.7	(Olivera et al. 2005)
Bacillus neizhouensis	Sea anemone	6.5–10 (8.5)	4–30 (25)	Aerobic	Grows at 0.5–10% NaCl	39.8	(Chen et al. 2010)

Bacillus horti	Soil	7–10 (about 10)	15–40 (ND)	Aerobe	Grows at 10% NaCl	40.2–40.9	(Yumoto et al. 1998)
Bacillus arsenicoselenatis	Bottom sediment of soda lake	7.5–10 (ND)	ND	Obligately anaerobe	Nonmotile, respiratory growth with Se(VI), As (V), Fe(III), nitrate, and fumarate	40.0	(Switzer Blum et al. 1998)
Bacillus marmarensis	Mushroom compost	8.0–12.5	ND	Aerobic	Catalase positive, oxidase negative	40.2	(Denizci et al. 2010)
Bacillus krulwichiae	Soil	8–10 (ND)	20–45 (ND)	Facultatively anaerobe	Grows at 0–14% NaCl	40.6–41.5	(Yumoto et al. 2003)
Bacillus gibsonii	Soil	8–10 (ND)	10–37 (ND)	Aerobe	Grows at 9% NaCl	40.6–41.7	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus macauensis	Influent of a water treatment plant	6.0–10.0 (8.5)	20–40 (30)	Facultatively anaerobe	Grows at 5–10% NaCl	40.8	(Zhang et al. 2006)
Bacillus okhensis	Saltpan	7–10 (9)	25–40 (37)	Aerobe	Grows at 0–10% NaCl	40–42	(Nowlan et al. 2006)
Bacillus lehensis	Soil	7.0–11.0 (8)	10–37 (25)	Aerobe	Grows at 0–12% NaCl	41.4	(Ghosh et al. 2007)
Bacillus solisalsi	Saline soil	5–13 (7–10)	15–53 (35–42)	Facultatively anaerobe	Grows at 15% NaCl	41.8	(Liu et al. 2009)
Bacillus okuhidaensis	Hot spa	6.0–11.0 (ND)	30–60 (ND)	Aerobe	Grows at 10% NaCl	41.0–41.1	(Li et al. 2002; Nogi et al. 2005)
Bacillus plakoridis	Sea	6.5–10 (8.5)	4–30 (15–20)	Aerobe	Grows at 0–12% NaCl	41.1	(Borchert et al. 2007)
Bacillus horikoshii	Soil	7–10 (ND)	10–40 (ND)	Aerobe	Grows at 8–9% NaCl	41.1–42.0	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus polygoni	Indigo ball	8–12 (9)	5–47 (29–30)	Aerobe	Grows at 3–14% NaCl	42.9	(Aino et al. 2008)
Bacillus aurantiacus	Extremely shallow, alkaline soda lakes	8.0–12.0 (9.5–10.0)	10–45 (28)	Aerobe	3–7% NaCl	42.9	(Borsodi et al. 2008)

Table 2.3.2 (Continued)

Species	Isolated location	pH range (opt.^{a}) (pH)	Temp. range (opt.) (°C)	Aerobe or anaerobe	Other characteristics	G+C content of DNA (%)	References
Bacillus clausii	Soil, broiler gastrointestinal tract, wastewater, abalone	7–10 (about 8)	15–50 (ND)	Aerobe	Grows at 10% NaCl	41.7–43.5	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus halodurans	Soil, animal manure, bird manure	7–10 (ND)	15–55 (ND)	Aerobe	Grows at 12% NaCl	42.1–43.9	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus clarkii	Soil	8–11 (ND)	15–45 (ND)	Aerobe	Grows at 16% NaCl	42.4–43.0	(Nielsen et al. 1995; Nogi et al. 2005)
Bacillus saliphilus	Algal mat from mineral pool	7–10 (9.0)	4–50 (37)	Aerobe	Grows at 1–25% NaCl	48.4	(Romano et al. 2005)
Bacillus selenitireducens	Bottom sediment of soda lake	8–11 (ND)	ND	Facultatively anaerobe	nonmotile, reducing selenite	49.0	(Switzer Blum et al. 1998)
Bacillus chagannorensis	Soda lake (Lake Chagannor)	5.8–11.0	6–40 (37)	Facultatively anaerobe	Grows at 3–20% NaCl	53.8	(Carrasco et al. 2007)

ND: no data; opt.^{a} = optimum

Two strains belonging to group E have been isolated from soda lakes in Africa. Until now, no approved species in group E has been reported. Therefore, there is no information on the detailed characteristics. Group F includes five strains isolated from soda lakes or environments related to soda lakes. Although growth characteristics of isolates from soda lakes are unknown, it seems that most of the strains belonging to this group are obligate alkaliphiles. The strains belonging to this group are related to water environments except *Bacillus agaradhaerens*. In group J, three strains isolated from soda lakes are located in similar phylogentic positions. On the other hand, other strains are isolated from soil or mud and artificial fermentation product. It is considered that most of the strains belonging to this group are obligate alkaliphiles. Groups F and J belong to rRNA group 7 (Nielsen et al. 1994; Yumoto 2007). Group K contains mostly facultative alkaliphiles that were isolated from soil or soil-related environments. This group corresponds to rRNA group 1 (Ash et al. 1991).

Groups L and M are new groups. Two strains belonging to group L are facultative alkaliphiles. It is understandable that group L is in an isolated phylogenetic position because the two strains belonging to the group were isolated from specific environments. On the other hand, two strains belonging to group M are highly adapted to high pH; both strains were isolated from soil. Strains belonging to group M were isolated from common sources. It is difficult to find the reason why the group exhibits an isolated position in the phylogenetic tree.

Conclusions and Perspective

In the past decade, various alkaliphilic Fermicutes species have been isolated and many species of alkaliphiles have been approved. As described above, alkaliphiles are distributed in various phylogenetic positions in the phylum Fermicutes. Therefore, it is considered that alkaliphiles are not a very particular group of bacteria. Certain alkaliphiles can be isolated from conventional environments. In addition, various alkaliphilic bacteria exist in soil or soil-related environments. However, it seems that specific alkaliphilic bacteria live in particular environments. It is considered that there are certain relationships between phylogentic position and environmental distribution in alkaliphilic bacteria. Actually, it is very difficult to define soil environments because it is considered that several microenvironments exist in a three-dimensional structure and there is no uniformity even in relatively small environments. It can be assumed that there is a mosaic distribution for alkaline environments of a very limited size. Hence, it is considered that a variety of alkaliphiles thrive in such complicated environments that harbor a variety of bacterial niches in a certain size of space. This may be one of the reasons why alkaliphiles exist in ordinary soil.

On the other hand, several certain alkaliphilic species have been isolated from various environments. In such cases, these microorganisms have high adaptability to various environments. Therefore, a wide range of adaptability in certain microorganisms is also one of the reasons for the wide range of distribution of the alkaliphiles. Actually, inserted gene sequences of transposase were more frequently observed only in alkaliphiles than in alkaliphilic *B. halodurans*, *O. iheyensis*, and neutralophilic *Bacillus subtilis* (Takami et al. 2000, 2002). It is assumed that some facultative alkaliphiles may have evolved from neutralophiles while some facultative alkaliphiles may have evolved from obligate alkaliphiles. Actually, several neutralophiles exhibit alkaliphilic characteristics and vice versa. Furthermore, certain alkaliphilic *Bacillus* spp. contain both obligate and facultative alkaliphiles.

In the future, we should further our understanding of the environmental and taxonomic distribution of alkaliphilic bacteria not only by the isolation of new species but also by metagenomic approaches to the study of several environments. In addition, genetic analyses of numerous sites of genes using numerous bacterial strains on the basis of population genetics will contribute to the clarification of the relationship between evolutional process and environmental distribution in alkaliphilic bacteria. Through these approaches we may clarify the ecological function and environmental exchange in the world of alkaliphilic bacteria.

Cross-References

- 2.1 Introduction and History of Alkaliphiles
- 2.2 Distribution and Diversity of Soda Lake Alkaliphiles
- 2.4 Anaerobic Alkaliphiles and Alkaliphilic Poly-Extremophiles

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