APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2008, p. 4535–4538 Vol. 74, No. 14
0099-2240/08/$08.00+0 doi:10.1128/AEM.00491-08
Copyright © 2008, American Society for Microbiology. All Rights Reserved.

Isolation of Bacteria Whose Growth Is Dependent on High Levels of CO_2 and Implications of Their Potential Diversity▽†

Kenji Ueda,* Yudai Tagami, Yuka Kamihara, Hatsumi Shiratori, Hideaki Takano, and Teruhiko Beppu

Life Science Research Center, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa 252-8510, Japan

Received 28 February 2008/Accepted 12 May 2008

Although some bacteria require an atmosphere with high CO_2 levels for their growth, CO_2 is not generally supplied to conventional screening cultures. Here, we isolated 84 bacterial strains exhibiting high-CO_2 dependence. Their phylogenetic affiliations imply that high-CO_2 culture has potential as an effective method to isolate unknown microorganisms.

It has been known for a long time that some bacteria require an atmosphere with high CO_2 levels for their growth. In 1952, Tuttle and Scherp (21) reported that *Neisseria meningitidis* grew effectively under an atmosphere containing 4% CO_2. Since then, the dependence of a number of pathogens (3, 8, 12, 17) and ruminal bacteria (5) on an atmosphere with high levels of CO_2 has been demonstrated; these microorganisms are called "capnophiles." Recently, several instances of a high-CO_2 requirement with regard to the commensal growth of some bacteria have been reported. For example, Diaz et al. (6) reported that the growth of *Porphyromonas gingivalis* depends on CO_2 supplied by coexisting *Fusobacterium nucleatum*. Bringel and Hubert (2) revealed the presence of a number of CO_2-dependent auxotrophs in a community of lactic acid bacteria. Recently, we revealed that *Symbiobacterium thermophilum*, a taxonomically unique syntrophic bacterium whose growth depends on coculture with cognate *Geobacillus stearothermophilus* strain S (22) demonstrated marked pure growth when CO_2 was introduced into the culture (24).

Despite such wide occurrence of capnophilic bacteria, CO_2 is not conventionally supplied to cultures used to isolate microorganisms from environmental samples. This made us speculate that some microorganisms dependent on high CO_2 levels have not yet been isolated and that they remain uncharacterized despite their culturability. Hence, we screened for bacteria whose isolation depended on the presence of an atmosphere with high levels of CO_2 by focusing on soil and water samples and studied the phylogenetic affiliations of these bacteria.

Prior to isolation, the effect of an atmosphere with high levels of CO_2 on the colony formation efficiency of bacteria from environmental samples was studied by microcolony counting. An appropriately diluted environmental sample was applied to a 0.45-μm nitrocellulose membrane disk (F4 cm; Advantec, Tokyo, Japan), which was divided into two pieces. The pieces were placed separately on two Luria-Bertani (LB) medium-containing agar plates containing tryptone (Difco Laboratories, Detroit, MI; 10 g/liter), yeast extract (Difco; 5 g/liter), NaCl (Kokusan, Tokyo, Japan; 5 g/liter), and agar (Kokusan; 10 g/liter). The pH of the medium was adjusted to 7.0 and 9.0 for neutral and alkaline samples using 50 mM phosphate and bicarbonate buffer, respectively. One plate was incubated under ambient air, and the other was incubated under an atmosphere with high levels of CO_2 (air supplied with 5% CO_2) using a CO_2 incubator (model 5400; Napco, OH). After an 8-h incubation at 28°C, a filter was stained with methylene blue, and the microcolony content was determined by direct counting under a stereo microscope (model SZH10; Olympus, Tokyo, Japan).

Using the above method, we preliminarily assessed the microcolony formation frequencies of conventional soil and water samples (neutral/alkaline) collected from several sites in Kanagawa Prefecture, Japan. The pH ranges of the neutral and alkaline samples were 6.9 to 7.6 and 9.1 to 9.4, respectively. Cultivation of the neutral samples under 5% CO_2 yielded 4×10^6 to 5×10^6 colonies per gram of soil and 2×10^3 to 3×10^3 colonies per milliliter of water, values which were slightly higher (7 to 10% increases) than the values obtained for cultures cultivated under ambient air. On the other hand, cultivation of the alkaline samples under 5% CO_2 yielded 1×10^6 to 6×10^6 colonies per gram of soil and 1×10^3 to 3×10^3 colonies per milliliter of water, values which were markedly higher (three- to fivefold increases) than those obtained by cultivation under ambient air. Hence, it appeared that the introduction of an atmosphere with high levels of CO_2 promotes the growth of microorganisms, particularly of those that exist in alkaline pH samples.

Further, the effect of the atmosphere with high levels of CO_2 on the diversity of the bacteria grown from environmental samples was roughly estimated by denaturing gradient gel electrophoresis (DGGE) analysis. Similar to procedures described above, the soil and water samples (neutral/alkaline) were inoculated into LB liquid medium (pH 7.0 or 9.0) and cultured at 28°C for 8 h. Then, the microbial cells were harvested by centrifugation and subjected to DNA extraction. The resulting DNA fraction containing the genomes of various bacteria was analyzed by DGGE using primers that extensively amplified the bacterial 16S rRNA

* Corresponding author. Mailing address: Life Science Research Center, College of Bioresource Sciences, Nihon University, 1866 Kameino, Fujisawa 252-8510, Japan. Phone: 81-466-84-3937. Fax: 81-466-84-3935. E-mail: ueda@brs.nihon-u.ac.jp.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 16 May 2008.

gene. The methods used for DNA extraction and DGGE analysis have been described previously (19). The obtained DGGE profiles showed a marked difference in the populations of cultures cultivated under 5% CO_2 and those cultivated under ambient air (for examples, see Fig. S1 in the supplemental material), suggesting that the atmospheric CO_2 content affects not only the number but also the diversity of the bacteria that grow from environmental samples.

The above preliminary observations suggested that cultivation under an atmosphere with high levels of CO_2 permits the cultivation of bacteria different from those obtained by cultivation under ambient air and that alkaline samples are an excellent source for the isolation of bacteria dependent on high CO_2 levels. Hence, we screened for bacteria dependent on high CO_2 levels by using the alkaline samples mentioned above as isolation sources. After appropriate suspension and dilution with sterile water, the samples were plated onto LB agar medium or 1/100 LB agar medium (1/100 dilution of LB medium added to 1% agar) and cultured at 30°C for 2 to 5 days under 5% CO_2. The pH of the medium was adjusted to 9.0 by adding $NaCO_3$. The poor nutritional content of the latter medium reduced the growth rate of fast growers and enabled the isolation of slow growers. Next, the bacterial colonies were replica plated onto two LB agar plates and separately cultured under 5% CO_2 and ambient air. Those exhibiting marked growth under only the former condition were selected. Since cultivation using a CO_2 incubator affected pH and humidity, the dependence of the selected isolates on high levels of CO_2 was carefully checked under several pH and humidity conditions before the isolates were finally described as being dependent on high CO_2 levels. For example, the pH of the above alkaline medium used for isolation (initial pH 9.0) was reduced to 8.3 during 5 days of incubation in a CO_2 incubator; hence, the isolates were checked for their growth defect under normal air using medium adjusted to pH 8.3 or lower. We did not obtain any isolate that depended on the slight acidification of media.

The isolates dependent on high CO_2 levels were phylogenetically characterized by a 16S rRNA gene-based analysis as described previously (19). The nucleotide sequence of the nearly complete (1.5-kb) 16S rRNA gene of each isolate was compared with the sequences in the GenBank/EMBL/DDBJ nucleotide sequence databases by using the BLASTN program (http://www.ncbi.nlm.nih.gov/BLAST/) and the SEQUENCE_MATCH program from the Ribosomal Database Project database (4). All nucleotide sequences were confirmed not to be chimeric using the CHECK_CHIMERA program from the Ribosomal Database Project website (http://rdp8.cme.msu.edu/html/). The sequences were aligned using the Clustal W program (20). Neighbor-joining phylogeny (18) was elucidated using the NJ plot program (16), and bootstrapping (7) was used to estimate the reliability of the phylogenetic reconstructions (1,000 replicates). The reference nucleotide sequences used in the tree construction were obtained from the GenBank/EMBL/DDBJ nucleotide sequence databases.

We studied approximately 600 colonies of mesophilic bacteria and obtained 77 strains (13%) that exhibited a distinct dependence on high levels of CO_2. Table 1 summarizes the phylogenetic affiliations of the isolates based on the 16S rRNA gene sequence. The result indicated that these isolates were affiliated with 19 species (isolates exhibiting sequence identities of 99% or more were classified as the same species; the names of representative strains are listed in Table 1). Among these, eight species exhibited low scores (<97%) for sequence identity to their closest identified relatives, suggesting that these isolates belonged to new taxa. The distinct taxonomic position of these isolates could also be inferred from the phylogenetic tree (see Fig. S2A in the supplemental material).

The 19 species mentioned above were affiliated with four bacterial classes (*Bacilli*, *Alphaproteobacteria*, *Betaproteobacteria*, and *Actinobacteria*). This implies that the high-CO_2 requirement occurs in bacteria belonging to various taxa. The most frequently obtained species (29 isolates) showed a marked similarity with regard to the 16S rRNA gene to *Yonghaparkia alkaliphila*. *Yonghaparkia* is a genus of *Actinobacteria* that has been proposed recently based on the isolation of *Y. alkaliphila*, which is the only species included in this genus (26). The two strains belonging to this species were described as dependent on alkaline pH but not on high levels of CO_2; however, the medium used for the cultivation of this bacterium contains 1% carbonate (http://www.dsmz.de/microorganisms/). It is possible that the high concentration of carbonate fulfils the CO_2 requirement of this bacterium and enables its cultivation under ambient air.

We also carried out a similar screening study by using neutral pH samples. The composition of the isolation medium was the same as that described above, except that the pH was adjusted to 7.0 by using NaOH. In contrast to isolation from alkaline pH samples, the isolation of bacteria dependent on high CO_2 levels from neutral pH samples was inefficient; almost all the colonies that appeared on the isolation medium were those of bacteria that did not require high levels of CO_2 for their growth. We succeeded in obtaining only seven strains dependent on high CO_2 levels after examining approximately 3,000 colonies (0.2%). These isolates, however, exhibited low 16S rRNA gene sequence identities with their closest identified relatives (91 to 97.7%; Table 1) (see also Fig. S2B in the supplemental material). This suggests that the neutral pH samples also have potential as effective sources for isolation of unknown bacteria that require high levels of CO_2, although an effective selective isolation method needs to be developed.

The successful isolation of taxonomically distinct bacterial strains from conventional samples reinforces the hypothesis that some microorganisms remain unidentified since they are dependent on high CO_2 levels. This in turn suggests that cultivation upon feeding CO_2 may become an effective method to isolate new microorganisms. The frequent occurrence of bacteria dependent on high CO_2 levels in the alkaline samples may be due to the high solubility of inorganic carbon in alkaline pH. Alkaline environments could be a favorable niche for microorganisms dependent on high CO_2 levels.

The requirement for an atmosphere with high levels of CO_2 is known to be a phenotype of carbonic anhydrase (enzyme catalyzing interconversion between CO_2 and bicarbonate) (EC 4.2.1.1) knockout mutants of some microorganisms such as *Ralstonia eutropha* (11), *Escherichia coli* (10, 13), *Corynebacterium glutamicum* (14), and *Saccharomyces cerevisiae* (1). These mutants are unable to grow under ambient air but grow under an atmosphere with high levels of CO_2. Carbonic anhydrase is probably involved in the acquisition of bicarbonate in nonphototrophic microorganisms. Strains that retain carbonic anhy-

TABLE 1. Phylogenetic characteristics of the isolates

Representative strain	Accession no.	Total no. of isolates	Colony appearance[a]	Class	Closest cultured relative		Closest GenBank relative	
					Species or strain (accession no.)	Similarity (%)	Species, strain, or clone (accession no.)	Similarity (%)
Alkaline pH isolates								
YT1101	AB362828	2	T	*Bacilli*	*Paenibacillus alkaliterrae* KSL-134 (AY960748)	91.8	Uncultured synthetic wastewater bacterium tmbr11-6 (AF30981)	93.1
YT0311	AB362830	1[b]	T	*Bacilli*	*Virgibacillus carmonensis* (AJ316302)	92.8	*Virgibacillus carmonensis* (AJ316302)	92.8
YT0039	AB362823	2[b]	T	*Bacilli*	*Paenibacillus campinasensis* (AB073187)	93.5	*Paenibacillus* sp. strain MB 2039 (AY257871)	94.6
YT0003	AB362821	1[c]	T	*Bacilli*	*Paenibacillus curdlanolyticus* (AB073202)	95.4	*Paenibacillus curdlanolyticus* (AB073202)	95.4
YT0011	AB362822	2	T	*Bacilli*	*Paenibacillus agaridevorans* (AJ345023)	95.9	*Paenibacillus* sp. strain DSM 6358 (AJ345018)	96.1
YT0095	AB362825	2[b]	W, s	*Alphaproteobacteria*	*Paracoccus versutus* (AY014175)	96.4	Uncultured alpha proteobacterium clone MTAF47 (AJ619069)	97.9
YT0001	AB362827	4	T	*Bacilli*	"*Paenibacillus thailandensis*" (AB265205)	96.6	"*Paenibacillus thailandensis*" (AB265205)	96.6
YT0099	AB362826	1[c]	T	*Betaproteobacteria*	*Leptothrix discophora* (L33974)	96.6	Uncultured *Aquabacterium* sp. clone J-21 (AF522998)	98.9
YT0315	AB362832	2	Y, c	*Bacilli*	*Geobacillus thermoglucosidasius* MH403 (DQ350831)	97.3	Uncultured compost bacterium clone 0B15 (DQ345468)	97.5
YT0164	AB376080	2	G	*Bacilli*	*Geobacillus thermoglucosidasius* MH403 (DQ350831)	97.9	Uncultured compost bacterium clone 0B15 (DQ345468)	98.1
YT0027	AB362829	2	Y	*Bacilli*	*Bacillus halmapalus* DSM 8723 (X76447)	97.9	*Bacillus* sp. strain KSM-KP43 (AB055093)	98.7
YT0042	AB362831	1	T	*Bacilli*	*Bacillus massiliensis* (DQ350816)	97.9	*Bacillus massiliensis* (DQ350816)	97.9
YT0047	AB376087	1	LY	*Actinobacteria*	*Yonghaparkia alkaliphila* KSL-133 (DQ256088)	98.3	*Yonghaparkia alkaliphila* KSL-133 (DQ256088)	98.3
YT0066	AB376081	11	W	*Actinobacteria*	*Microbacterium schleiferi* (Y17237)	98.4	*Microbacterium* sp. strain KV-483 (AB234026)	99.7
YT0620	AB376082	3	Y, c	*Actinobacteria*	*Microbacterium aurum* (Y17229)	98.5	*Microbacterium* sp. strain KV-492 (AB234028)	99.4
YT0611	AB376083	1	B	*Actinobacteria*	*Microbacterium schleiferi* (Y17237)	98.5	*Microbacterium* sp. strain R1 (AY974047)	99.5
YT0147	AB376084	2	T	*Bacilli*	*Paenibacillus daejeonensis* (AF290916)	98.8	Uncultured bacterium clone AKIW499 (DQ129244)	98.9
YT0045	AB376085	8	LY	*Actinobacteria*	*Yonghaparkia alkaliphila* KSL-133 (DQ256088)	99.1	Gram-positive bacterium strain 12-8 (AB008510)	99.5
YT0006	AB376086	29	LY	*Actinobacteria*	*Yonghaparkia alkaliphila* KSL-133 (DQ256088)	99.6	*Yonghaparkia alkaliphila* KSL-133 (DQ256088)	99.6
Neutral pH isolates								
YT0021	AB362776	2	LY	*Sphingobacteria*	*Niastella yeongjuensis* (DQ244076)	91	Uncultured lake bacterium S10.23 (AY752132)	91.5
YT0325	AB362779	1	T	*Gammaproteobacteria*	*Tolumonas auensis* (X92889)	93.9	*Tolumonas auensis* (X92889)	93.9
YT0023	AB362777	2	W	*Alphaproteobacteria*	*Agrobacterium vitis* (AB118158)	95.3	*Agrobacterium vitis* (AB118158)	95.3
YT0017	AB376088	1[c]	LY	*Betaproteobacteria*	*Pseudomonas saccharophila* (AF368755)	97	Antarctic bacterium strain R-8875 (AJ440994)	98
YT0136	AB362778	1	Y	*Alphaproteobacteria*	*Sphingomonas subterranea* (AB025014)	97.7	Uncultured bacterium clone BY14 (DQ494790)	99.2

[a] T, transparent; W, white; Y, yellow; LY, light yellow; B, brown; s, shiny; c, creamy.
[b] These strains lost clear dependence on high CO_2 after successive cultivation.
[c] This strain did not recover from freezing storage at −80°C.

drase can generate sufficient bicarbonate for growth from environmental CO_2 by catalytic conversion and supply it to bicarbonate-dependent enzymes such as phosphoenolpyruvate carboxylase and acetyl-coenzyme A carboxylase; hence, they can grow even under ambient air that contains low levels of CO_2 (0.035%). On the other hand, microbial strains lacking this enzyme do not initiate growth unless they are supplied with a high concentration of bicarbonate; hence, they cannot

grow under ambient air but can grow under an atmosphere with high levels of CO_2 by utilizing the bicarbonate that is generated to maintain natural equilibrium.

Recently, we showed, by cocultivation with *Bacillus subtilis*, that an *E. coli* carbonic anhydrase mutant grew under ambient air, probably due to the CO_2/bicarbonate generated by the growth of *B. subtilis* (24). This suggests that bacteria dependent on high CO_2 levels can grow in commensal situations in the natural environment despite lacking carbonic anhydrase. This hypothesis is reinforced by our observation that none of the isolates obtained in this study exhibited carbonic anhydrase activity in an activity staining assay (15) (data not shown). Currently, however, we should be careful about the negative correlation between the occurrence of microorganisms dependent on high CO_2 levels and the presence of carbonic anhydrase. This conventional enzyme assay may not be sufficiently sensitive to detect weak activities although the activity staining successfully detected *E. coli* carbonic anhydrase activity with a high intensity. In addition, we observed that the growth of the isolates under ambient air was not restored by the addition of palmitic acid, arginine, and uracil, which are known products of bicarbonate-dependent metabolism (data not shown). This is inconsistent with the knowledge that supplying these substances partially restores the growth of a carbonic anhydrase mutant of *S. cerevisiae* under ambient air (1).

Another possibility is that high levels of CO_2 serve as a signal that stimulates the germination of spores or the propagation of dormant cells. It is known that, at high levels, CO_2 induces spore germination in *Clostridium botulinum* (25) and *Streptomyces viridochromogenes* (9) spp. High levels of CO_2 can be an indicator of nutrition content; hence, it is reasonable for microbial cells to break their dormant state and initiate proliferation upon sensing high levels of CO_2. In this case, an atmosphere with high levels of CO_2 may need to be supplied for their isolation but not for cultivation after isolation. Actually, we observed that some isolates lost their dependence on high levels of CO_2 during successive cultivations (corresponding to the three species indicated in Table 1). Such isolates, however, may also be unknown strains since their isolation is difficult unless the screening culture is cultivated under an atmosphere with high levels of CO_2. A detailed understanding of the reason underlying the high-CO_2 requirement may help in developing culture techniques for the mining of new microorganisms.

We thank David Hopwood for his helpful insights.

This study was supported by The High-Tech Research Center Project; a Grant-In-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan; and grants from Noda Institute for Scientific Research and Kyowa Hakko Kogyo.

REFERENCES

1. **Aguilera, J., J. P. Van Dijken, J. H. De Winde, and J. T. Pronk.** 2005. Carbonic anhydrase (Nce103p): an essential biosynthetic enzyme for growth of *Saccharomyces cerevisiae* at atmospheric carbon dioxide pressure. Biochem. J. **391:**311–316.
2. **Bringel, F., and J. C. Hubert.** 2003. Extent of genetic lesions of the arginine and pyrimidine biosynthetic pathways in *Lactobacillus plantarum*, *L. paraplantarum*, *L. pentosus*, and *L. casei*: prevalence of CO_2-dependent auxotrophs and characterization of deficient *arg* genes in *L. plantarum*. Appl. Environ Microbiol. **69:**2674–2683.
3. **Burns, B. P., S. L. Hazell, and G. L. Mendz.** 1995. Acetyl-CoA carboxylase activity in *Helicobacter pylori* and the requirement of increased CO_2 for growth. Microbiology **141:**3113–3118.
4. **Cole, J. R., B. Chai, R. J. Farris, Q. Wang, S. A. Kulam, D. M. McGarrell, G. M. Garrity, and J. M. Tiedje.** 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res. **33:**D294–D296.
5. **Dehority, B. A.** 1971. Carbon dioxide requirement of various species of rumen bacteria. J. Bacteriol. **105:**70–76.
6. **Diaz, P. I., P. S. Zilm, and A. H. Rogers.** 2002. *Fusobacterium nucleatum* supports the growth of *Porphyromonas gingivalis* in oxygenated and carbon-dioxide-depleted environments. Microbiology **148:**467–472.
7. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.
8. **Field, M. F., and H. C. Lichstein.** 1958. Growth stimulating effect of autoclaved glucose media and its relationship to the CO_2 requirement of propionibacteria. J. Bacteriol. **76:**485–490.
9. **Grund, A. D., and J. C. Ensign.** 1978. Role of carbon dioxide in germination of spores of *Streptomyces viridochromogenes*. Arch. Microbiol. **118:**279–288.
10. **Hashimoto, M., and J. Kato.** 2003. Indispensability of the *Escherichia coli* carbonic anhydrases YadF and CynT in cell proliferation at a low CO_2 partial pressure. Biosci. Biotechnol. Biochem. **67:**919–922.
11. **Kusian, B., D. Sultemeyer, and B. Bowien.** 2002. Carbonic anhydrase is essential for growth of *Ralstonia eutropha* at ambient CO_2 concentrations. J. Bacteriol. **184:**5018–5026.
12. **Liu, P.** 1954. Carbon dioxide requirement of group F and minute colony G hemolytic streptococci. J. Bacteriol. **68:**282–288.
13. **Merlin, C., M. Masters, S. McAteer, and A. Coulson.** 2003. Why is carbonic anhydrase essential to *Escherichia coli*? J. Bacteriol. **185:**6415–6424.
14. **Mitsuhashi, S., J. Ohnishi, M. Hayashi, and M. Ikeda.** 2004. A gene homologous to beta-type carbonic anhydrase is essential for the growth of *Corynebacterium glutamicum* under atmospheric conditions. Appl. Microbiol. Biotechnol. **63:**592–601.
15. **Patterson, B. D., C. A. Atkins, D. Graham, and R. B. Wills.** 1971. Carbonic anhydrase: a new method of detection on polyacrylamide gels using low-temperature fluorescence. Anal. Biochem. **44:**388–391.
16. **Perriere, G., and M. Gouy.** 1996. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie **78:**364–369.
17. **Platt, D. J.** 1976. Carbon dioxide requirement of *Neisseria gonorrhoeae* growing on a solid medium. J. Clin. Microbiol. **4:**129–132.
18. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
19. **Shiratori, H., H. Ikeno, S. Ayame, N. Kataoka, A. Miya, K. Hosono, T. Beppu, and K. Ueda.** 2006. Isolation and characterization of a new *Clostridium* sp. that performs effective cellulosic waste digestion in a thermophilic methanogenic bioreactor. Appl. Environ Microbiol. **72:**3702–3709.
20. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
21. **Tuttle, D. M., and H. W. Scherp.** 1952. Studies on the carbon dioxide requirement of *Neisseria meningitidis*. J. Bacteriol. **64:**171–182.
22. **Ueda, K., and T. Beppu.** 2007. Lessons from studies of *Symbiobacterium thermophilum*, a unique syntrophic bacterium. Biosci. Biotechnol. Biochem. **71:**1115–1121.
23. Reference deleted.
24. **Watsuji, T. O., T. Kato, K. Ueda, and T. Beppu.** 2006. CO_2 supply induces the growth of *Symbiobacterium thermophilum*, a syntrophic bacterium. Biosci. Biotechnol. Biochem. **70:**753–756.
25. **Wynne, E. S., and J. W. Foster.** 1948. Physiological studies on spore germination, with special reference to *Clostridium botulinum*. III. Carbon dioxide and germination, with a note on carbon dioxide and aerobic spores. J. Bacteriol. **55:**331–339.
26. **Yoon, J. H., S. J. Kang, P. Schumann, and T. K. Oh.** 2006. *Yonghaparkia alkaliphila* gen. nov., sp. nov., a novel member of the family *Microbacteriaceae* isolated from an alkaline soil. Int. J. Syst. Evol. Microbiol. **56:**2415–2420.