Gut of Higher Termites as a Niche for Alkaliphiles as Shown by Culture-Based and Culture-Independent Studies

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We screened alkaliphilic bacteria from the first proctodaeal region (P1) of the hindgut, which is known to show high alkalinity and K⁺ richness, of several species of higher termites. Phylogenetic analyses based on the 16S rDNA sequence revealed that most isolates were affiliated with known alkaliphilic bacilli, frequently isolated from soil. Though many physiological characteristics of the isolated strains were similar to those of soil alkaliphilic bacilli, some strains showed a distinctive NaCl sensitivity. Many strains grew better in an alkaline medium containing K₂CO₃ than one containing Na₂CO₃. One strain, closely related to the xylanolytic and alkaliphilic *Paenibacillus* sp. SM-XY60 from the soil-feeding termite *Sinocapritermes muthae*, was also isolated. Culture-independent analysis showed that bacteria closely related to our isolated alkaliphiles widely inhabit the termite gut. Our results also suggested that some alkaliphilic bacilli in soil share ecological niches in the termite gut.

Key words: alkaliphile, termite, *Bacillus*, *Paenibacillus*, niche

Termites harbor an abundance and diversity of microbes in their gut. The symbiotic association of gut microbiota with termites contributes to the conversion of several kinds of organic matter and the control of carbon and nitrogen fluxes in semiarid and humid ecosystems⁴. Recent culture-independent molecular approaches have been revealing the microbial diversity and community structure of the termite gut ecosystem^{10,20,22}. Termites are ordinarily divided into two groups, higher and lower termites. Higher termites (the family Termitidae) show variety in their feeding behavior and have a more complicated gut structure than lower termites²¹. The gut of higher termites comprises compart-

ments including the midgut, first proctodaeal segment (P1), main hindgut (P3), colon (P4a and P4b), and rectum (P5) sections of the hindgut (see Fig. 1), which show different physicochemical conditions. The P1 region of higher termites, especially that of the subfamily Termitinae, generally shows high alkalinity (pH 10–12)^{2,5}, and is also stationary anoxic in spite of a continuous oxygen supply through the gut epithelium¹⁵. The function of the elevated pH in the P1 region has been long obscure. Recent studies with soil-feeding termites have shown that the extreme alkalinity in the P1 region, combined with autoxidative processes, facilitates the transformation of organic substances and increases their solubility^{13,14,15}. The transformation in the P1 region seems to be largely due to a chemical reaction rather than a biological one because the number of microorganisms was

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Fig. 1. The gut structure of *Termes comis*. M (midgut and mixed segment); P1 (first proctodaeal segment); P3 (main hindgut); P4a/b (colon); P5 (rectum).

estimated to be much lower in the extremely alkaline P1 region than the other gut regions¹⁴.

We expected to find alkaliphiles in the P1 region of the gut, which utilize solubilized or released parts of organic matter and contribute to further transformation. Ohkuma *et al.* has reported the isolation of an alkaliphilic and xylanolytic *Paenibacillus* from soil-feeding *Sinocapritermes mushae* in the accompanying report²³. To obtain more ecological and physiological information on alkaliphiles in the termite gut, we screened bacteria from the P1 section of various kinds of higher termites in alkaline and K⁺-rich conditions that mimic the gut environment, and examined properties including phylogenetic position, growth, and several hydrolytic activities.

Materials and Methods

Bacterial isolation and cultivation

Higher termites (Termitidae), *Pericapritermes latignathus* (Termitinae, soil-feeding), *Amitermes longignathus* (Termitinae, soil-feeding), *Termes comis* (Termitinae, soil/wood-interface), *Microcerotermes crassus* (Termitinae, wood-feeding), and *Speculitermes* sp. (Apicotermatinae, feeding unknown) were collected from Thailand. They belong to the subfamily Termitinae or Apicotermatinae which shows a defined P1 region². The P1 sections of their guts were gathered using forceps. The collected gut sections were homogenized in sterile distilled water, spread onto alkaline-K⁺ medium (1 g/l yeast extract, 1 g/l (NH₄)₂SO₄, 1 g/l K₂HPO₄, 0.2 g/l MgSO₄·7H₂O, 10 g/l K₂CO₃, and 10 g/l starch, pH 10.5), and incubated at 30°C for 3 days. Isolated strains were routinely cultivated in the alkaline-K⁺ medium at 30°C. For the growth tests in Fig. 4, 10 g/l K₂CO₃ in the alkaline-K⁺ medium was replaced with 0.1 M K₂CO₃ or Na₂CO₃ for alkaline conditions (pH 10.5) and 0.2 M KCl or NaCl for neutral conditions (pH 7). Finally, 15 g/l of agar was added for agar medium.

DNA extraction from P1 sections

DNA was extracted from the P1 gut sections using a combination of the ISOPLANT II kit (Nippon Gene Co., Tokyo, Japan) and DNeasy Tissue kit (QIAGEN K.K., Tokyo, Japan) according to the manufacturer's instructions. The guts of termites, *P. latignathus*, *T. comis*, *M. crassus*, and *Speculitermes* sp., were dissected with sterile, fine-tipped forceps on the agarose, and approximately 10 pieces of P1 sections were collected into a sterile 1.5-ml tube in ice. The gut sections were gently crushed using a pestle in 300 μl of solution I of ISOPLANT II, and processed according to the directions supplied with the kit. The supernatant containing nucleic acids after centrifugation was treated with 1 mg/ml of RNase A, and a half volume of pure ethanol was added for purification of DNA using the DNeasy spin column.

16S rDNA analysis

The procedures of PCR amplification and sequencing were mostly described in Ohkuma *et al.*²³ PCR was performed using the primers 27F and 1392R under conditions of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (2 min at 72°C) for 25 cycles. Approximately 720 bp of the 16S rDNA clones were sequenced with primer EUB750R (5'-TACCAGGGTATCTAATCC-3'), and analyzed by BLAST 2.0¹ to find the closest phylogenetic neighbors. Sequences were aligned using CLUSTAL X1.8²⁵. The region corresponding to positions 50–795 in *E. coli* (J01695) was used for the alignment. A neighbor-joining tree⁷ of the aligned sequences was constructed using the PHYLIP package version 3.5c⁶. The tree topology was tested statistically by bootstrap resampling²⁴ 1000 times. Seven representative strains (S23, T31, T32, T41, A62, A63, and A64) were determined for the whole sequence of the 16S rDNA clone. For culture-independent analysis, 16S rDNA fragments (approximately 1.3 kb) were amplified by PCR using primers 27F and BAC1300R. Template DNAs were prepared from the P1 sections of *P. latignathus*, *T. comis*, *M. crassus*, and *Speculitermes* sp., collected from the same nest as used for bacterial isolation. Then, 9 or 10 independent clones were picked and sequenced with EUB750R after cloning into pGEM-T vector (Promega, Madison, WI, USA).

Sporulation

Cells were grown in modified 2×SG sporulation medium (16 g/l Difco nutrient broth, 1 g/l KCl, 1 mM MgSO₄, 1 mM Ca(NO₃)₂, 1 mM FeSO₄, 10 mM MnCl₂, and 1 g/l

glucose)¹⁶⁾ adjusted to pH 7, 8, 9, or 10 with KOH. The number of spores was examined as the number of CFU on the alkaline-K⁺ plates after heat treatment (80°C, 15 min).

Substrate utilization

Carbon utilization, hydrolysis of starch and gelatin, and nitrate reduction were examined using API50CHB/E and API20E kits (Biomérieux Inc., Durham, NC, USA) after cells were grown on tryptic soy agar (Difco laboratories, Sparks, MD, USA), as recommended by the provider. Strains T41 and A62 were grown on the alkaline-K⁺ medium, because the two showed poor growth on tryptic soy agar. The hydrolysis of casein, xylan and carboxy methyl-cellulose (CMC) was examined based on the formation of a halo on alkaline-K⁺ and neutral-K⁺ agar plates that include casein, xylan or CMC instead of starch. In the case of CMC, halo formation was determined after staining with 0.1% of congo-red followed by two washes with 1 M NaCl.

Nucleotide sequence accession numbers

All the nucleotide sequences of 16S rDNA determined in this study have been submitted to the DDBJ/EMBL/GenBank databases under accession number AB111932–AB111938 (the 7 representative strains), AB111968–111204 (16S rDNA clones), and AB112005–112018 (the other 14 isolated strains).

Results

Phylogenetic affiliation of isolated strains based on partial 16S rDNA sequence

We successfully isolated 21 bacterial strains under alkaline conditions with K₂CO₃ from the P1 gut sections of several species of higher termites; 4 strains from *P. latignathus* (designated as P strain), 4 strains from *A. longignathus* (A strain), 8 strains from *T. comis* (T strain), 2 strains from *M. crassus* (M strain), and 3 strains from *Speculitermes* sp. (S strain). All strains except strain A63 were affiliated with the genus *Bacillus* in the phylogenetic analysis based on partial 16S rDNA sequences (Fig. 2). They were classified into 6 groups and each clustered with known alkaliphilic *Bacillus*; 8 strains (P121, S22, T31, T42, T43, T44, T46, M51, underlined is the representative, see below) with *Bacillus* sp. DSM 8717¹⁹⁾; 6 strains (P13, P14, T32, T45, A61, S21) with *B. gibsonii* or *B. horikoshii*¹⁹⁾; strains P15 and S23 with *B. clausii*¹⁹⁾; strains A62 and M52 with *B. horti*²⁰⁾; strain A64 with *B. halmapalus*¹⁹⁾; and strain T41 with *Bacillus* sp. N-1⁸⁾. The percent identities were 97.4 to 99.5%. Strain A63 was closest to *Paenibacillus* sp. SM-XY60, which was iso-

lated from the gut of soil-feeding *Sinocapritermes mushae* (described in the accompanying paper²³⁾), and next closest to *P. lautus*^{9,18)}. Since the strains in a group showed similar colony morphologies and growth properties, we selected representative strains, T31, T32, T41, S23, A62, A63, and A64, from each group and characterized then further.

Detection of alkaliphilic *Bacillus*-related bacteria using a culture-independent approach

Though bacterial diversity and communities in the termite gut ecosystem have been studied extensively, almost no bacilli clones have been detected^{10,22)}. We developed a primer BAC1300 from an alignment of 16S rDNAs of alkaliphilic bacilli and our 7 representative strains (Fig. 3). This primer was expected to detect specifically *B. clausii*, *B. gibsonii*, *B. horikoshii*, *Bacillus* sp. DSM 8714 and strains S23 and T32. The specificity of primer BAC1300R was checked using the Probe Match program on the Ribosomal Database Project (RDP) web site (<http://rdp.genes.nig.ac.jp/>). As shown in Fig. 2, many clones amplified with the specific primer were clustered with the above targeted species and also with *Bacillus* sp. DSM 8717 and strains T31 and A63, in spite that there was not a complete match between the target and primer sequences (see Fig. 3). The frequent detection of T31-related clones may reflect a significant population in the termite gut communities. Our successful detection of 16S rDNA clones closely related to our isolates suggested that these groups of bacilli indeed exist in the termite gut. Clones not clustered in Fig. 2 were hits for the following species: *Desulfotobacterium metallireducens* (85.6% identity, 2 clones) and *Burkholderia* sp. (98.3%, 1 clone) from *T. comis*; *Simonsiella muelleri* (88.4%, 1 clone) from *M. crassus*; *Micrococcus luteus* (97.6%, 1 clone) and an uncultured bacterium (AF371536) (92.8%, 1 clone) from *Speculitermes* sp. Their amplification was probably because the PCR conditions used were not optimized and the primer specificity was lower than expected.

Growth properties and Physiological characterization of isolated alkaliphiles

We examined growth properties using alkaline (pH 10.5) and neutral (pH 7.0) media containing 0.2 M K⁺ or Na⁺ (Fig. 4). All strains except T41 and A62 grew under both alkaline and neutral conditions, and showed a facultative alkaliphilic phenotype. Strains S23, A63, and A64 required K⁺ ion for their growth at alkaline pH, while strains T31 and T32 did not show such a preference. T41 and A62 grew well only under alkaline-K⁺ conditions and showed a strictly alkaliphilic and K⁺ preferable phenotype. We next determined

Fig. 2. Phylogenetic tree showing positions of 21 isolated strains and PCR clones in relation to *Bacillus* and *Paenibacillus* species based on partial 16S rDNA sequences. The *Paenibacillus* species was used as an outgroup. The isolated strains and PCR clones were classified into 7 clusters. The DDBJ/EMBL/GenBank database accession numbers are shown in parentheses. Strains assigned as alkaliphilic *Bacillus* are shown with asterisks (*). Isolated strains are shown with the first letter of the name of the termite from which they originated in upper case. The seven representative strains are underlined. PCR clones amplified with a specific primer are shown with the first letter of the name of the termite from which they originated in lower case. Bootstrap values above 500 from 1,000 resamplings are shown for each node. The scale bar represents 0.01 substitutions per nucleotide position.

<i>B. alcalophilus</i> (DSM485)	1240	GCGAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1299
<i>B. agaradhaerens</i> (DSM8721)	1256	GCGAGACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1315
<i>B. clarkii</i> (DSM8720)	1257	GCAAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1316
<i>B. cohnii</i> (DSM6307)	1241	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1300
<i>B. halmapalus</i> (DSM8723)	1239	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1298
<i>B. horti</i> (K13)	1250	GCGAA-GCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1308
<i>B. pseudocalophilus</i> (DSM8725)	1240	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1299
<i>B. pseudofirmus</i> (DSM8715)	1241	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1300
<i>B. clausii</i> (DSM8716)	1240	GCGAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1299
<i>B. gibsonii</i> (DSM8722)	1241	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1300
<i>B. horikoshii</i> (DSM8719)	1241	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1300
<i>B. halodurans</i> (DSM8718)	1239	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1298
<i>Bacillus</i> sp. (DSM8714)	1240	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1299
<i>Bacillus</i> sp. (DSM8717)	1237	GCGAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1296
S23	1263	GCGAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1322
T31	1264	GCGAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1323
T32	1268	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1327
T41	1266	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1325
A62	1279	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1338
A63	1268	GCGAAGCCGCGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1328
A64	1265	GCAAAACCGGAGGTGAGCCAATCCCATAAA-GCCATTCT	CAGTTGCGATTGTAGGCTGC	1324

Primer sequence

GAAGCCAATCCCATAAA GCCATTCT

Fig. 3. Alignment of 16S rDNA sequences of alkaliphilic *Bacillus* species and representative strains. Positions are shown according to nucleotide number in the DDBJ/EMBL/GenBank database described in Fig. 1. Bases not identical to the primer sequence are shown in boxes.

Fig. 4. Growth of representative strains in alkaline and neutral medium containing 0.2 M K^+ or Na^+ . Growth was examined by measuring OD_{660} at 6h after inoculation of precultured cells grown in alkaline- K^+ medium into alkaline medium containing 0.2 M K_2CO_3 (filled columns), alkaline medium containing 0.2 M Na_2CO_3 (hatched columns), neutral medium containing 0.2 M KCl (dotted columns), or neutral medium containing 0.2 M NaCl (open columns).

the effect of pH on sporulation of the representative strains in 2×SG sporulation medium. All strains except T31 and A63 showed a more efficient sporulation at pH 9 or 10 than pH 7 (Fig. 5).

Physiological characteristics of the seven representative

Fig. 5. Effect of pH on sporulation. Cells were precultured in alkaline- K^+ and inoculated into 2×SG medium of pH 7 (open columns), 8 (dotted columns), 9 (hatched columns), or 10 (filled columns). Numbers of spores were examined 3 days after inoculation as described in Materials and Methods.

strains are summarized in Table 1. Many characteristics were identical between isolates and their closest relatives, but some variations were observed. Strain T32 was very similar to *B. gibsonii* and *B. horikoshii* in 16S rDNA sequence (99.5% identities to both), and in substrate utilization was more similar to *B. gibsonii* than *B. horikoshii*. However, strain T32 was sensitive to 5% NaCl while both *B. gibsonii* and *B. horikoshii* are tolerant to 8–9% NaCl¹⁹.

Table 1. Characteristics of representative strains in comparison with a reference strain.

Cluster [†]	7		5			2		6		4		3		1	
Characteristics	T31	DSM 8717 ^a	T32	<i>B. gibsonii</i> ^a	<i>B. horikoshii</i> ^a	T41	<i>Bacillus</i> N-1 ^b	S23	<i>B. clausii</i> ^a	A62	<i>B. horti</i> ^c	A64	<i>B. halmapalus</i> ^a	A63	SM-XY60 ^d
Color of colonies	yellow	ND	cream-y	yellow	cream-w	cream-w	cream-w	white	white	white	white	white	cream-w	white	white
Growth:															
at pH 7	+	+	+	+	+	+ ^W	+	+	+	+ ^W	+	+	+	+	+
at pH 10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
in 5% NaCl	+	+	–	+	+	–	+	–	+	–	+	–	–	–	–
Spore formation	+	+	+	+	+	+	ND	+	+	+	+	+	+	+	+
Utilization of:															
Glycerol	+ ^W	+	–	+	+	–	ND	+ ^W	+	–	ND	–	+	–	ND
L-Arabinose	+ ^W	–	+ ^W	+	–	–	ND	+ ^W	+	–	ND	–	–	+ ^W	+
Ribose	+ ^W	–	+ ^W	+	–	–	ND	+ ^W	+	–	+	–	–	+ ^W	+
D-Xylose	+ ^W	–	+ ^W	–	–	–	+	+ ^W	+	–	+	+ ^W	–	+ ^W	+
Galactose	–	+	–	–	–	–	ND	–	+	–	ND	–	–	–	+
Glucose	+	+	+ ^W	+	+	–	+	+	+	–	+	+	+	+ ^W	+
Fructose	+	+	+ ^W	+	+	–	ND	+	+	–	+	+	+	+	+
Mannose	+	+	+ ^W	+	–	–	–	+	+	–	+ ^W	+	+	+ ^W	ND
Rhamnose	–	–	–	–	–	–	ND	+ ^W	+	–	–	–	–	+ ^W	+
Inositol	+ ^W	+	–	–	–	–	ND	–	–	–	–	–	–	+ ^W	–
Sorbitol	–	–	–	–	–	–	–	+	+	–	–	–	–	–	+
Maltose	+	+	+ ^W	+	+	–	ND	+ ^W	+	–	ND	+	+	+	+
Lactose	–	–	+ ^W	+	–	–	ND	+ ^W	+	+ ^W	+ ^W	–	–	+	ND
Melibiose	+	+	+	+	–	–	–	+ ^W	+	–	–	–	–	+ ^W	+
Sucrose	+	+	+ ^W	+	+	–	ND	+	+	–	–	+	+	+ ^W	+
Trehalose	+	+	+	+	+	–	ND	+	+	–	ND	+	+	+	+
Raffinose	+	+	+ ^W	+	–	–	–	+ ^W	+	–	–	–	–	+ ^W	+
Hydrolysis of:															
Starch	+	+	+ ^W	–	+	–	ND	+ ^W	+	+	+	+	+	+	+
Gelatin	+	+	–	+	+	–	–	+	+	+	+	+ ^W	+	–	+
Casein	+	+	+	+	+	+	–	+	+	+	+	+	+	–	+
CMC	–	ND	–	ND	ND	–	+	–	ND	–	ND	–	ND	+	+
Xylan	–	ND	–	ND	ND	–	ND	–	ND	–	ND	–	ND	+	+
Reduction of nitrate	–	–	–	–	–	–	+	–	+	–	+	–	–	–	–
16SrDNA sequence identity	99.4%		99.5%/99.5%			97.4%		98.8%		98.4%		98.7%		99.3%	

[†] Cluster number shown in Figure 1.+, positive reaction; –, negative reaction; +^W, weakly positive; ND, not determined.

For colony color, cream-w, cream white and cream-y, cream yellow.

^a Data from ^aNielsen *et al.*¹⁹⁾.^b Data from Fritze *et al.*⁸⁾.^c Data from Yumoto *et al.*²⁶⁾.^d Data from Ohkuma *et al.*²³⁾ and Ohkuma, M. (unpublished results).

Similar observations were found in the cases of strains T41, S23, A62, and A63. This Na⁺ sensitivity was inconsistent with the general observation that most alkaliphilic bacilli require 1–5% NaCl for growth and tolerate to NaCl at up to 5–10%^{11,19}.

Hydrolytic activities of starch, gelatin, casein, CMC, and xylan were examined (Table 1). Strain A63 was positive for hydrolysis of CMC and xylan, whereas the other 20 isolated strains were negative. Most strains were positive for hydrolysis of starch and casein, which was more highly induced at alkaline pH than neutral pH (data not shown).

Discussion

We successfully isolated several kinds of alkaliphilic *Bacillus* and *Paenibacillus* strains from the alkaline P1 section of the termite gut. We also detected 16S rDNA clones closely related to our isolates by a culture-independent approach. Our results strongly suggested that the isolated alkaliphiles widely inhabit the gut of higher termites and that the termite gut is an ecological niche for alkaliphiles. Though the alkaliphiles from the termite gut showed characteristics similar to those of alkaliphilic bacilli from soil, they mostly showed a remarkable difference in NaCl sensitivity. Some of them also showed a preference for K⁺ to Na⁺ in their growth at alkaline pH, as described in the accompanying paper²³. We consider this unexpected property due to physiological adaptation to a gut environment that is rich in K⁺. Since most alkaliphiles are known to utilize Na⁺/H⁺ antiporters for the control of pH^{11,12}, it would be worth studying how alkaliphiles sensitive to Na⁺ control cytoplasmic pH.

Strains T31 and A63 showed inefficient sporulation at pH 10, suggesting that these types of bacteria exist as vegetative cells rather than spores and actively contribute to the digestion of organic matter in the extremely alkaline gut region. T31-related bacteria seem to exist in termite guts to no small extent (Fig. 2). A63-related bacteria also seem to be widely distributed in guts of termites from Thailand and Japan²³. The T31 strain, as well as the other isolated bacilli, showed casein-hydrolyzing activity under alkaline conditions. It has been recently shown with soil-feeding termites that peptidic components of humic substances as well as structural proteins of plants and bacteria are mineralized and/or transformed during gut passage^{13,14,15}. Thus, it is considered that the casein-hydrolyzing activity of strain T31 may contribute to the degradation of such proteinous compounds partially solubilized by the gut alkalinity in soil-feeding termites. On the other hand, the CMC and xylan-hydrolyzing activity of strain A63 may contribute to diges-

tion of hemicellulosic compounds, as described in the accompanying report²³.

Margulis *et al.* has described spore-forming filamentous bacilli, named *Arthromitus*, in the guts of termites and many other arthropods¹⁷. The *Arthromitus* was identified as *B. cereus*, which is well-known as soil bacilli. They argued that the *Arthromitus* is one symbiotic life stage of bacilli that cycle between arthropod guts and soil¹⁷. It could be that spore-forming alkaliphilic bacilli inhabit the arthropod guts and cycle between the alkaline gut and soil, as in the case of *B. cereus*. This idea may explain why alkaliphiles can be frequently isolated from conventional soils that are not alkaline^{11,12}.

Brune³) reported that most alkaliphiles isolated from the gut of soil-feeding termites were also found in the surrounding soil but gave no description of the isolates. This observation is consistent with our report and could be fully explained if the soil alkaliphiles have one life stage in the termite gut. The species of isolated strains or clones were not correlated with the species or feeding behavior of termites (see Fig. 2). This result would also be reasonable if cycling between the gut and soil occurs.

Concerning salt preference, some termite alkaliphiles showed a sensitivity to NaCl which distinguished them from soil alkaliphilic bacilli. Termite guts also contained alkaliphiles that showed a salt preference similar to that of soil alkaliphilic bacilli, such as strain T31. As soil alkaliphiles have been isolated and characterized mostly using sodium-rich media, studies of alkaliphiles in relation to potassium are very rare. In fact, we found colonies that grew on a K₂CO₃-containing alkaline medium from the surrounding soil, the number of which was at least as large as that grown on Na₂CO₃-containing medium (data not shown). The soil may potentially contain many alkaliphiles that prefer K⁺ to Na⁺ similar to our isolates.

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